

Supplementary Information

Vaginal formulations selectively target *Lactobacillus iners* and *Candida* spp. while preserving *Lactobacillus crispatus*

Francesco Di Pierro, Valeria Sagheddu, Serena Galletti, Alice Casaroli, Sara Soldi, Alexander Bertuccioli, Chiara Maria Palazzi, Roberto Senatori, Andrea Carugno, Maxim Olegovich Antipov, Nikolay Ivanovich Briko, Nicola Zerbinati

Supplementary Methods

Clinical observation and ethical considerations

A single longitudinal clinical observation was used to explore the translational consistency of the in vitro findings. Previously issued reports from serial vaginal microbiota analyses and two endometrial microbiota analyses, prescribed during the patient's assisted reproductive care, were reviewed retrospectively. The source data were the final diagnostic reports issued to the patient by the external laboratories. The present clinical analysis used these reported results without obtaining or reanalyzing patient biological samples or raw sequencing data. The methodological descriptions below concern the diagnostic procedures underlying the reports. The clinical history, therapeutic interventions and microbiological sampling time points are summarized in Supplementary Table S7. The observation was reported according to the CARE guidelines [35]. Written informed consent was obtained for the analysis and publication of the anonymized clinical and microbiological data.

Vaginal microbiota analysis

Vaginal swabs were collected in sterile tubes containing 1 mL DNA/RNA Shield (Zymo Research, Irvine, CA, USA) and stored until bacterial DNA extraction. Total DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit according to the manufacturer's instructions (Zymo Research). The V3 region of the bacterial 16S rRNA gene was amplified using the Probio_Uni/Probio_Rev primer pair [36]. Illumina adapter overhang sequences were added to the amplicons, and sequencing libraries were prepared according to the 16S Metagenomic Sequencing Library Preparation protocol (Part No. 15044223 Rev. B; Illumina, San Diego, CA, USA). Amplification was performed using a Veriti Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Amplicon integrity was assessed using a 2200 TapeStation instrument (Agilent Technologies, Santa Clara, CA, USA), and PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter Genomics, Bernried, Germany). Library concentrations were determined using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Amplicons were normalized to 4 nM, pooled in equal volumes and sequenced using an Illumina MiSeq instrument with MiSeq Reagent Kit v3 chemistry. Paired-end reads were processed using a custom pipeline based on QIIME [37]. Read pairs were assembled to reconstruct the complete Probio_Uni/Probio_Rev amplicons. Quality control retained sequences 140–400 bp in length with a mean Phred quality score >20. Reads containing homopolymers >7 bp or mismatched primer sequences were removed. Amplicon sequence variants, reported in the analytical reports as operational taxonomic units (OTUs), were inferred at single-nucleotide resolution using DADA2 [38]. Features represented by fewer than two reads within the same sample were excluded. Taxonomic classification was performed to the lowest supported rank using QIIME 2 [39], the q2-feature-classifier plugin [40] and the SILVA reference database [41]. Within-sample richness was expressed as the

number of observed OTUs. Species-level profiling of *Lactobacillus* was performed by amplifying the internal transcribed spacer between the 16S and 23S rRNA genes using the Probio-lac_Uni/Probio-lac_Rev primer pair [42]. Library preparation and sequencing were performed as described for the bacterial 16S rRNA analysis. ITS reads 100–400 bp in length with a mean Phred quality score >20 were retained, whereas reads containing homopolymers >7 bp or mismatched primers were removed. ITS sequences were clustered at 100% sequence identity using UCLUST [43] and classified against the curated *Lactobacillus* ITS reference database [42]. Community state types were assigned according to the predominant *Lactobacillus* species [1,2]. Because the species-level *Lactobacillus* analysis reports the distribution of species within the total *Lactobacillus* population, the relative abundance of each species within the entire bacterial community was calculated as follows: Relative abundance of the *Lactobacillus* species in the total community (%) = total *Lactobacillus* relative abundance (%) × proportion of the species within the *Lactobacillus* population (%) / 100. All longitudinal comparisons were based on these recalculated whole-community relative abundances.

Endometrial microbiota analysis

Endometrial-fluid collection and microbiota analysis were performed according to the procedure described by Moreno et al. [44]. With the patient in the lithotomy position, the cervix was cleansed using a sterile cotton swab. Approximately 20–80 µL of endometrial fluid was aspirated using a sterile transcervical catheter (Wallace; Smiths Medical International, Ashford, UK) introduced into the uterine cavity without contact with the vaginal walls. To minimize potential contamination, cervical mucus was aspirated before endometrial-fluid recovery, and suction was discontinued at the internal cervical os during catheter withdrawal. Bacterial DNA was extracted using the MagNa Pure Compact Nucleic Acid Isolation Kit I (Roche, Madison, WI, USA). Before automated extraction, bacterial cell-wall digestion was enhanced by incubating the sample with 50 µL lysozyme at 50 mg/mL (Sigma, Dorset, UK) and bacterial lysis buffer (Roche) for 30 min at 37 °C. Proteinase K digestion, enzyme inactivation and DNA purification were subsequently performed according to the manufacturer's protocol. Extracted DNA was quantified using the Quant-iT PicoGreen DNA Assay (Invitrogen, Carlsbad, CA, USA). The V3–V5 region of the bacterial 16S rRNA gene was amplified using key-tagged eubacterial primers and subjected to unidirectional pyrosequencing using a 454 GS FLX+ instrument (Roche). Reads with a Phred quality score ≤20 were removed. Primer and sequencing-adaptor sequences were trimmed, reads shorter than 300 nucleotides were excluded and chimeric sequences were removed. QIIME [37] was used for downstream processing, and sequences were clustered into OTUs at 97% identity using UCLUST [43]. Taxonomic assignment was performed using the Ribosomal Database Project classifier. Genus-level assignments supported by no more than two reads or having a mean classifier score <0.9 were excluded. Endometrial microbiota profiles were classified as *Lactobacillus*-dominated when *Lactobacillus* represented >90% of the bacterial community and as non-*Lactobacillus*-dominated

when its relative abundance was <90%, with >10% of the community represented by other bacterial taxa [44].

