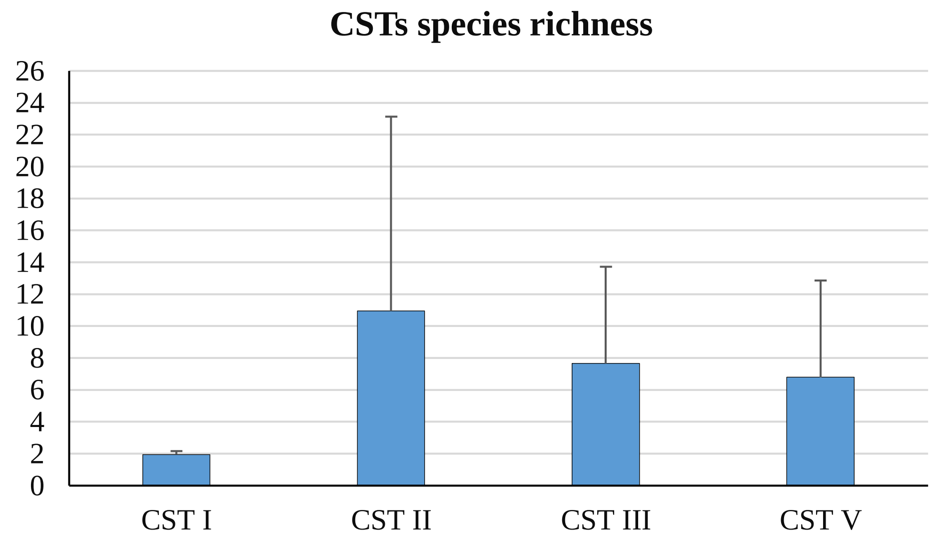
a)


CSTs species richness
26
24
22
20
18
16
14
12
10
8
6
4
2
0
CST I
CST II
CST III
CST V

b)

