Supplementary Information

Exopolysaccharides of *Lactobacillus crispatus* mediate key balancing interactions with the vaginal mucosa

Vanessa Croatti[1,2], Caroline Dricot[1], Tom Eilers[1], Jelle Dillen[1], Tim Van Rillaer[1], Eline Cauwenberghs[1], Ilke Van Tente[1], Sam Bakelants[1], Dieter Vandenheuvel[1], Camille Allonsius[1], Isabel Pintelon[3,4], Sofie Thys[3,4], Wendy Mensah[5], Marina Naldi[5], Peter A. Bron[1], Stijn Wittouck[1], Irina Spacova[1], Carola Parolin[2], Beatrice Vitali[2], Sarah Lebeer[1*]

1 – Laboratory of Applied Microbiology and Biotechnology (LAMB), Department of Bioscience Engineering, University of Antwerp, Antwerp, Belgium.

2 – Beneficial Microbes Laboratory, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy

3 – Laboratory of Cell Biology and Histology, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Wilrijk, Belgium

4 – Antwerp Centre for Advanced Microscopy (ACAM), University of Antwerp, Wilrijk, Belgium

5 – Bio-Pharmaceutical and Pharmaceutical Analysis, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy

* corresponding author: sarah.lebeer@uantwerpen.be

Table S1

Species tested	***Strains***	***Growth in MRS media (OD_{600}) Cut-off 1.5***	***Growth in MRS+glycine2%+ sucrose2% (OD_{600}) Cut-off 1***	***Chloramphenicol MIC (µg/mL) Cut-off 10 µg/mL***
Lactobacillus crispatus	BC1	5.33 ± 1.01	1.53 ± 0.02	5
	BC3	1.67 ± 0.12	1.25 ± 0.10	5
	BC5	5.10 ± 0.52	1.64 ± 0.04	5
	AMBV-0006	4.31 ± 1.14	1.28 ± 0.11	5
	AMBV-0815	3.26 ± 0.09	1.64 ± 0.05	5
	AMBV-1513	2.42 ± 0.67	1.14 ± 0.02	5
	AMBV-2491	2.03 ± 0.88	1.44 ± 0.20	2.5
Lactiplantibacillus plantarum	WCFS1	3.04 ± 0.32	1.50 ± 0.05	10

Table S1. Selection of in-house L. crispatus strains stuitable for genetic manipulation. Data are reported as mean ± SD (n=2). *L. plantarum* WCFS1 was used as a control strain for electrocompetent cells preparation due to its high genetic accessibility using glycine-sucrose-based protocols.

Figure S1

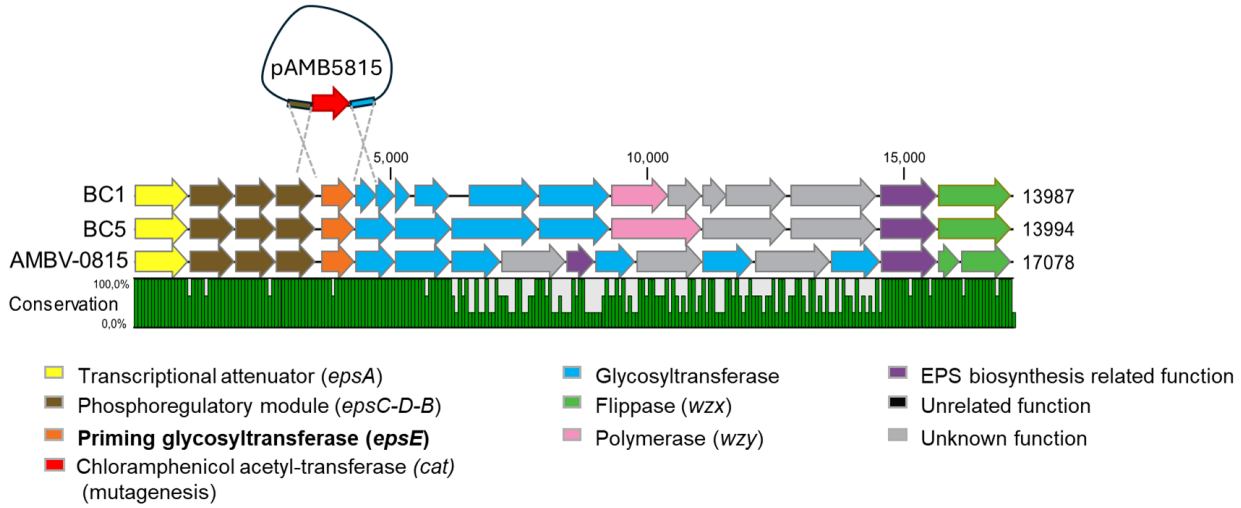

Figure S1. Schematic representation of the EPS gene cluster in L. crispatus strains with highly conserved epsE neighboring genes and the construction of the semi-generic mutagenesis plasmid (pAMB5815). *Nucleotide sequence conservation between the strains is reported in the line plot (%).*