Supplementary Table S1. API 50 CH carbohydrate-utilization profiles of the tested *Lactobacillus* strains.

A positive symbol indicates detectable utilization at one or both readings (24 and/or 48 h); a negative symbol indicates no detectable utilization at either reading.

***L. crispatus* strains**

Substrate	M247	SS	SG	DC	DCL
Water	–	–	–	–	–
Glycerol	–	–	–	–	–
Erythritol	–	–	–	–	–
D-arabinose	–	–	–	–	–
L-arabinose	–	–	–	–	–
D-ribose	–	–	–	–	–
D-xylose	–	–	–	–	–
L-xylose	–	–	–	–	–
D-adonitol	–	–	–	–	–
Methyl-β-D-xylopyranoside	–	–	–	–	–
D-galactose	+	+	+	+	+
D-glucose	+	+	+	+	+
D-fructose	+	+	+	+	+
D-mannose	–	+	–	–	–
L-sorbose	–	–	–	–	–
L-rhamnose	–	–	–	–	–
Dulcitol	–	–	–	–	–
Inositol	–	–	–	–	–
D-mannitol	+	–	–	–	–
D-sorbitol	–	–	–	–	–
Methyl-α-D- mannopyranoside	–	–	–	–	–
Methyl-α-D- glucopyranoside	–	–	–	–	–
N-acetylglucosamine	+	+	+	+	+
Amygdalin	–	–	–	–	–
Arbutin	–	–	–	–	–
Esculin ferric citrate	–	+	–	+	+
Salicin	–	+	–	+	+

Substrate	M247	SS	SG	DC	DCL
D-cellobiose	+	+	–	+	+
D-maltose	+	+	+	+	+
D-lactose	+	+	+	+	+
D-melibiose	–	–	–	+	–
Sucrose	+	+	+	+	+
D-trehalose	+	+	–	+	–
Inulin	–	–	–	–	–
D-melezitose	–	–	–	–	–
D-raffinose	–	–	–	–	–
Starch	+	+	–	–	+
Glycogen	+	+	–	–	–
Xylitol	–	–	–	–	–
Gentiobiose	–	–	–	–	–
D-turanose	–	–	–	–	–
D-lyxose	–	–	–	–	–
D-tagatose	–	–	–	–	–
D-fucose	–	–	–	–	–
L-fucose	–	–	–	–	–
D-arabitol	–	–	–	–	–
L-arabitol	–	–	–	–	–
Potassium gluconate	–	–	–	–	–
Potassium 2- ketogluconate	–	–	–	–	–
Potassium 5- ketogluconate	–	–	–	–	–

***L. iners* strains**

Substrate	LMG 18913	LMG 18914T	LMG 18915	LMG 18916	LMG 14328
Water	-	-	-	-	-
Glycerol	-	-	-	-	-
Erythritol	-	-	-	-	-
D-arabinose	-	-	-	-	-
L-arabinose	-	-	-	-	-
D-ribose	-	-	-	-	-
D-xylose	-	-	-	-	-
L-xylose	-	-	-	-	-
D-adonitol	-	-	-	-	-
Methyl- β -D-xylopyranoside	-	-	-	-	-
D-galactose	-	-	-	-	-
D-glucose	+	+	+	+	+
D-fructose	-	+	-	-	-
D-mannose	-	+	+	-	-
L-sorbose	-	-	-	-	-
L-rhamnose	-	-	-	-	-
Dulcitol	-	-	-	-	-
Inositol	-	-	-	-	-
D-mannitol	-	-	-	-	-
D-sorbitol	-	-	-	-	-
Methyl- α -D- mannopyranoside	-	-	-	-	-
Methyl- α -D- glucopyranoside	-	-	-	-	-
N-acetylglucosamine	-	+	-	-	-
Amygdalin	-	-	-	-	-
Arbutin	-	-	-	-	-
Esculin ferric citrate	-	-	-	-	-
Salicin	-	-	-	-	-
D-cellobiose	-	-	-	-	-
D-maltose	+	+	+	+	+
D-lactose	+	+	-	-	-
D-melibiose	-	-	-	-	-
Sucrose	-	-	-	-	-
D-trehalose	-	-	-	-	-
Inulin	-	-	-	-	-

Substrate	LMG 18913	LMG 18914T	LMG 18915	LMG 18916	LMG 14328
D-melezitose	-	-	-	-	-
D-raffinose	-	-	-	-	-
Starch	-	+	-	-	-
Glycogen	+	+	-	+	+
Xylitol	-	-	-	-	-
Gentiobiose	-	-	-	-	-
D-turanose	-	-	-	-	-
D-lyxose	-	-	-	-	-
D-tagatose	-	-	-	-	-
D-fucose	-	-	-	-	-
L-fucose	-	-	-	-	-
D-arabitol	-	-	-	-	-
L-arabitol	-	-	-	-	-
Potassium gluconate	-	-	-	-	-
Potassium 2- ketogluconate	-	-	-	-	-
Potassium 5- ketogluconate	-	-	-	-	-

Supplementary Table S2. API ZYM enzymatic-activity scores of the tested *Lactobacillus* strains.