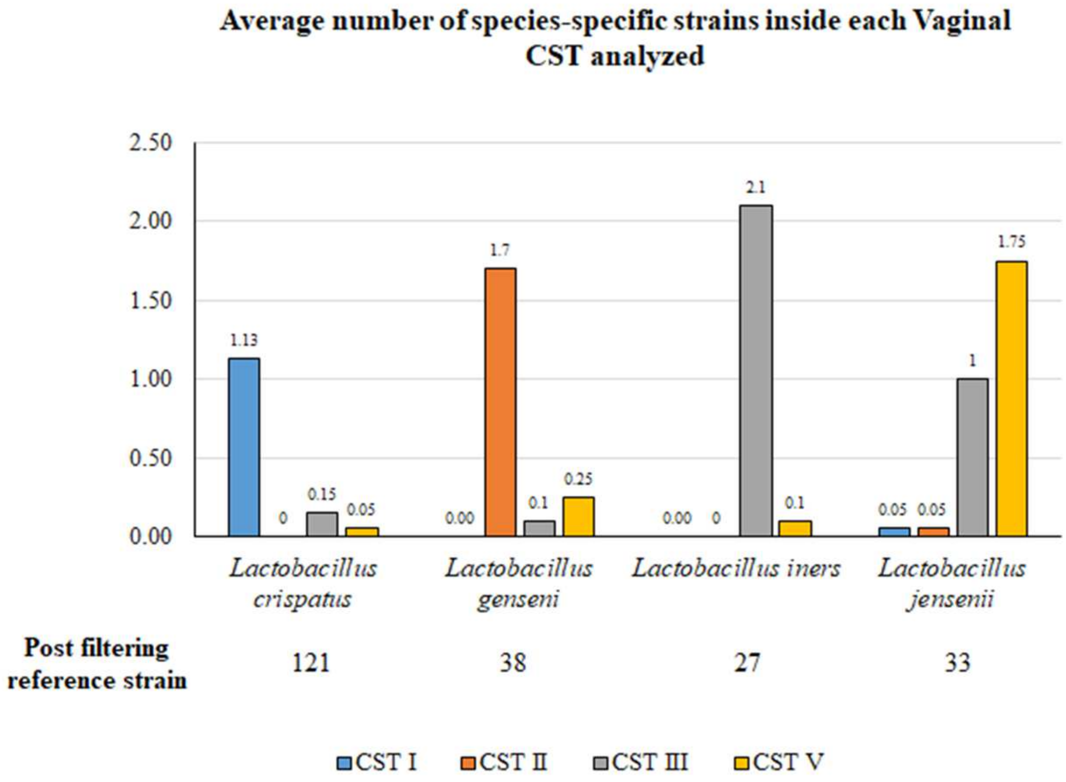
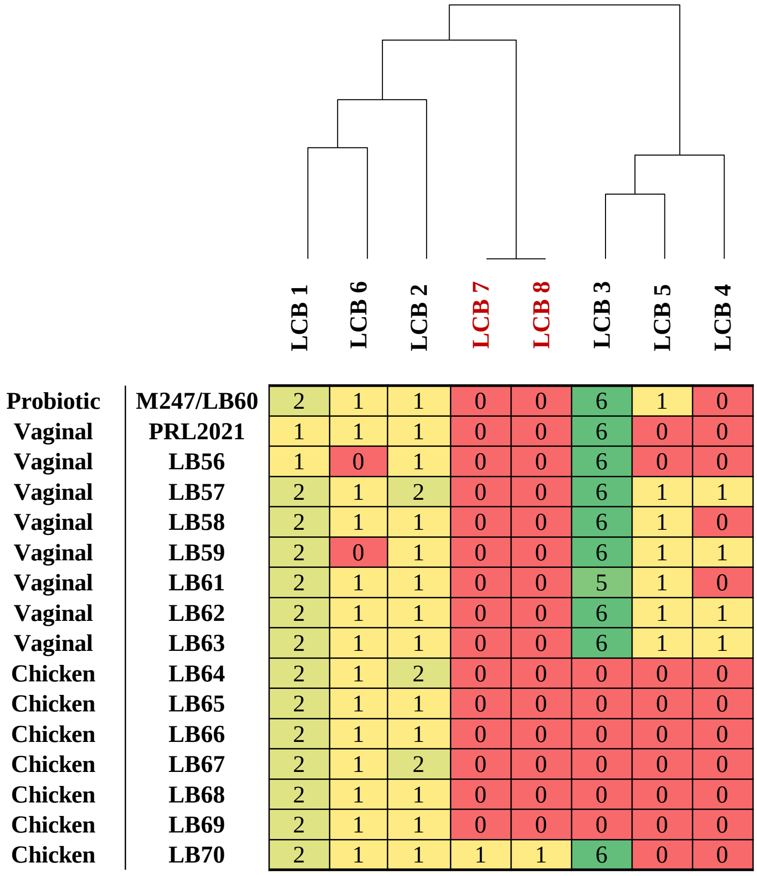

Average number of species-specific strains inside each Vaginal CST analyzed
2.50
2.00
1.50
1.00
0.50
0.00
1.13
0
0.15
0.05
0.00
1.7
0.1
0.25
0.00
0
2.1
0.1