Table S2

Species tested	***Strains***	***Transformation efficiency (CFU/µg pNZ123)***	***Mutagenesis efficiency (pAMB5815) (CFU)***
Lactobacillus crispatus	BC1	$<10^3$	0
	BC5	$<10^3$	0
	AMBV-0815	$1.5x10^4$	1
Lactiplantibacillus plantarum	WCFS1	$8.4x10^3$	/

Table S2. Genetic accessibility of selected strains (pNZ123) and mutagenesis efficiency (pAMB5815). *L. plantarum* WCFS1 was used as a control strain for electrocompetent cells preparation due to its high genetic accessibility using glycine-sucrose-based protocols.

Figure S2

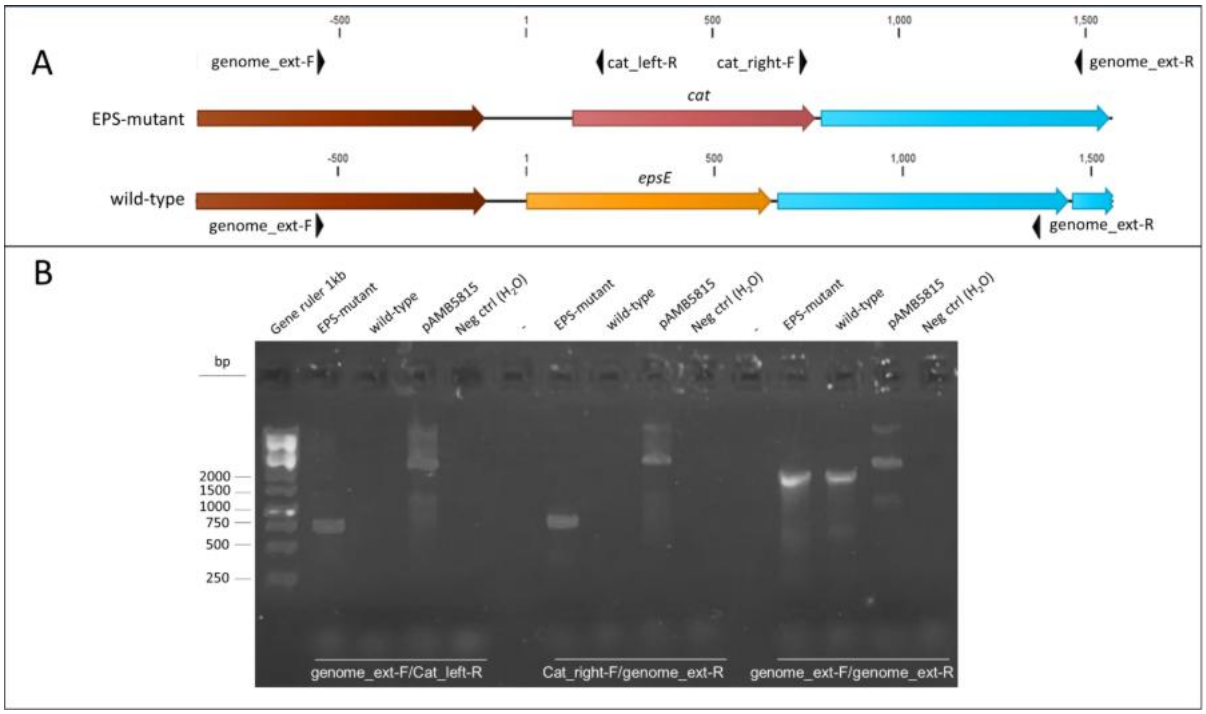

Figure S2. A. Schematic representation of the primers used to check the double homologous recombination in L. crispatus AMBV-0815 ΔepsE (EPS-mutant). B. Gel electrophoresis (agarose 1% w/v) from colony PCR. *All samples were amplified with PCR using three different primer pairs, indicated in white. EPS-mutant and wild-type genome was obtained from colony PCR. Mutagenesis plasmid pAMB5815 and H_2O were used as negative controls. Nucleotides position is reported starting from the start codon (ATG) of epsE gene in the wild-type strain. Fragments expected in EPS-mutant strain: genome_ext-F/Cat_left-R: 766 bp; Cat_right-F/genome_ext-R: 756 bp and genome_ext-F/genome_ext-R: 2048 bp. Fragments expected in wild-type strain: genome_ext-F/Cat_left-R: / ; Cat_right-F/genome_ext-R: / and genome_ext-F/genome_ext-R: 1920 bp. PCR amplicons obtained for EPS-mutant were Sanger sequenced.*

