

**Table S1** Primers and amplified DNA fragments

Primers	Sequence	Target sequence	Size of PCR product (bp)	References
L R	5'-TGGAAACAGGTGCTAATACCG-3' 5'-CCATTGTGGAAGATTCCC-3'	<i>Lactobacillus</i> 16 SRNA	230	McOrist <i>et al.</i> 2002
P0 P6	5'-GAGAGTTTGATCCTGGCTCAG-3' 5'-CTACGGCTACCTTGTACGA-3'	Eubacterial 16 SRNA	1 507	Di Cello and Fani, 1996
scr-Rhmn-2Fw scr-Rhmn-2Rv	5'- AAAGTCGTGTATATGTAGCCGG -3' 5'- GAACCGTCTCGTCTTCCAATAG -3'	CRISPR array	562	This study
scr-gass-1Fw scr-gass-1Rv	5'-CCTATTGTCGAACCCTTACGAA-3 5'- GTGCTGAAATGCTTGGTGTAG -3'	CRISPR array	392	This study

**Table S2** *Lactobacillus* strains used to test the primers specificity

<b>Strain</b>	<b>Origin of the strain</b>	<b>Reference</b>
<i>L. gasseri</i> S1011RA2	Vaginal, South Africa	Penharkar <i>et al.</i> 2013 <sup>a</sup>
<i>L. gasseri</i> S1013RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. gasseri</i> S1040RAS3	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. gasseri</i> S1025RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. gasseri</i> S1184RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. gasseri</i> S1196RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. gasseri</i> 16R5	Vaginal, Sweden	Larsson <i>et al.</i> 2011 <sup>b</sup>
<i>L. gasseri</i> 6M7	Vaginal, Sweden	Larsson <i>et al.</i> 2011
<i>L. gasseri</i> 15M9	Vaginal, Sweden	Larsson <i>et al.</i> 2011
<i>L. gasseri</i> 7B1	Vaginal, Sweden	Larsson <i>et al.</i> 2011
<i>L. gasseri</i> 20M39	Vaginal, Sweden	Larsson <i>et al.</i> 2011
<i>L. gasseri</i> 6M9	Vaginal, Sweden	Larsson <i>et al.</i> 2011
<i>L. gasseri</i> 20M37	Vaginal, Sweden	Larsson <i>et al.</i> 2011
<i>L. gasseri</i> 16B1	Vaginal, Sweden	Larsson <i>et al.</i> 2011
<i>L. gasseri</i> E9B4-1	Intestinal, Estonia	Koll <i>et al.</i> 2010 <sup>c</sup>
<i>L. gasseri</i> 177-3	Intestinal, Estonia	Koll <i>et al.</i> 2010
<i>L. gasseri</i> E16B7	Intestinal, Estonia	Koll <i>et al.</i> 2010
<i>L. gasseri</i> E101G2-4-2	Intestinal, Estonia	Koll <i>et al.</i> 2010
<i>L. rhamnosus</i> 16.5.2-1	Vaginal, Sweden	Penharkar <i>et al.</i> 2015 <sup>d</sup>
<i>L. rhamnosus</i> 17.3.2-3	Vaginal, Sweden	Penharkar <i>et al.</i> 2015
<i>L. rhamnosus</i> 23.2.2-1	Vaginal, Sweden	Penharkar <i>et al.</i> 2015
<i>L. rhamnosus</i> GG ATCC 53103	Intestinal, US	Gorbach and Goldin, 1983
<i>L. rhamnosus</i> 35-DLB-5	Oral, Estonia	Koll <i>et al.</i> 2008 <sup>e</sup>
<i>L. rhamnosus</i> 14-DLB-8	Oral, Estonia	Koll <i>et al.</i> 2008
<i>L. rhamnosus</i> 19-DLB-2A	Oral, Estonia	Koll <i>et al.</i> 2008
<i>L. rhamnosus</i> 19-DLB-5	Oral, Estonia	Koll <i>et al.</i> 2008
<i>L. rhamnosus</i> 21-DLB-5B	Oral, Estonia	Koll <i>et al.</i> 2008
<i>L. rhamnosus</i> 21-DLB-7	Oral, Estonia	Koll <i>et al.</i> 2008
<i>L. rhamnosus</i> 23-DLB-2	Oral, Estonia	Koll <i>et al.</i> 2008
<i>L. rhamnosus</i> 23-DLB-5	Oral, Estonia	Koll <i>et al.</i> 2008
<i>L. rhamnosus</i> 23-DLB-6	Oral, Estonia	Koll <i>et al.</i> 2008
<i>L. mucosae</i> S1002RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. paracasei</i> S1002RA4	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. coleohominis</i> S1003RA4S	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. crispatus</i> S1003RA1B	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. crispatus</i> S1040RAS1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. crispatus</i> S1098RAR1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. crispatus</i> S1056RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. crispatus</i> S1083RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. crispatus</i> S1178RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. crispatus</i> S1181RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. crispatus</i> S1182RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. crispatus</i> S1185RA3	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. crispatus</i> S1197RA3	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. vaginalis</i> S1004RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. vaginalis</i> S1008RA1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. vaginalis</i> S1040RAS2	Vaginal, South Africa	Penharkar <i>et al.</i> 2013
<i>L. vaginalis</i> S1098RAP1	Vaginal, South Africa	Penharkar <i>et al.</i> 2013