0.05
0.05
1
1.75
Lactobacillus crispatus
Lactobacillus genseni
Lactobacillus iners
Lactobacillus jensenii
Post filtering reference strain
121
38
27
33
CST I
CST II
CST III
CST V

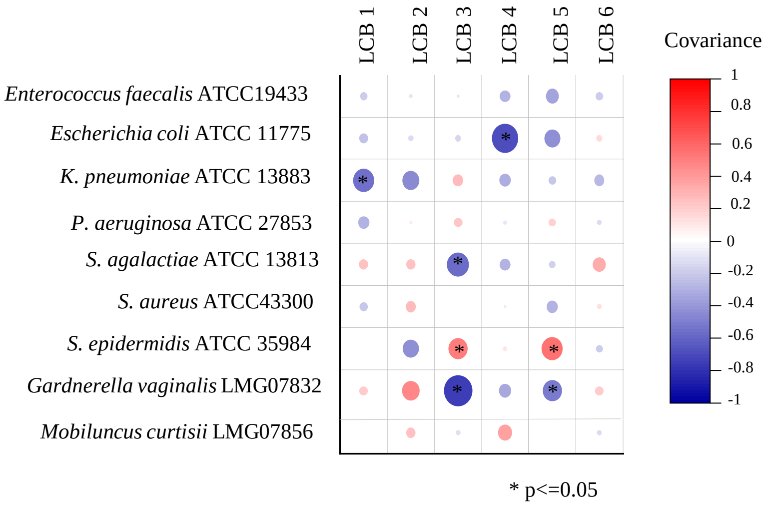
Figure S1

a)