Figure S3

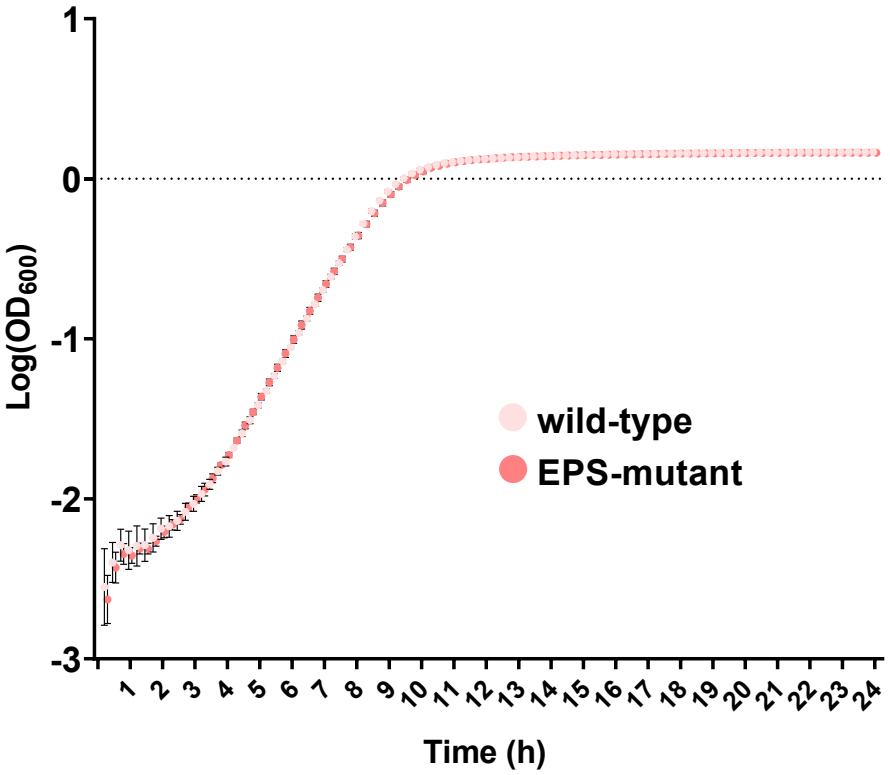

Figure S3. Growth curve in MRS liquid of L. crispatus AMBV-0815 wild-type and EPS-mutant (24 h). *Data are reported as mean ± SD (n = 3).*

