

LAB PROTOCOL

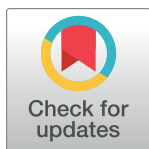
'From women for women': A citizen science approach engaging women in the isolation and application of the vaginal health-associated bacterium *Lactobacillus crispatus*

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Abstract

A vaginal microbiome rich in *Lactobacillus crispatus* is associated with good reproductive and sexual health outcomes. Dysbiosis, indicated by the loss of *Lactobacillus crispatus*, is a risk factor for urogenital infections, such as the clinical diagnosis of bacterial vaginosis (BV) or urinary tract infections. While many scientists have explored probiotics using a conventional pharmaceutical approach, concerns about accessibility and affordability prompt an investigation into a preventive approach using this naturally occurring bacterium. Our study aimed to explore a potential woman-friendly vaginal probiotic product using the naturally occurring bacterium, *Lactobacillus crispatus*. Citizen scientists actively participated in a two-day practicum and successfully performed the procedures using self-collected vaginal swabs. The practicum received positive responses from participants who demonstrated notable engagement and enthusiasm. With expert guidance, participants without a laboratory background were able to execute assigned tasks successfully. From the Dutch *crispatus* Citizen Science Collective of 48 women, 22 succeeded in isolating their own *Lactobacillus crispatus* strains using a Loop-Mediated Isothermal Amplification (LAMP) protocol for identification. Additionally, 48 metagenomes and 54 whole genomes from 22 individuals were sequenced for comparative analysis. This project effectively engaged a community of women in the isolation of *Lactobacillus crispatus* strains from their vaginal microbiota, followed by *in vitro* characterization experiments and a hackathon for the development of a probiotic product. Our citizen science approach opens up collaboration possibilities and new avenues for exploration of vaginal health, facilitating community involvement and the development of targeted interventions to enhance women's well-being.

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Competing interests: Authors R. Hertzberger and R. Kort are unpaid board members of the non-profit organization stichting crispatus. In this capacity, they oversee communication and represent the citizen scientists without receiving any additional pay, benefits, or gifts. It is important to note that this policy is in place to maintain the integrity and credibility of our work. The authors declare that they have no financial or personal interests that could be perceived as influencing the objectivity of this research. The other authors declare no competing interests. The authors declare that they adhere to PLOS ONE policies on sharing data and materials, and have no financial or personal interests that could be perceived as influencing the objectivity of this research.

Introduction

Studies have shown that a vaginal microbiome rich in *Lactobacillus crispatus* is associated with good reproductive and sexual health outcomes, such as a reduced risk of preterm births and urogenital infections. These bacteria are able to degrade and grow on glycogen present in the vagina [1, 2], and produce inhibitory substances such as lactic acid, hydrogen peroxide and bacteriocins [3], which curbs the growth of other organisms that would otherwise cause an infection [4].

A loss of *Lactobacillus* dominance indicates a state of dysbiosis, that is, an overgrowth of anaerobic bacteria and high species diversity in the vagina. Dysbiosis is a risk factor for urogenital infections such as the clinical diagnosis of bacterial vaginosis (BV) or urinary tract infection [5–7]. BV is associated with an increased risk of acquiring and transmitting not only bacterial STD's such as Chlamydia and Gonorrhea, but also viral infections, such as HIV, HSV, and HPV [7–9]. While a diverse collection of bacteria is desirable for good health, in the gut, the inverse is true for the vagina [7, 10].

Antibiotics are often prescribed to women experiencing adverse health effects owing to imbalances in the vaginal microbiome. Many scientists have explored the potential of probiotics, which are live biotherapeutic products based on bacteria, such as *L. crispatus*, as an alternative treatment for BV. This shift is motivated by concerns regarding the disruptive effect of antibiotics on the healthy microbiome of the body [11]. These probiotics are currently being developed using a conventional pharmaceutical approach. As a result, these products are protected by intellectual property, financially supported by venture capital, and geared towards distribution through prescription-dependent medical channels.

The pharmaceutical route raises concerns regarding accessibility and affordability for women seeking the effective prevention of serious health problems. We also question the appropriateness of this approach for a product that utilizes a naturally occurring bacterium abundantly present in the bodies of women and is likely to have a preventive rather than a curative effect.

The primary goal of this project is to gain a deeper understanding of the bacteria in the vaginal flora, specifically focusing on the subtle differences among *L. crispatus* bacteria. Additionally, we aim to collect *L. crispatus* bacteria to investigate the possibility that these bacteria colonize the vagina of other women who do not possess the bacteria. Based on previous studies, we anticipate that these bacteria may help prevent infections [12]. We aim to create a woman-friendly vaginal probiotic that could help prevent urogenital infections by developing a multi-strain over-the-counter probiotic that is accessible to everyone and has long-lasting colonization.

For this purpose, we asked citizen scientists to culture, identify, and isolate *L. crispatus*. We conducted a two-day practicum during which participants collected vaginal swabs, examined their own vaginal microbiota under a microscope, inoculated agar plates, and after three to seven days of incubation, received guidance in recognizing *L. crispatus* colonies. Subsequently, utilizing the loop-mediated isothermal amplification method (LAMP) [13, 14], they identified and isolated colonies of vaginal *L. crispatus* strains on fresh agar plates.