<i>L. vaginalis</i> S1131RAB1	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. iners</i> S1183BA1	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. iners</i> S1006BA2	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. iners</i> S1008BA4	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. iners</i> S1116BA1	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. ruminis</i> S1002RA9	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. ruminis</i> S1174RA1	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. ruminis</i> S1175RA1	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. jensenii</i> S1014RA1	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. jensenii</i> S1132RA1	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. jensenii</i> S1185RA1	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. jensenii</i> S1196RA3	Vaginal, South Africa	Pendharkar <i>et al.</i> 2013
<i>L. plantarum</i> TU196	Intestinal, Estonia and Sweden	Koll <i>et al.</i> 2010
<i>L. fermentum</i> 338-1-1	Intestinal, Estonia and Sweden	Koll <i>et al.</i> 2010
<i>L. acidophilus</i> 821-3	Intestinal, Estonia and Sweden	Koll <i>et al.</i> 2010

<sup>a</sup> Pendharkar S, Magopane T, Larsson PG, de Bruyn G, Gray GE, Hammarström L, Marcotte H. Identification and characterisation of vaginal lactobacilli from South African women. *BMC Infect Dis.* 2013 Jan 26; 13:43.

<sup>b</sup> Larsson PG, Brandsborg E, Forsum U, Pendharkar S, Andersen KK, Nasic S, Hammarström L, Marcotte H. Extended antimicrobial treatment of bacterial vaginosis combined with human lactobacilli to find the best treatment and minimize the risk of relapses. *BMC Infect Dis.* 2011 Aug 19; 11:223.

<sup>c</sup> Kõll P, Mändar R, Smidt I, Hütt P, Trusalu K, Mikelsaar RH, Shchepetova J, Krogh-Andersen K, Marcotte H, Hammarström L, Mikelsaar M. Screening and evaluation of human intestinal lactobacilli for the development of novel gastrointestinal probiotics. *Curr Microbiol.* 2010 Dec; 61(6):560-6.

<sup>d</sup> Pendharkar S, Brandsborg E, Hammarström L, Marcotte H, Larsson PG. Vaginal colonisation by probiotic lactobacilli and clinical outcome in women conventionally treated for bacterial vaginosis and yeast infection. *BMC Infect Dis.* 2015 Jul 3; 15:255.

<sup>e</sup> Kõll P, Mändar R, Marcotte H, Leibur E, Mikelsaar M, Hammarström L. Characterization of oral lactobacilli as potential probiotics for oral health. *Oral Microbiol Immunol.* 2008 Apr; 23(2):139-47.