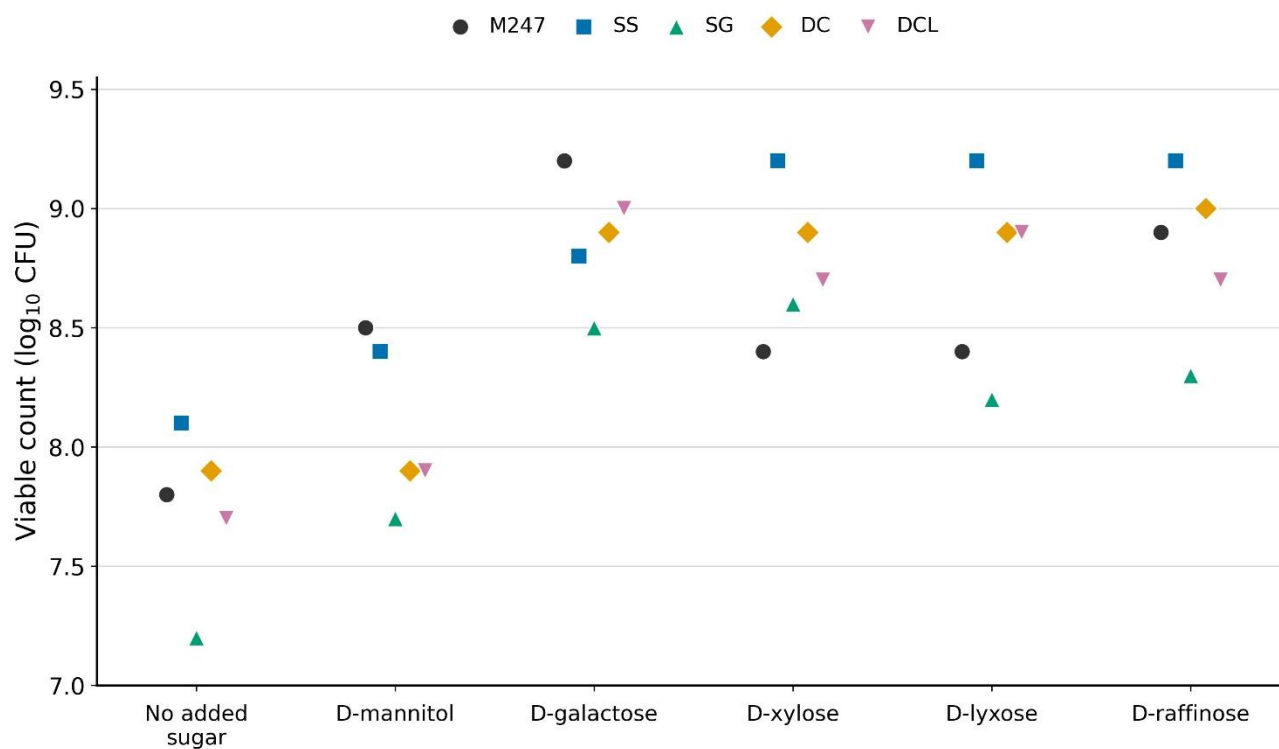
Scores were assigned from the manufacturer's color chart after 4 h of incubation. Enzymatic activity was considered positive at a score of 3 or higher.

***L. crispatus* strains**

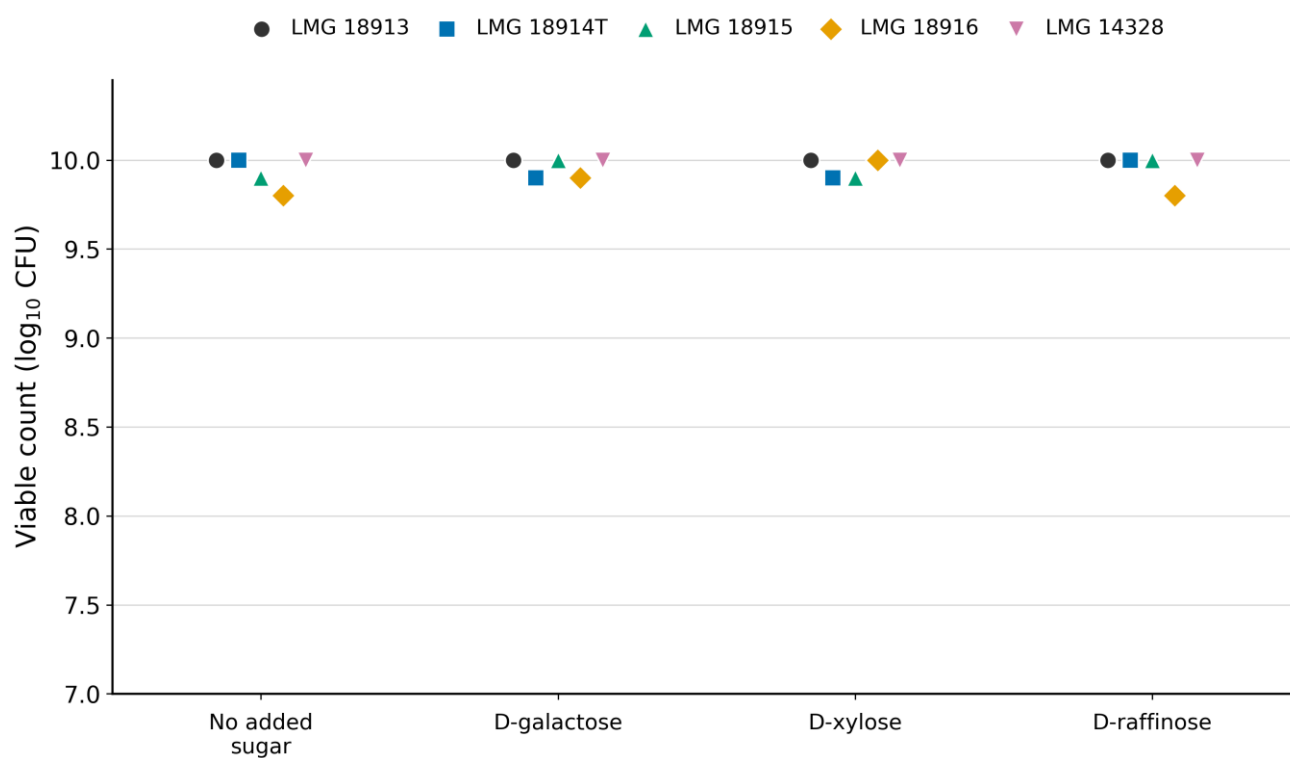
Enzyme	M247	SS	SG	DC	DCL
Alkaline phosphatase	0	3	0	0	0
Esterase (C4)	1	1	0	1	0
Esterase lipase (C8)	0	1	0	0	0
Lipase (C14)	0	0	0	0	0
Leucine arylamidase	3	5	3	4	4
Valine arylamidase	0	0	0	0	0
Cystine arylamidase	0	0	0	0	0
Trypsin	0	0	0	0	0
α -chymotrypsin	0	0	0	0	0
Acid phosphatase	1	5	2	1	1
Naphthol-AS-BI-phosphohydrolase	4	3	3	4	3
α -galactosidase	0	0	0	0	0
β -galactosidase	3	5	2	5	4
β -glucuronidase	0	0	0	0	0
α -glucosidase	2	3	1	4	1
β -glucosidase	0	3	2	4	2
N-acetyl- β -glucosaminidase	0	0	0	0	0
α -mannosidase	0	0	0	0	0
α -fucosidase	0	0	0	0	0