To account for their research, citizen scientists collectively constitute a group of authors. Additionally, participants form a donor panel within the *crispatus* foundation that has the right to, among other things, accept or reject new subcultures of isolated *L. crispatus* strains per majority vote. They were also invited to participate in a hackathon aimed at developing a probiotic product. Together, these aspects establish a platform where the lived experiences of donors inform the collaboration between them and scientists, which in turn fosters responsibility in relation to *L. crispatus* bacteria and future users of vaginal probiotics.

Materials and method

Human participants for the citizen science in this study were recruited from April 30 to September 1, 2023. A written informed consent was obtained from all participants. Citizen scientists actively participated in a two-day practicum, successfully performing procedures with self-collected vaginal swabs, including the isolation of *L. crispatus* by selective enrichment on nutrient agar plates, species identification by Loop-Mediated Isothermal Amplification (LAMP), microscopy, and pH measurements. The detailed protocol described in this peer-reviewed article is published on protocols.io, [[dx.doi.org/10.17504/protocols.io.81wgbzdkygp/v1](https://doi.org/10.17504/protocols.io.81wgbzdkygp/v1)] and is included as supporting information file 1 with this article.

Ethics statement

The Medical Ethics Committee (METC) of the Vrije Universiteit, Amsterdam reviewed the research protocol (Reference 2022.0791) and concluded that the Dutch Medical Research Involving Human Subjects Act—in Dutch: wet medisch-wetenschappelijk onderzoek met mensen (WMO) does not apply to this research protocol ('not subject to WMO').

Conclusion

The citizen scientists successfully cultivated, identified, and isolated *L. crispatus* following the instructions provided to them. We isolated *L. crispatus* strains from 22 of 48 women aged between 18 and 45 years. This achievement underscores that with proper guidance, we can make certain scientific practices accessible to a wider audience without compromising the quality of research.

This citizen science project raised awareness of vaginal health within the community and fostered open discussions between scientists and participants. Moreover, it successfully encouraged enthusiastic engagement from participants, whose feedback concretely shaped the practicum, license agreement, and this article. As a result, our citizen science approach opened up collaboration possibilities and new avenues for exploration of vaginal health, facilitating community involvement and the development of targeted interventions to enhance women's well-being.

Expected results

Research findings have highlighted a correlation between the abundance of *L. crispatus* and female reproductive hormone levels. Given that estrogen and progesterone levels significantly decrease in postpartum women, we hypothesized that isolating *Lactobacillus crispatus* during this period would be challenging [15]. This pattern was also observed in postmenopausal women.

A study conducted in Amsterdam on the prevalence of *Lactobacillus* dominance in the vaginal microbiota of women aged 18–34, indicated that approximately 60% of the population was dominated by *Lactobacillus* spp, including *L. crispatus*, *L. iners*, *L. gasseri*, and *L. jensenii* [16]. We expected similar results in the present study.

We also understand that low vaginal pH, attributed to the production of lactic acid, along with the observation of rod-shaped bacteria under light microscopy, serves as an indicator of the presence of *Lactobacillus* spp.

This citizen-science-based lab protocol allows the isolation of *Lactobacillus crispatus* strains from women, followed by the characterization of inter- and intra-individual differences between genotypes and phenotypes of these strains and product development for vaginal health. This citizen science initiative including a cultivation-based approach for the isolation

of *Lactobacillus crispatus* is complementary to a recent initiative that included cultivation-independent analysis of the vaginal microbiomes of 3,345 women, leading to a further understanding of the composition and function of the vaginal microbiome [17].

Supporting information

S1 File. Step-by-step protocol, also available on [[dx.doi.org/10.17504/protocols.io.81wgbzdkygp/v1](https://doi.org/10.17504/protocols.io.81wgbzdkygp/v1)].
(PDF)

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Author Contributions

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Funding acquisition: Remco Kort.

Investigation: Shardelice Illidge, Rosanne Hertzberger.

Methodology: Shardelice Illidge.

Supervision: Remco Kort.

Writing – original draft: Shardelice Illidge.

Writing – review & editing: Remco Kort, Rosanne Hertzberger.

References

1. 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 Jan; 23(10):5590. <https://doi.org/10.3390/ijms23105590> PMID: 35628398
2. van der Veer C, Hertzberger RY, Bruisten SM, Tytgat HLP, Swanenburg J, de Kat Angelino-Bart A, et al. Comparative genomics of human *Lactobacillus crispatus* isolates reveals genes for glycosylation and glycogen degradation: implications for in vivo dominance of the vaginal microbiota. *Microbiome*. 2019 Mar 29; 7(1):49. <https://doi.org/10.1186/s40168-019-0667-9> PMID: 30925932
3. Argentini C, Fontana F, Alessandri G, Lugli GA, Mancabelli L, Ossiprandi MC, et al. Evaluation of Modulatory Activities of *Lactobacillus crispatus* Strains in the Context of the Vaginal Microbiota. *Visca P*,