**Table S3** Factors associated with bacterial vaginosis during follow-up

<b>Variable</b>	<b>n or median (IQR)</b>	<b>Univariate</b>		<b>Multivariate</b>	
		<b>RR (95% CI)</b>	<b>p-value</b>	<b>RR (95% CI)</b>	<b>p-value</b>
<b>Study Arm</b>					
Healthy + probiotic (Group 1)	13 (33.3)	Ref		Ref	
BV + antibiotic + probiotic (Group 2)	12 (30.8)	5.03 (1.90-13.4)	0.0012	3.60 (1.32-9.81)	0.0123
BV + antibiotic (Group 3)	14 (35.9)	4.90 (1.75-13.7)	0.0025	4.39 (1.59-12.2)	0.0044
<b>Age-Group</b>					
18-29 years	19 (48.7)	Ref			
30-40 years	20 (51.3)	0.92 (0.43-1.95)	0.8277		
<b>Education</b>					
Secondary	33 (84.6)	Ref			
Tertiary	6 (15.4)	0.87 (0.27-2.77)	0.8160		
<b>Employment</b>					
Yes	9 (23.1)	1.61 (0.77-3.35)	0.2084		
No	30 (76.9)	Ref			
<b>Housing type</b>					
Shack	5 (12.8)	0.99 (0.43-2.25)	0.9789		
Brick house	34 (87.2)	Ref			
<b>Alcohol use</b>					
Yes	24 (61.5)	0.86 (0.41-1.80)	0.6811		
No	15 (38.5)	Ref			
<b>Tobacco use</b>					
Yes	12 (30.8)	1.73 (0.84-3.55)	0.1377	1.88 (0.91-3.87)	0.0876
No	27 (69.2)	Ref		Ref	
<b>Abnormal fishy discharge</b>					
Yes	14 (35.9)	1.26 (0.62-2.56)	0.5241		
No	25 (64.1)	Ref			
<b>Ever treated for an STI in the past 12 months</b>					
Yes	4 (10.5)	0.25 (0.07-0.86)	0.0288	0.40 (0.17-0.96)	0.0413
No	34 (89.5)	Ref		Ref	
<b>Ever treated for BV in the past 12 months</b>					
Yes	2 (5.1)	2.07 (1.37-3.12)	0.0005	2.31 (1.16-4.60)	0.0173
No	37 (94.9)	Ref		Ref	

<b>Variable</b>	<b>n or median (IQR)</b>	<b>Univariate</b>		<b>Multivariate</b>	
		<b>RR (95% CI)</b>	<b>p-value</b>	<b>RR (95% CI)</b>	<b>p-value</b>
<b>Sex debut age</b>					
< 18 years	15 (38.5)	Ref			
=> 18 years	24 (61.5)	1.05 (0.47-2.35)	0.9085		
<b>Condom use</b>					
Consistent	6 (15.4)	Ref			
Inconsistent	33 (84.6)	0.57 (0.16-2.07)	0.3942		
<b>Frequency of weekly vaginal sex</b>	2 (1-3)	1.03 (0.77-1.39)	0.8270		
<b>Do you use soap to wash your vagina?</b>					
Yes	17 (43.6)	0.84 (0.38-1.89)	0.6808		
No	22 (56.4)	Ref			
<b>Number of partners</b>	1 (1-1)	0.77 (0.48-1.21)	0.2532		
<b>Number of partners in the past 12 months</b>	1 (1-2)	0.89 (0.56-1.40)	0.6088		