***L. iners* strains**

Enzyme	LMG 18913	LMG 18914T	LMG 18915	LMG 18916	LMG 14328
Alkaline phosphatase	0	1	1	1	0
Esterase (C4)	4	3	3	3	3
Esterase lipase (C8)	3	3	1	0	4
Lipase (C14)	0	1	1	2	0
Leucine arylamidase	5	5	4	3	4
Valine arylamidase	2	2	1	1	2
Cystine arylamidase	2	2	1	1	2
Trypsin	0	1	0	0	0
α -chymotrypsin	0	1	1	1	0
Acid phosphatase	1	2	2	2	1
Naphthol-AS-BI-phosphohydrolase	3	5	1	1	3
α -galactosidase	0	1	2	1	0
β -galactosidase	0	1	1	2	0
β -glucuronidase	0	1	0	0	0
α -glucosidase	4	5	3	4	3
β -glucosidase	0	1	1	1	0
N-acetyl- β -glucosaminidase	0	1	2	0	0
α -mannosidase	0	1	1	2	0
α -fucosidase	0	1	0	1	0



Supplementary Figure S1. Endpoint viable counts of *Lactobacillus crispatus* strains in sugar-free MRS supplemented with selected carbohydrates. Viable counts were determined after 24 h of microaerophilic incubation at 37 °C. Each symbol represents one strain under the indicated condition. DCL corresponds to the strain designated DC smooth in the laboratory report. Values were reconstructed from the strain-level plots supplied in the analytical report.



Supplementary Figure S2. Endpoint viable counts of *Lactobacillus iners* strains in improved sugar-free MRS supplemented with selected carbohydrates. Viable counts were determined after 48 h of anaerobic incubation at 37 °C. The improved MRS medium contained 1% defibrinated horse blood. Each symbol represents one strain under the indicated condition. Values were reconstructed from the strain-level plots supplied in the analytical report.

Supplementary Table S3. Dependence of *Lactobacillus iners* replication on defibrinated horse blood.

Strain	Improved sugar-free MRS with 1% defibrinated horse blood	Same medium without defibrinated horse blood
LMG 18913	Replication detected	No detectable replication
LMG 18914T	Replication detected	No detectable replication
LMG 18915	Replication detected	No detectable replication
LMG 18916	Replication detected	No detectable replication
LMG 14328	Replication detected	No detectable replication

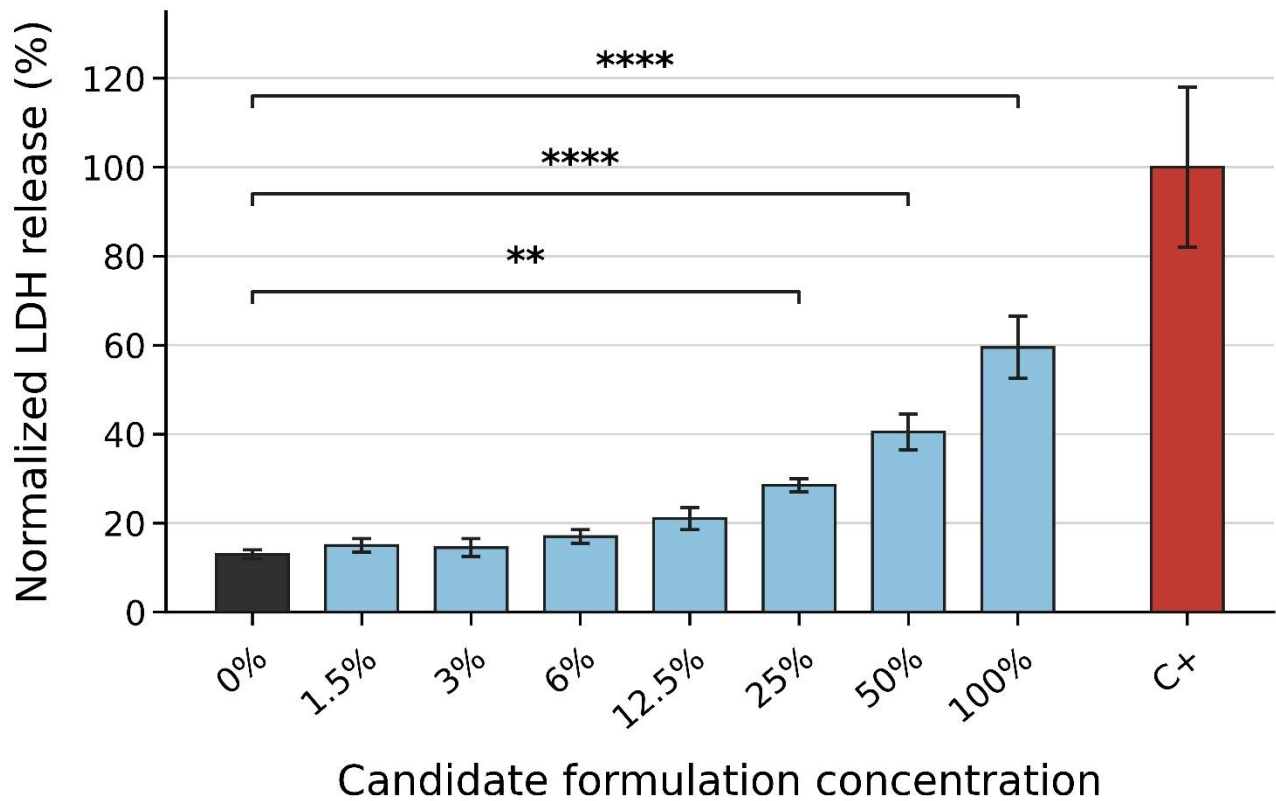
The analytical report provided a qualitative replication outcome for the blood-omission experiment; numerical viable counts were not reported for the blood-free condition.