Figure S4

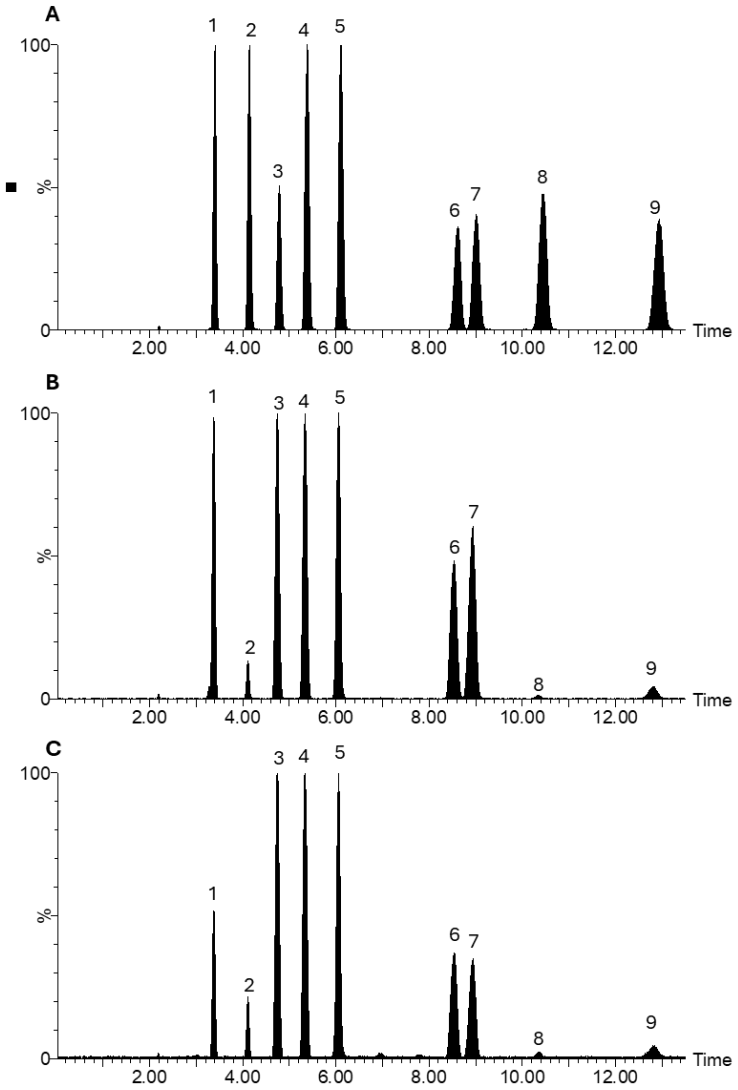

Figure S4. Extract Ion Chromatograms of identified PMP-monosaccharides (LC-MS)*. (A) mixture of nine standard PMP-monosaccharides; (B) PMP-monosaccharides from L. crispatus AMBV-0815; (C) EPS-mutant samples. Peaks:* ***1****: D-glucosamine;* ***2****: D-mannose;* ***3****: galactosamine;* ***4****: D-ribose;* ***5****: D-rhamnose;* ***6****: D-glucose;* ***7****: D-galactose;* ***8****: D-xylose;* ***9****: D-fucose.*

Figure S5

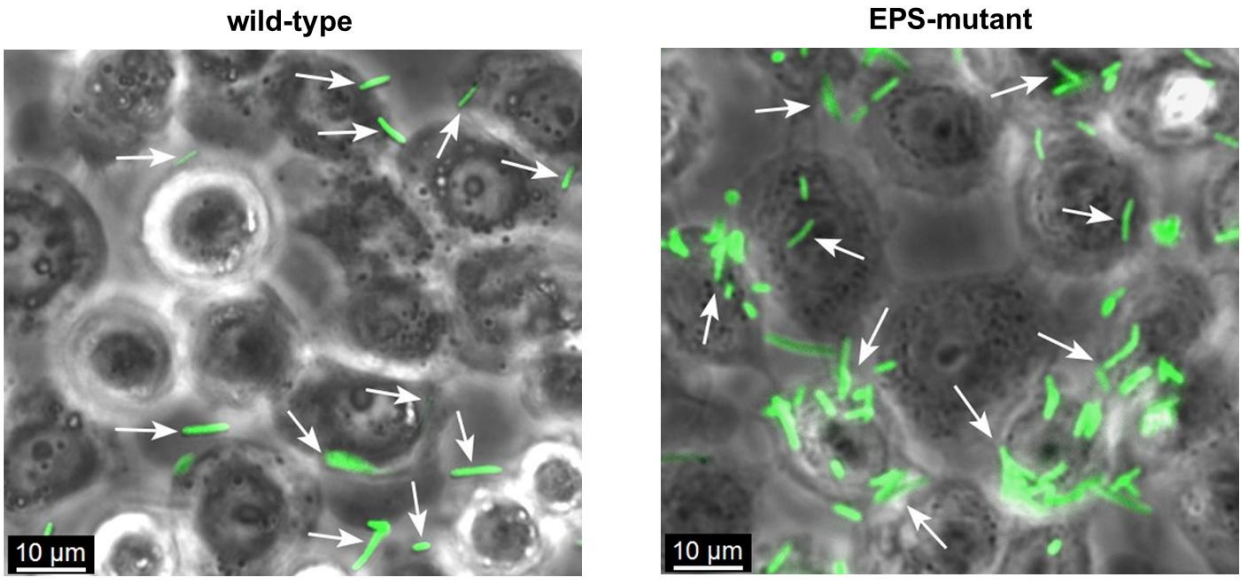

Figure S5. Fluorescence microscopy micrographs of L. crispatus AMBV-0815 wild-type and EPS-mutant adherent on vaginal epithelium cells monolayer (VK2/E6E7) (3h). *L. crispatus AMBV-0815 wild-type and EPS-mutant were fluorescence stained with FITC (0.1 µg/mL - green channel, indicated with white arrows).*