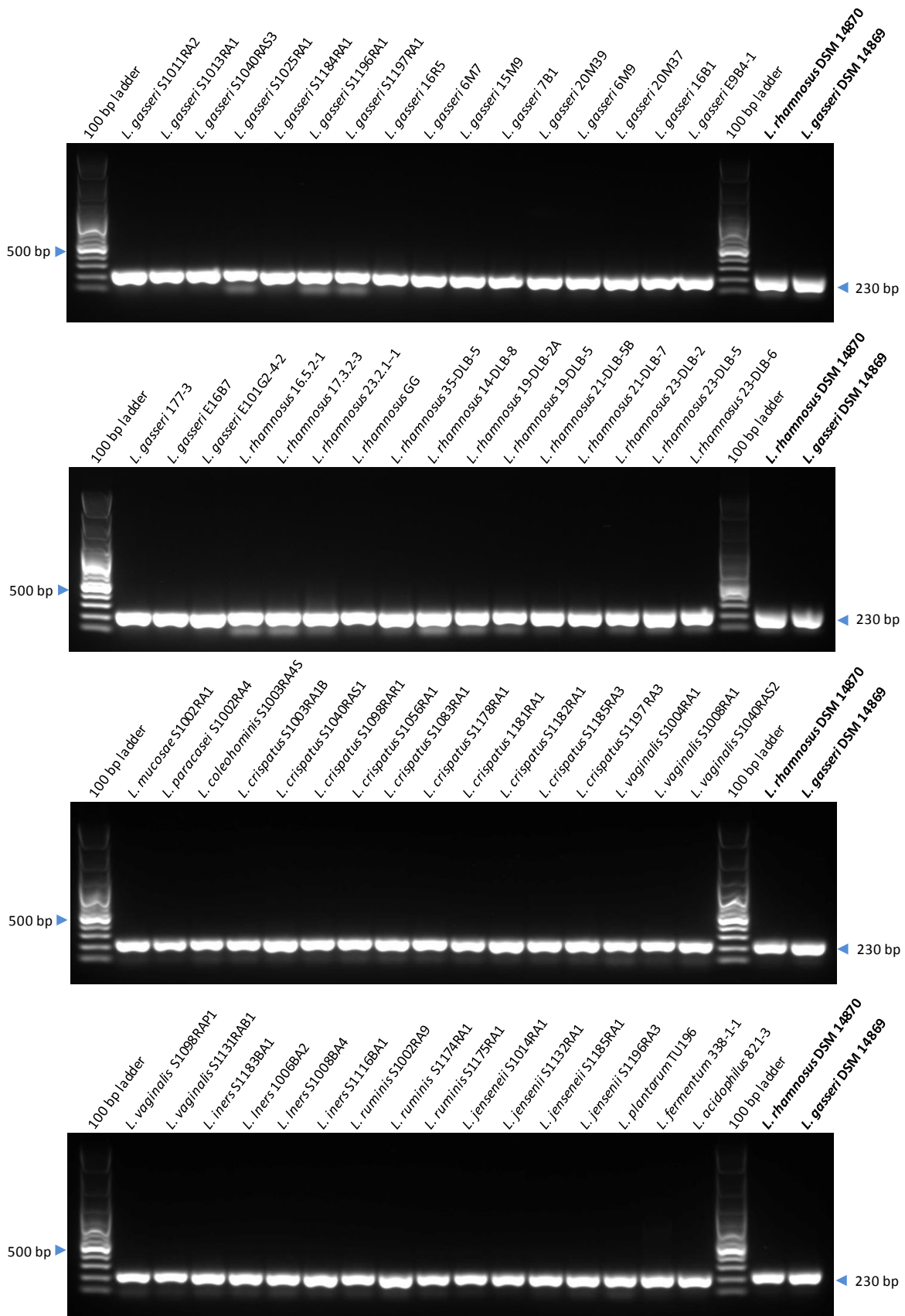
**A) *L. rhamnosus* DSM 14870**

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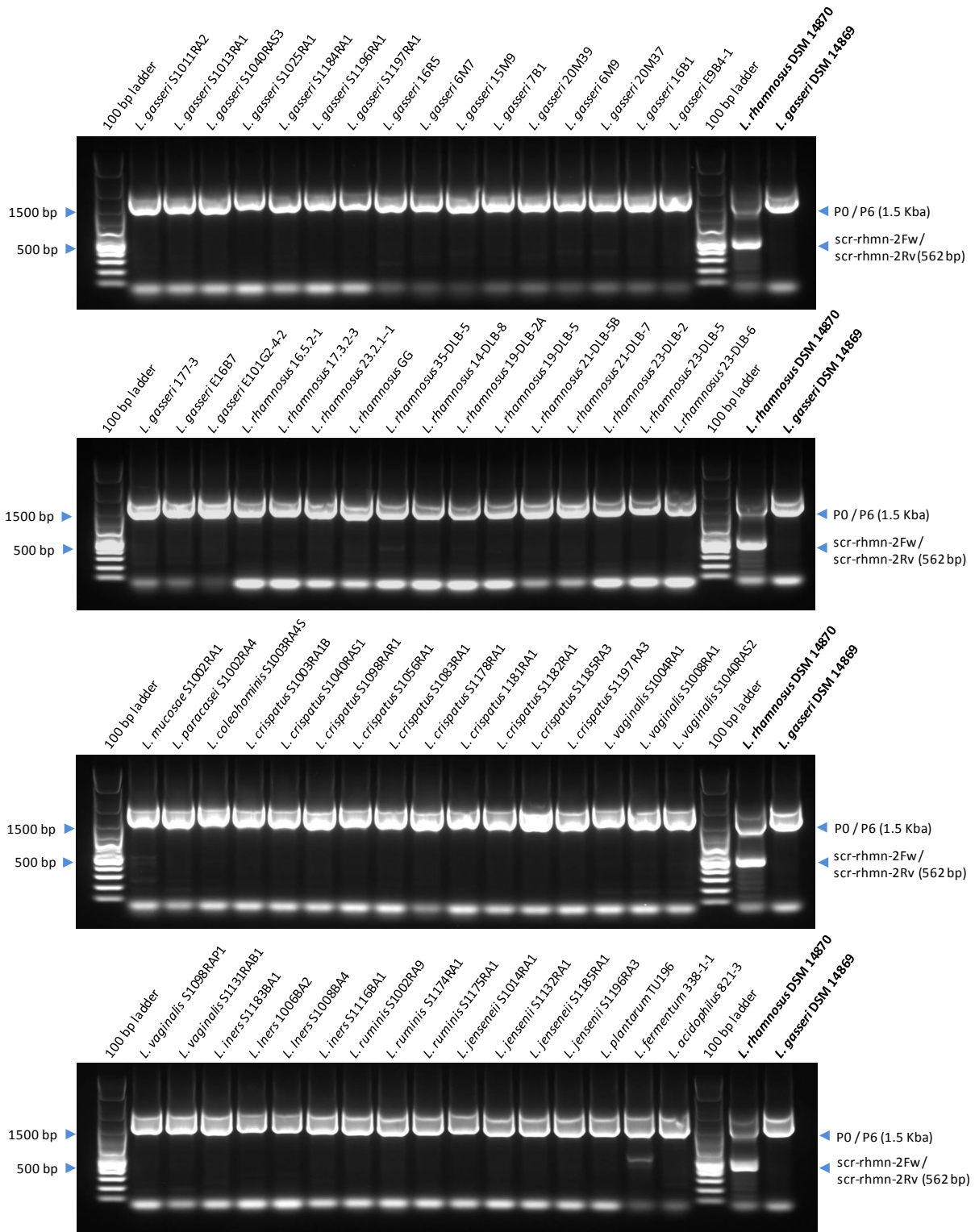
**B) *L. gasseri* DSM 14869**

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tattcccaattagcataggttgaaagtcgtattctccacgtatgtggagatgatcctattgtcgaaccttacgaaataccctag  
gttagcgtattctccacgtatgtggagatgatcctaaagtttgcctcccaaaagtcctggaatgtattcgtattctccacgtatg  
tggagatgatcctaacggagaagattgaagaaactatcaatcagattctccacgtatgtggagatgatccttaattcaga  
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atgaaagttagatagctggtgaaagtttttagtgggttaggtttggcaaatggcaaggtatagtagtttttagataa