Supplementary Table S4. Viability of *Lactobacillus crispatus* strains after 24 h exposure to OPCs, EDTA, and their combinations.

Candidate ingredient(s)	Nominal concentration	Viable count at 24 h (log ₁₀ CFU/mL)		
		M247	SS	DC
MRS control	–	8.80	9.06	8.94
OPCs	0.5%	8.37	7.74	8.21
OPCs	1%	2.96	3.96	4.72
OPCs	2%	2.86	3.00	3.32
EDTA	0.05%	8.70	8.81	8.74
EDTA	0.1%	8.80	5.93	7.89
OPCs + EDTA	0.25% + 0.05%	7.78	7.98	7.80
OPCs + EDTA	0.25% + 0.1%	7.86	7.12	7.63
OPCs + EDTA	0.5% + 0.05%	7.47	7.39	7.21
OPCs + EDTA	0.5% + 0.1%	6.96	7.03	7.44

Values are viable counts measured after 24 h and are expressed as log₁₀ CFU/mL. MRS, de Man, Rogosa and Sharpe medium; OPCs, oligomeric proanthocyanidins; EDTA, ethylenediaminetetraacetic acid.

Supplementary Figure S3. Concentration-dependent cytotoxicity of the candidate formulation in HeLa cells.



HeLa cervical epithelial cells were exposed for 16 h to serial dilutions of the selected three-component mixture containing 0.25% (w/v) oligomeric proanthocyanidins, 0.05% (w/v) EDTA and 6.4 mM oleic acid at full strength. Boric acid, raffinose and monacolins were not included. The percentages shown on the x-axis indicate dilutions of the complete mixture, with 100% corresponding to the full-strength preparation and 0% to untreated cells. Cytotoxicity was assessed by measuring lactate dehydrogenase (LDH) release into the culture supernatant. Cells treated with lysis buffer served as the maximum-release positive control (C+), normalized to 100%. Data are presented as mean $\pm$ SD from three replicate wells per condition ($n = 3$ technical replicates). Statistical comparisons with untreated cells were performed using one-way ANOVA followed by Dunnett's multiple-comparisons test. ** $P < 0.01$; **** $P < 0.0001$.

Supplementary Table S5. Minimum inhibitory and fungicidal concentrations of selected long-chain fatty acids against *Candida* spp.

Strain	Oleic acid		Linoleic acid		Palmitoleic acid	
	MIC	MFC	MIC	MFC	MIC	MFC
<i>C. albicans</i> MUCL 29903	>6.4	>6.4	>6.4	>6.4	>6.4	>6.4
<i>C. glabrata</i> MUCL 29833	>6.4	>6.4	>6.4	>6.4	>6.4	>6.4
<i>C. krusei</i> MUCL 29849	>6.4	>6.4	>6.4	>6.4	>6.4	>6.4
<i>C. parapsilosis</i> MUCL 29868	>6.4	>6.4	>6.4	>6.4	>6.4	>6.4

Values are expressed in mM. The tested concentration range was 0.012–6.4 mM; >6.4 indicates that the corresponding endpoint was not reached at the highest concentration tested. MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration. Each assay was performed in duplicate.

Supplementary Table S6. Viability of *Lactobacillus crispatus* strains after 24 h exposure to monacolin K and monacolin KA.

Candidate ingredient(s)	Nominal concentration	Viable count at 24 h (log ₁₀ CFU/mL)		
		M247	SS	DC
MRS vehicle control	-	8.80	9.06	8.94
Monacolin K	2.5%	8.36	8.46	8.34
Monacolin KA	2.5%	8.82	8.62	8.45
Monacolin K	5%	8.88	8.55	8.75
Monacolin KA	5%	7.88	8.35	8.18

Values are viable counts measured after 24 h and are expressed as log₁₀ CFU/mL. Nominal concentrations refer to volumetric dilutions of the supplied 1 mg/mL stock solutions and not to weight/volume percentages of pure monacolin. The vehicle control contained the same amount of ethanol used in the corresponding monacolin-treated condition. MRS, de Man, Rogosa and Sharpe medium; KA, hydroxy-acid form of monacolin K.