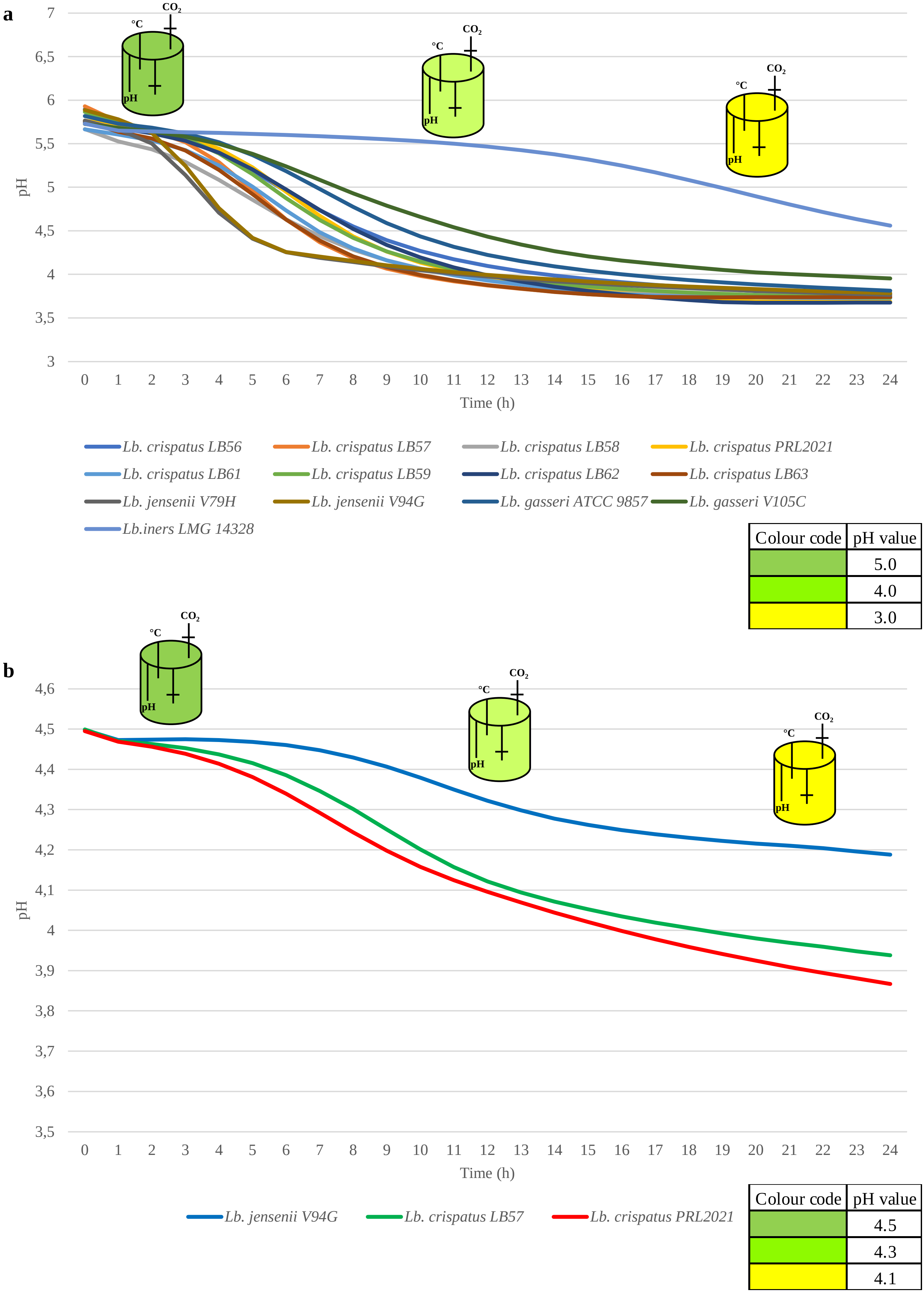
		LCB 1	LCB 6	LCB 2	LCB 7	LCB 8	LCB 3	LCB 5	LCB 4
Probiotic	**M247/LB60**	2	1	1	0	0	6	1	0
Vaginal	**PRL2021**	1	1	1	0	0	6	0	0
Vaginal	**LB56**	1	0	1	0	0	6	0	0
Vaginal	**LB57**	2	1	2	0	0	6	1	1
Vaginal	**LB58**	2	1	1	0	0	6	1	0
Vaginal	**LB59**	2	0	1	0	0	6	1	1
Vaginal	**LB61**	2	1	1	0	0	5	1	0
Vaginal	**LB62**	2	1	1	0	0	6	1	1
Vaginal	**LB63**	2	1	1	0	0	6	1	1
Chicken	**LB64**	2	1	2	0	0	0	0	0
Chicken	**LB65**	2	1	1	0	0	0	0	0
Chicken	**LB66**	2	1	1	0	0	0	0	0
Chicken	**LB67**	2	1	2	0	0	0	0	0
Chicken	**LB68**	2	1	1	0	0	0	0	0
Chicken	**LB69**	2	1	1	0	0	0	0	0
Chicken	**LB70**	2	1	1	1	1	6	0	0