**Figure S1** Clustered regularly interspaced short palindromic repeats (CRISPRs, in yellow) alternate with spacers of different sequence but similar length. A) *L. rhamnosus* DSM 14870 CRISPR locus, repeat region position between 992959 and 995240, B) *L. gasseri* DSM 14869 CRISPR locus 2 repeat region, position between 434451 and 436005. The primers sequences are indicated in red.

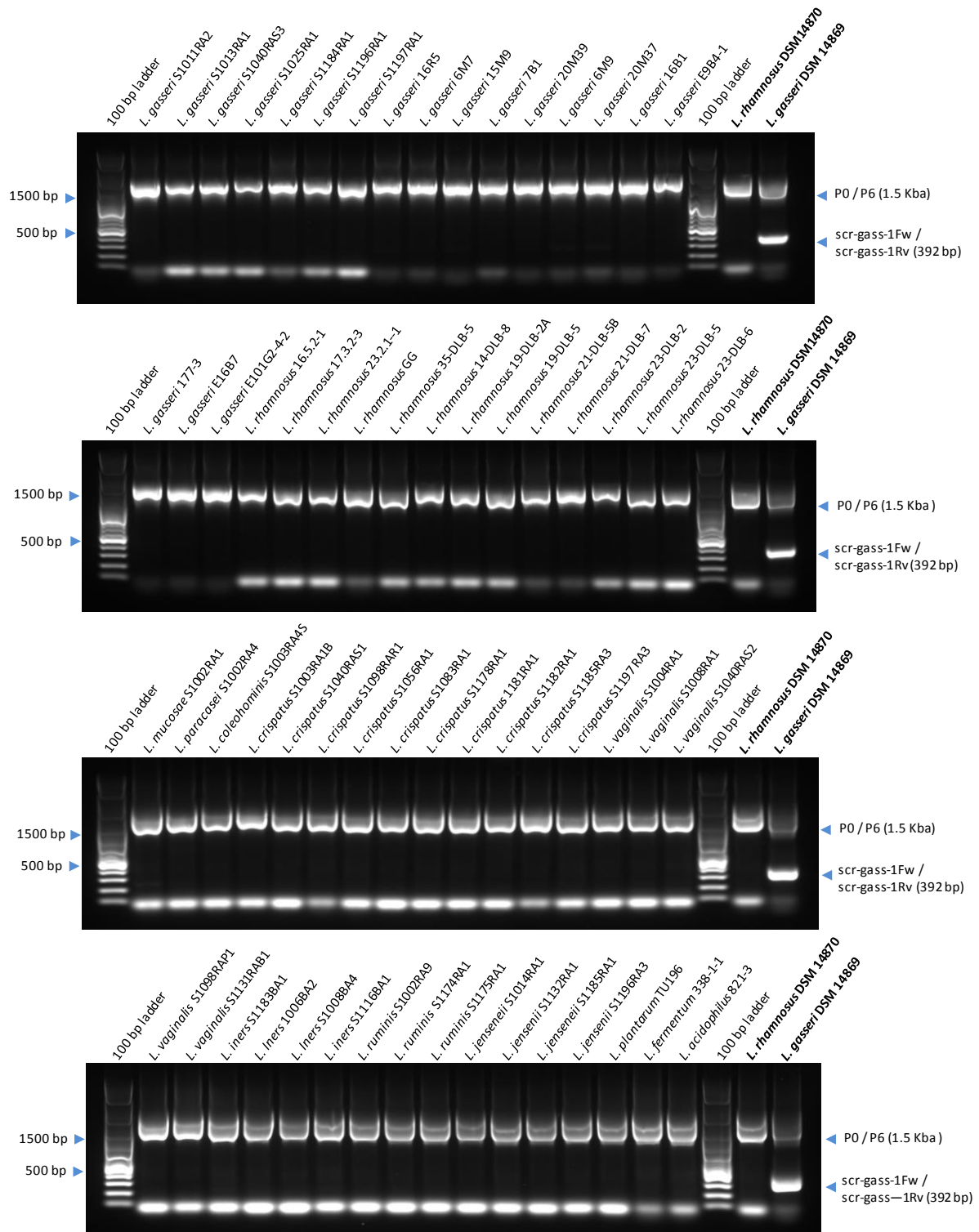


**Figure S2** Amplification of the *Lactobacillus* 16S rRNA gene segment with the genus-specific primers L and R. The theoretical size of the PCR product is indicated on the right of the gel pictures. The origin of the strains used is described in Table S2.



**Figure S3** Specificity of the multiplex PCR for the detection of *L. rhamnosus* DSM 14870. The amplification of the 16S rRNA gene (internal control) with primers P0 and P6 resulted in a 1.5 Kb PCR product for all the *Lactobacillus* strains tested. The amplification of the CRISPR DNA segment with primers scr-rhmn-2Fw and scr-rhmn-2Rv resulted in a ~550 bp PCR product for *L. rhamnosus* DSM 14870 only. An additional light band was observed for *L. fermentum* 338-1-1 but at ~900 bp (lower panel, line 16). The theoretical size of the PCR products is indicated on the right of the gel pictures.