Supplementary Results

Longitudinal clinical observation during assisted reproductive treatment

A 32-year-old woman with infertility underwent longitudinal vaginal and endometrial microbiota monitoring as part of an assisted-reproduction programme. The purpose of the microbiological assessments was to evaluate whether the vaginal and endometrial environments were suitable for embryo transfer and, when necessary, to guide interventions aimed at restoring a *Lactobacillus*-dominated condition. In January 2024, while awaiting embryo transfer, the patient underwent her first vaginal microbiota analysis. The profile was favourable, with 13 observed OTUs, a total *Lactobacillus* abundance of 95.728%, and *L. crispatus* accounting for 94.973% of the entire bacterial community, corresponding to CST I. As part of the pre-transfer evaluation, the assisted-reproduction centre also requested an endometrial microbiota analysis. Although the endometrial profile was classified as abnormal because *Lactobacillus* represented only 37% of the community, no bacteria associated with chronic endometritis were detected. The remaining community consisted predominantly of Sphingomonadaceae (41%) and other taxa (22%). Because the analysis did not indicate endometritis, no antibiotic was prescribed. The centre instead recommended oral supplementation with *L. rhamnosus* GR-1 and *L. reuteri* RC-14, one capsule daily for 90 days, to increase endometrial lactobacilli and promote a microbiological environment considered more favourable for embryo implantation. In April 2024, the patient developed symptomatic bacterial vaginosis. A vaginal swab performed because of the symptoms indicated frank gardnerellosis, and the second vaginal microbiota analysis produced a concordant result. The community had shifted from CST I to CST IV: *Gardnerella* represented 69.628%, *Atopobium* 19.183%, and total *Lactobacillus* only 0.036%. The patient was treated with metronidazole 250 mg intravaginal ovules once daily for 10 days, after which the vaginal symptoms resolved. In May 2024, the assisted-reproduction centre requested a second endometrial microbiota analysis before reconsidering embryo transfer. The endometrial microbiota remained abnormal and non-*Lactobacillus*-dominated, with 24% *Lactobacillus*, 28% *Gardnerella vaginalis*, 45% Bifidobacteriaceae, and 3% other taxa. In contrast to the first endometrial assessment, the laboratory reported the presence of bacteria associated with chronic endometritis. The patient was therefore treated with oral metronidazole 250 mg three times daily for 14 days, followed by a further three-month course of the oral probiotic containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14. On 2 July 2024, the third vaginal microbiota analysis—the first performed after the two metronidazole treatments—showed a marked transition to CST III. The community had very low richness, with only four observed OTUs, and was almost entirely composed of lactobacilli; however, *L. iners*, rather than *L. crispatus*, represented the dominant species. Total *Lactobacillus* abundance was 99.893%, of which *L. iners* accounted for 99.087% of the entire bacterial community. This post-antibiotic transition from *L. crispatus* dominance to almost complete *L. iners* dominance was consistent with the post-antibiotic enrichment of *L. iners*

reported in previous microbiota studies [10–12]. At the same time, the patient developed symptomatic cystitis associated with *E. coli* at >100,000 CFU/mL. Ciprofloxacin 500 mg twice daily for 3 days was prescribed, and the urinary symptoms resolved within 3 days. After completion of ciprofloxacin, a probiotic containing *L. crispatus* M247 was administered orally once daily and simultaneously by the intravaginal route. Intravaginal administration was performed nightly outside menstruation for 1 month and subsequently every other day for a further 2 months. The objective was to replace the post-antibiotic *L. iners*-dominated community with a more stable *L. crispatus*-dominated vaginal microbiota. Despite this intervention, the fourth vaginal microbiota analysis, performed in November 2024, showed renewed dysbiosis. *Gardnerella* represented 77.844% of the community, total *Lactobacillus* 17.591%, and *L. iners* 16.594%, whereas *L. crispatus* accounted for only 0.020%. A concomitant vaginal swab was positive for fungal infection, although the patient had no mycotic symptoms. She received one commercially available boric acid with polycarbophil vaginal capsule (AB-300; Farmitalia S.r.l., Italy) nightly for 7 days. Oral and alternate-day intravaginal supplementation with *L. crispatus* M247 was continued. On evenings alternating with the intravaginal probiotic, a prototype pro-*L. crispatus* ovule containing 0.25% oligomeric proanthocyanidins and 0.05% EDTA in an extra-virgin olive-oil vehicle was introduced. No monacolins were present in this clinical formulation. In January 2025, the fifth vaginal microbiota analysis showed 114 observed OTUs. *Gardnerella* remained dominant at 67.713%, but *L. iners* had decreased from 16.594% to 0.348%, while *L. crispatus* had increased from 0.020% to 5.695%. The patient reported only mild symptoms compatible with bacterial vaginosis. Because of their limited severity, no additional culture-based vaginal swab was performed and no antibiotic was prescribed. Oral *L. crispatus* M247 and one prototype intravaginal ovule every 3 days were continued. In June 2025, the sixth vaginal analysis showed 18 observed OTUs. *Gardnerella* still represented 73.236% of the community, but total *Lactobacillus* had increased to 25.341% and was entirely represented by *L. crispatus*. *L. iners* was no longer detected. Mild bacterial-vaginosis symptoms were again managed without vaginal swabbing or antibiotic treatment, and oral *L. crispatus* M247 together with one prototype ovule every 3 days was maintained. In December 2025, the seventh vaginal microbiota analysis showed a clear inversion of the previous relationship between *Gardnerella* and *L. crispatus*. The community displayed very low richness, with three observed OTUs. Total *Lactobacillus* abundance had increased to 68.608%, with *L. crispatus* accounting for 68.338% of the entire bacterial community, whereas *Gardnerella* had decreased to 31.386%. *L. iners* remained undetectable, and the patient was asymptomatic. Oral *L. crispatus* M247 and the prototype ovule every 3 days were therefore continued. At the final assessment in April 2026, the eighth vaginal microbiota analysis showed restoration of a CST I profile. Total *Lactobacillus* abundance was 97.777%, and *L. crispatus* represented 96.862% of the entire bacterial community. *Gardnerella* was no longer detected, while *L. iners* remained present only at a minor relative abundance of 0.337%. The patient remained asymptomatic,

and both the oral probiotic and the prototype intravaginal formulation were discontinued. No treatment-related adverse events were reported during the observation period.

Supplementary Table S7. Longitudinal clinical, microbiological, and therapeutic history during assisted reproductive treatment.