- editor. *Microbiol Spectr*. 2022 Apr 27; 10(2):e02733–21. <https://doi.org/10.1128/spectrum.02733-21> PMID: 35266820
4. France M, Alizadeh M, Brown S, Ma B, Ravel J. Towards a deeper understanding of the vaginal microbiota. *Nat Microbiol*. 2022 Mar; 7(3):367–78. <https://doi.org/10.1038/s41564-022-01083-2> PMID: 35246662
 5. van Teijlingen NH, Helgers LC, Zijlstra-Willems EM, van Hamme JL, Ribeiro CMS, Strijbis K, et al. Vaginal dysbiosis associated-bacteria *Megasphaera elsdenii* and *Prevotella timonensis* induce immune activation via dendritic cells. *J Reprod Immunol*. 2020 Apr; 138:103085. <https://doi.org/10.1016/j.jri.2020.103085> PMID: 32004804
 6. Dols JAM, Molenaar D, van der Helm JJ, Caspers MPM, de Kat Angelino-Bart A, Schuren FHJ, et al. Molecular assessment of bacterial vaginosis by *Lactobacillus* abundance and species diversity. *BMC Infect Dis*. 2016 Apr 23; 16:180. <https://doi.org/10.1186/s12879-016-1513-3> PMID: 27107961
 7. van der Veer C, Hertzberger RY, Bruisten SM, Tytgat HLP, Swanenburg J, de Kat Angelino-Bart A, et al. Comparative genomics of human *Lactobacillus crispatus* isolates reveals genes for glycosylation and glycogen degradation: implications for in vivo dominance of the vaginal microbiota. *Microbiome*. 2019 Mar 29; 7(1):49. <https://doi.org/10.1186/s40168-019-0667-9> PMID: 30925932
 8. Abou Chacra L, Ly C, Hammoud A, Iwaza R, Mediannikov O, Bretelle F, et al. Relationship between Bacterial Vaginosis and Sexually Transmitted Infections: Coincidence, Consequence or Co-Transmission? *Microorganisms*. 2023 Oct 1; 11(10):2470. <https://doi.org/10.3390/microorganisms11102470> PMID: 37894128
 9. Coudray MS, Madhivanan P. Bacterial vaginosis-A brief synopsis of the literature. *Eur J Obstet Gynecol Reprod Biol*. 2020 Feb; 245:143–8. <https://doi.org/10.1016/j.ejogrb.2019.12.035> PMID: 31901667
 10. Deng F, Li Y, Zhao J. The gut microbiome of healthy long-living people. *Aging*. 2019 Jan 15; 11(2):289–90. <https://doi.org/10.18632/aging.101771> PMID: 30648974
 11. Webb L. Probiotics for preventing recurrent bacterial vaginosis. *JAAPA Off J Am Acad Physician Assist*. 2021 Feb 1; 34(2):19–22. <https://doi.org/10.1097/01.JAA.0000731484.81301.58> PMID: 33448711
 12. DE Leo V, Lazzeri E, Governini L, Cuppone AM, Colombini L, Teodori L, et al. Vaginal colonization of women after oral administration of *Lactobacillus crispatus* strain NTCVAG04 from the human microbiota. *Minerva Obstet Gynecol*. 2023 Oct; 75(5):432–9. <https://doi.org/10.23736/S2724-606X.22.05087-4> PMID: 35686637
 13. Higashide S, Cho O, Matsuda Y, Ogishima D, Kurakado S, Sugita T. Rapid detection of *Lactobacillus crispatus* and *Lactobacillus iners* in vaginal specimens by loop-mediated isothermal amplification. *J Microbiol Methods*. 2019 Mar; 158:18–20. <https://doi.org/10.1016/j.mimet.2019.01.011> PMID: 30682384
 14. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res*. 2000 Jun 15; 28(12):E63. <https://doi.org/10.1093/nar/28.12.e63> PMID: 10871386
 15. Odogwu NM, Onebunne CA, Chen J, Ayeni FA, Walther-Antonio MRS, Olayemi OO, et al. *Lactobacillus crispatus* thrives in pregnancy hormonal milieu in a Nigerian patient cohort. *Sci Rep*. 2021 Sep 13; 11(1):18152. <https://doi.org/10.1038/s41598-021-96339-y> PMID: 34518588
 16. Borgdorff H, van der Veer C, van Houdt R, Alberts CJ, de Vries HJ, Bruisten SM, et al. The association between ethnicity and vaginal microbiota composition in Amsterdam, the Netherlands. *PLOS ONE*. 2017 Jul 11; 12(7):e0181135. <https://doi.org/10.1371/journal.pone.0181135> PMID: 28700747
 17. Lebeer S, Ahannach S, Gehrman T, Wittouck S, Eilers T, Oerlemans E, et al. A citizen-science-enabled catalogue of the vaginal microbiome and associated factors. *Nat Microbiol*. 2023 Oct 26; 8(11):2183–95. <https://doi.org/10.1038/s41564-023-01500-0> PMID: 37884815