**Figure S4** Specificity of the multiplex PCR for the detection of *L. gasseri* DSM 14869. The amplification of the 16S rRNA gene (internal control) with primers P0 and P6 resulted in a 1.5 Kba PCR product for all the *Lactobacillus* strains tested. The amplification of the CRISPR DNA segment with primers scr-gass-1Fw and scr-gass-1Rv resulted in a ~400 bp PCR product for *L. gasseri* DSM 14869 only. The theoretical size of the PCR products is indicated on the right of the gel pictures.

**A) Healthy, probiotic capsules (Group 1, 13 participants)**

	0	2	3	4	5	6	7	
001								BV visit 5
002								
009								BV visit 2
013								
040								
075								
079								BV visit 7
103								BV visit 2
131								
136								
147								
154								
165								

**B) BV, antibiotic with probiotic capsules (Group 2, 12 participants)**

	0	2	3	4	5	6	7	
004								
099								cured
104								
107								cured
109								
113								
119								cured
129								
130								cured, relapse visit 3
142								
157								cured, relapse visit 4
170								

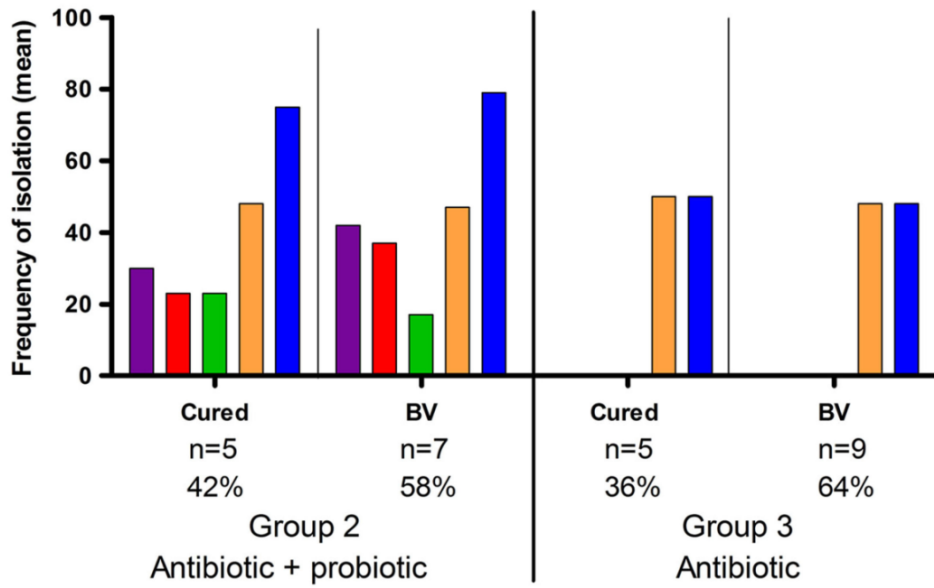
**C) BV, antibiotic (Group 3, 14 participants)**