Date and assessment	Clinical indication	Microbiological findings	Management and subsequent course
20 Jan 2024 Vaginal analysis 1	Baseline evaluation during assisted reproductive treatment while awaiting embryo transfer.	13 observed OTUs. Total <i>Lactobacillus</i> , 95.728%; <i>L. crispatus</i> , 94.973%. CST I.	No vaginal treatment was required.
31 Jan 2024 Endometrial analysis 1	Assessment of endometrial suitability before embryo transfer.	Abnormal non- <i>Lactobacillus</i> -dominated endometrial microbiota, without bacteria associated with chronic endometritis: <i>Lactobacillus</i> , 37%; Sphingomonadaceae, 41%; other taxa, 22%.	No antibiotic. Oral <i>L. rhamnosus</i> GR-1 plus <i>L. reuteri</i> RC-14, one capsule daily for 90 days, to optimize the endometrial environment before embryo transfer.
22 Apr 2024 Vaginal analysis 2	Symptomatic bacterial vaginosis; concomitant vaginal swab indicated frank gardnerellosis.	20 observed OTUs. <i>Gardnerella</i> , 69.628%; <i>Atopobium</i> , 19.183%; total <i>Lactobacillus</i> , 0.036%. CST IV.	Metronidazole 250 mg intravaginal ovule once daily for 10 days. Vaginal symptoms resolved.
10 May 2024 Endometrial analysis 2	Repeat pre-transfer assessment requested by the assisted-reproduction centre.	Abnormal endometrial microbiota in the presence of bacteria associated with chronic endometritis: <i>Lactobacillus</i> , 24%; <i>Gardnerella vaginalis</i> , 28%; Bifidobacteriaceae, 45%; other taxa, 3%.	Oral metronidazole 250 mg three times daily for 14 days, followed by oral <i>L. rhamnosus</i> GR-1 plus <i>L. reuteri</i> RC-14 for 3 months.
2 Jul 2024 Vaginal analysis 3	First vaginal assessment after the two metronidazole courses. Concomitant symptomatic cystitis with <i>Escherichia coli</i> >100,000 CFU/mL.	4 observed OTUs. Total <i>Lactobacillus</i> , 99.893%; <i>L. iners</i> , 99.087%. CST III.	Ciprofloxacin 500 mg twice daily for 3 days; urinary symptoms resolved. Subsequently, a probiotic containing <i>L. crispatus</i> M247 was given orally once daily and intravaginally nightly outside menstruation for 1 month and every other day for a further 2 months.
11 Nov 2024 Vaginal analysis 4	Assessment after oral and intravaginal <i>L. crispatus</i> M247 administration. Concomitant vaginal swab positive for fungal infection, without mycotic symptoms.	24 observed OTUs. <i>Gardnerella</i> , 77.844%; total <i>Lactobacillus</i> , 17.591%; <i>L. iners</i> , 16.594%; <i>L. crispatus</i> , 0.020%.	AB-300, one intravaginal ovule nightly for 7 days. Oral and alternate-day intravaginal <i>L. crispatus</i> M247 were continued. A prototype pro- <i>L. crispatus</i> ovule was introduced on evenings alternating with the intravaginal probiotic.
31 Jan 2025 Vaginal analysis 5	Assisted-reproduction follow-up. Mild symptoms compatible with bacterial vaginosis; no additional culture-based vaginal swab was performed.	114 observed OTUs. <i>Gardnerella</i> , 67.713%; total <i>Lactobacillus</i> , 6.956%; <i>L. crispatus</i> , 5.695%; <i>L. iners</i> , 0.348%.	No antibiotic. Oral <i>L. crispatus</i> M247 and one prototype intravaginal ovule every 3 days were continued.

Date and assessment	Clinical indication	Microbiological findings	Management and subsequent course
27 Jun 2025 Vaginal analysis 6	Assisted-reproduction follow-up. Mild bacterial-vaginosis symptoms; no additional culture-based vaginal swab was performed.	18 observed OTUs. <i>Gardnerella</i> , 73.236%; total <i>Lactobacillus</i> , 25.341%, entirely represented by <i>L. crispatus</i> ; <i>L. iners</i> not detected.	No antibiotic. Oral <i>L. crispatus</i> M247 and one prototype intravaginal ovule every 3 days were continued.
23 Dec 2025 Vaginal analysis 7	Assisted-reproduction follow-up; patient asymptomatic.	3 observed OTUs. Total <i>Lactobacillus</i> , 68.608%; <i>L. crispatus</i> , 68.338%; <i>Gardnerella</i> , 31.386%; <i>L. iners</i> not detected.	Oral <i>L. crispatus</i> M247 and one prototype intravaginal ovule every 3 days were continued.
Apr 2026 Vaginal analysis 8	Final assisted-reproduction follow-up; patient asymptomatic.	18 observed OTUs. Total <i>Lactobacillus</i> , 97.777%; <i>L. crispatus</i> , 96.862%; <i>L. iners</i> , 0.337%; <i>Gardnerella</i> not detected. CST I.	Oral probiotic and prototype intravaginal formulation discontinued. No treatment-related adverse events were reported during follow-up.

The prototype intravaginal ovule contained 0.25% oligomeric proanthocyanidins and 0.05% EDTA in an extra-virgin olive-oil vehicle. No monacolins were included in the clinical formulation. CST, community state type; CFU, colony-forming units.

Supplementary Table S8. Longitudinal vaginal bacterial-community composition and recalculated whole-community abundances of *Lactobacillus* species.