Figure S6

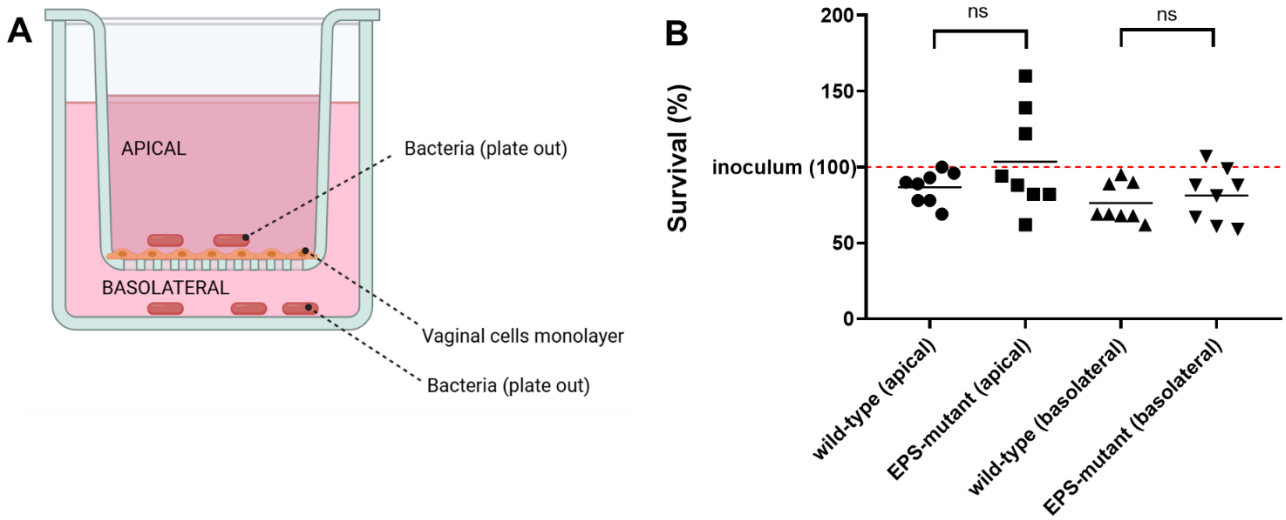

Figure S6. Survival of bacterial cells in presence of vaginal cells on apical side (direct contact) and on basolateral side (indirect contact) of a transwell model (3h). *(A) Schematic representation of the assay. (B) Survival rates (%) of L. crispatus AMBV-0815 wild-type and EPS-mutant. The survival was calculated basing on the CFU plated out after 3h exposure in respect to the starting inoculum (CFU before exposure –100% survival). Two-way ANOVA with Tukey correction (n=8, ns =non significance, p > 0.05)*

Figure S7

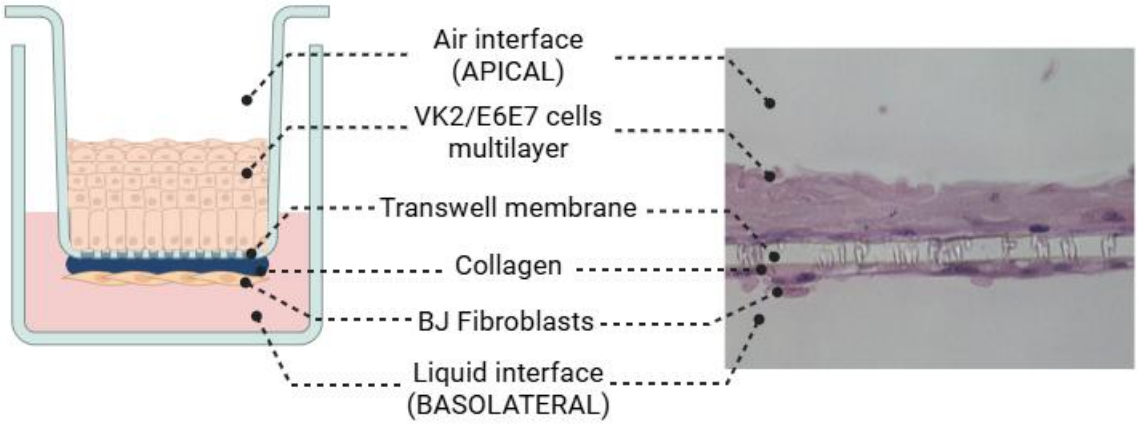

Figure S7. Representation and micrograph of cross section of the 3D vaginal transwell model (hematoxylin and eosin stained) used in the present study.