	0	2	3	4	5	6	7	
049								Cured, LTF visit 5
088								
089								
102								cured
106								
108								
112								
126								
128								cured
134								cured, relapse visit 3
151								
152								cured
159								
161								LTF visit 3

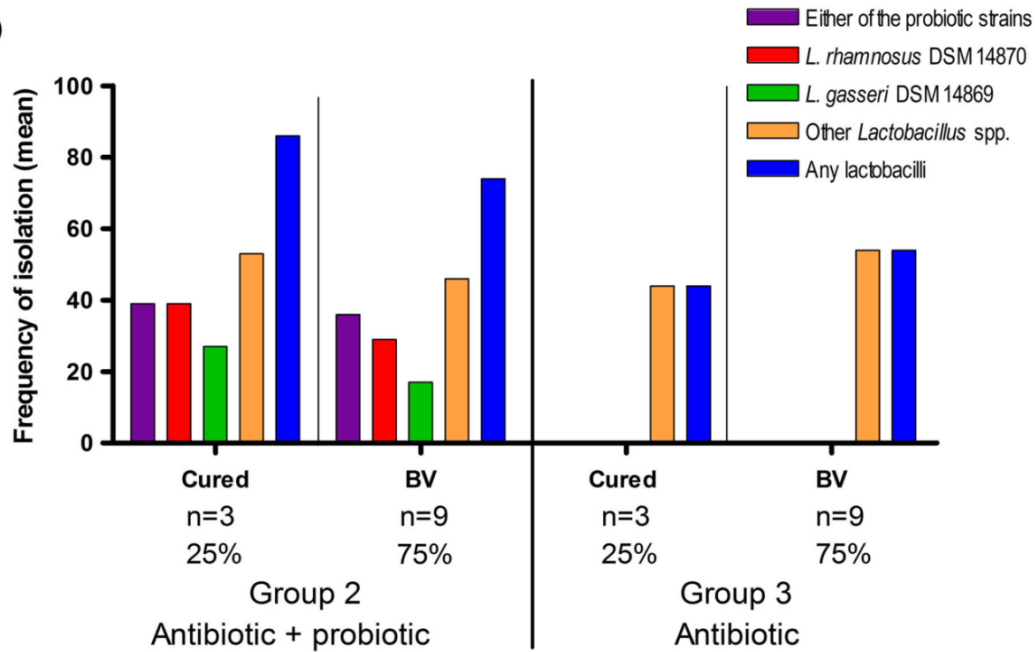
■ *L. rhamnosus* 14870 ■ *L. gasseri* 14869 ■ *Lactobacillus* ■ No *Lactobacillus* ■ No samples LTF: Lost to follow-up

**Figure S5** Isolation of *L. rhamnosus* DSM 14870 and *L. gasseri* DSM 14869 in vaginal samples.

A)



B)



**Figure S6** Association between the mean frequency of isolation of probiotic strains and other lactobacilli with the 1-month (A) and 6-month (B) cure rates. The frequency of isolation (y axis) was determined as the percentage of samples positive for probiotic strains and other lactobacilli on the total number of samples for each individual (cured or BV positive) during the entire follow up. The results are presented as a mean. No statistical difference in frequency of isolation of probiotic strains or other lactobacilli was observed between cured and BV positive women within Group 2 (antibiotic + probiotic) and Group 3 (antibiotic) using a t-test and a p-value cutoff of 0.05 (two tailed).