Date and CST	OTUs	Lactobacilli (%)	<i>Lactobacillus</i> species in the entire bacterial community (%)	Leading non- <i>Lactobacillus</i> taxa in the entire bacterial community (%)
20 Jan 2024 CST I	13	95.728	<i>L. crispatus</i> , 94.973; <i>L. jensenii</i> , 0.318; <i>L. iners</i> , 0.248; <i>L. paracasei</i> , 0.096; <i>L. gasseri</i> , 0.065; <i>L. reuteri</i> , 0.028	<i>Bacteroides</i> , 1.074; Unclassified Lachnospiraceae, 0.822; <i>Faecalibacterium</i> , 0.571; Unclassified Prevotellaceae, 0.297; <i>Eubacterium eligens</i> group, 0.297; <i>Blautia</i> , 0.274; Christensenellaceae R-7 group, 0.274; Unclassified Ruminococcaceae, 0.228; Unclassified BRC1 phylum, 0.160; <i>Alistipes</i> , 0.137; <i>Ruminococcus</i> , 0.137
22 Apr 2024 CST IV	20	0.036	<i>L. gasseri</i> , 0.029; <i>L. backii</i> , 0.005; <i>L. crispatus</i> , 0.003	<i>Gardnerella</i> , 69.628; <i>Atopobium</i> , 19.183; <i>Dialister</i> , 9.652; <i>Prevotella</i> , 0.771; <i>Ureaplasma</i> , 0.361; <i>Howardella</i> , 0.088; <i>Haemophilus</i> , 0.085; <i>Fingoldia</i> , 0.046; <i>Campylobacter</i> , 0.033; <i>Faecalibacterium</i> , 0.033; <i>Bacteroides</i> , 0.030; <i>Fusobacterium</i> , 0.030; <i>Anaerococcus</i> , 0.015
2 Jul 2024 CST III	4	99.893	<i>L. iners</i> , 99.087; <i>L. jensenii</i> , 0.762; <i>L. paracasei</i> , 0.043	<i>Faecalibacterium</i> , 0.058; <i>Gardnerella</i> , 0.049
11 Nov 2024 CST IV	24	17.591	<i>L. iners</i> , 16.594; <i>L. jensenii</i> , 0.976; <i>L. crispatus</i> , 0.020	<i>Gardnerella</i> , 77.844; <i>Bacteroides</i> , 0.796; <i>Faecalibacterium</i> , 0.714; <i>Lachnoclostridium</i> , 0.702; <i>Dialister</i> , 0.632; <i>Streptococcus</i> , 0.257; <i>Corynebacterium</i> , 0.176; <i>Eubacterium eligens</i> group, 0.176; <i>Staphylococcus</i> , 0.176; <i>Veillonella</i> , 0.164; <i>Alistipes</i> , 0.152; <i>Escherichia-Shigella</i> , 0.140; <i>Prevotella</i> , 0.105; <i>Fusobacterium</i> , 0.094; <i>Haemophilus</i> , 0.094; <i>Parabacteroides</i> , 0.082; <i>Pseudomonas</i> , 0.070; <i>Phascolarctobacterium</i> , 0.035
31 Jan 2025 CST IV	114	6.956	<i>L. crispatus</i> , 5.695; <i>L. rhamnosus</i> , 0.459; <i>L. gasseri</i> , 0.359; <i>L. iners</i> , 0.348; <i>L. jensenii</i> , 0.071; <i>L. paracasei</i> , 0.014; <i>L. acidophilus</i> , 0.010; <i>L. reuteri</i> , 0.002	<i>Gardnerella</i> , 67.713; <i>Prevotella</i> , 4.552; <i>Roseburia</i> , 2.330; unclassified Ruminococcaceae, 2.082; <i>Bacteroides</i> , 1.619; unclassified Prevotellaceae, 1.603; Christensenellaceae R-7 group, 1.570; unclassified Lachnospiraceae, 1.421; <i>Alistipes</i> , 1.264; <i>Barnesiella</i> , 0.884; <i>Parabacteroides</i> , 0.876; <i>Ruminococcus</i> , 0.843; <i>Ruminiclostridium</i> , 0.752; <i>Lachnoclostridium</i> , 0.512; <i>Dialister</i> , 0.479; <i>Sutterella</i> , 0.438; <i>Megasphaera</i> , 0.281; <i>Paraprevotella</i> , 0.264
27 Jun 2025 CST IV	18	25.341	<i>L. crispatus</i> , 25.341; <i>L. iners</i> , not detected	<i>Gardnerella</i> , 73.236; <i>Pediococcus</i> , 0.904; <i>Streptococcus</i> , 0.158; <i>Bacteroides</i> , 0.086; <i>Faecalibacterium</i> , 0.061; <i>Prevotella</i> , 0.048; <i>Lachnospira</i> , 0.036; <i>Blautia</i> , 0.023; <i>Alistipes</i> , 0.015; <i>Eubacterium coprostanoligenes</i> group, 0.010;

Date and CST	OTUs	Lactobacilli (%)	<i>Lactobacillus</i> species in the entire bacterial community (%)	Leading non- <i>Lactobacillus</i> taxa in the entire bacterial community (%)
				<i>Megasphaera</i> , 0.008; unclassified Ruminococcaceae, 0.008; <i>Escherichia-Shigella</i> , 0.008; unclassified Prevotellaceae, 0.005; <i>Ruminococcus</i> , 0.005
23 Dec 2025 CST I	3	68.608	<i>L. crispatus</i> , 68.338; <i>L. paracasei</i> , 0.270; <i>L. iners</i> , not detected	<i>Gardnerella</i> , 31.386
Apr 2026 CST I	18	97.777	<i>L. crispatus</i> , 96.862; <i>L. iners</i> , 0.337; <i>L. paracasei</i> , 0.329; <i>L. reuteri</i> , 0.144; <i>L. jensenii</i> , 0.106	<i>Clostridium sensu stricto</i> 1, 2.037; <i>Faecalibacterium</i> , 0.039; <i>Pseudobutyrvibrio</i> , 0.032; <i>Bacteroides</i> , 0.029; <i>Staphylococcus</i> , 0.026; <i>Ureaplasma</i> , 0.026; <i>Fingoldia</i> , 0.013; <i>Peptoniphilus</i> , 0.011; <i>Campylobacter</i> , 0.005; <i>Ruminococcus</i> , 0.005

Species-level *Lactobacillus* abundances were recalculated relative to the entire bacterial community by multiplying total *Lactobacillus* abundance by the within-genus proportion of each species. Values are rounded to three decimal places. Minor differences between total and species-level values reflect rounding or unresolved minor fractions. The table lists the named non-*Lactobacillus* taxa shown among the 20 most abundant taxa in each analytical report; aggregate categories such as ‘other bacteria’ were excluded. CST, community state type; OTU, operational taxonomic unit.