b)



* p<=0.05

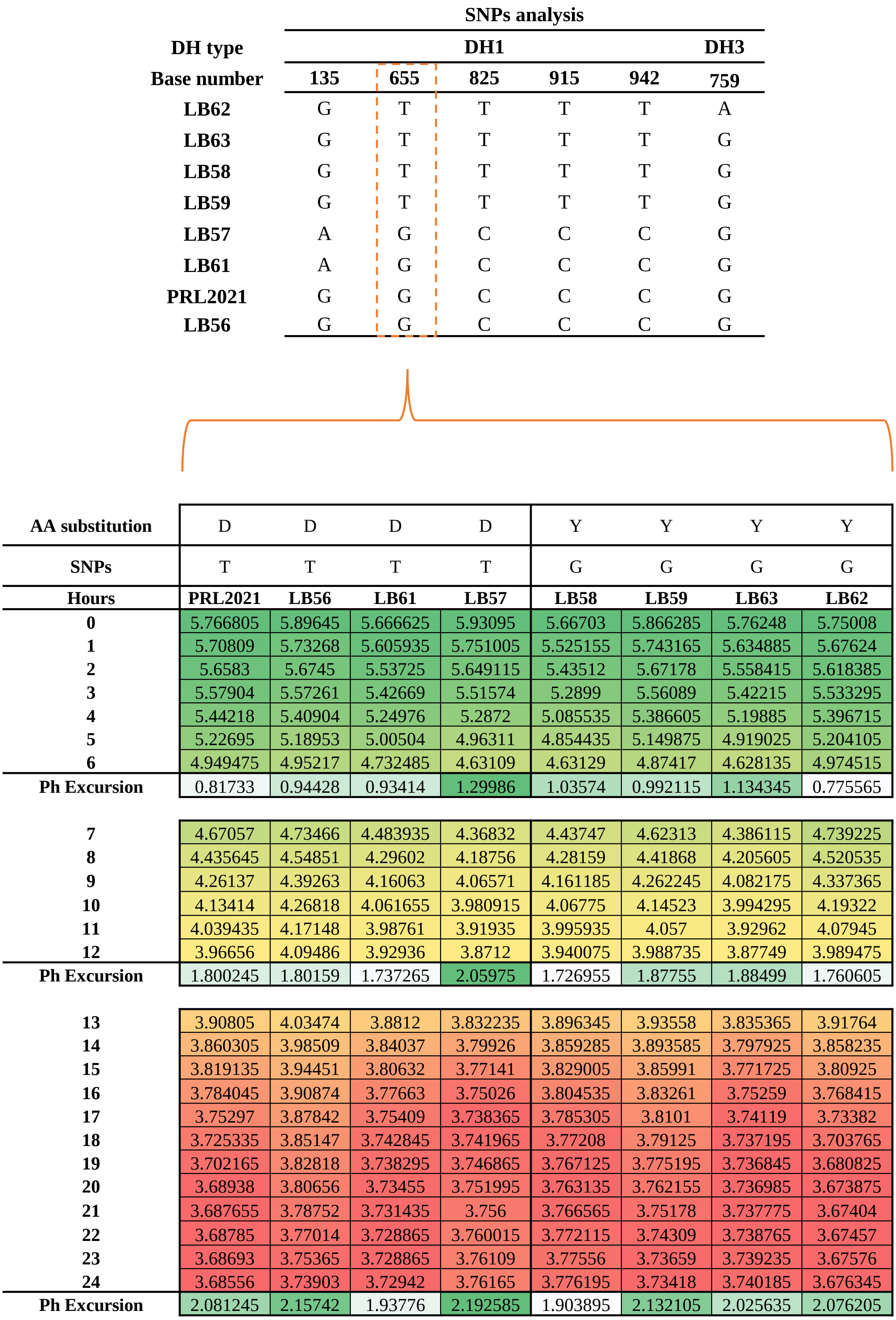
Figure S2



Colour code	pH value
🟩	5.0
🟩	4.0
🟨	3.0

Colour code	pH value
🟩	4.5
🟩	4.3
🟨	4.1

Figure S3



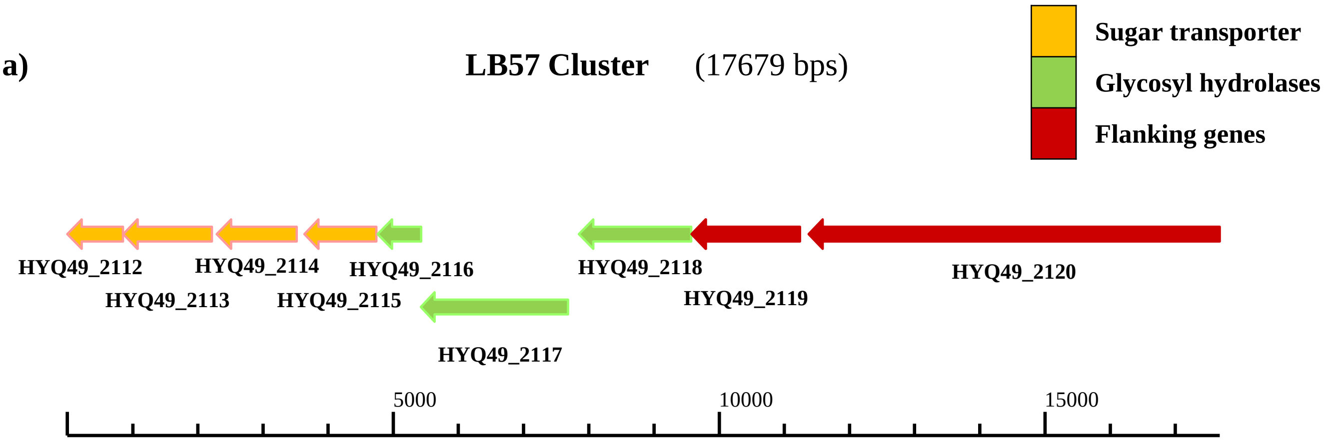
SNPs analysis

DH type	DH1					DH3
Base number	**135**	**655**	**825**	**915**	**942**	**759**
LB62	G	T	T	T	T	A
LB63	G	T	T	T	T	G
LB58	G	T	T	T	T	G
LB59	G	T	T	T	T	G
LB57	A	G	C	C	C	G
LB61	A	G	C	C	C	G
PRL2021	G	G	C	C	C	G
LB56	G	G	C	C	C	G

AA substitution	D	D	D	D	Y	Y	Y	Y
SNPs	T	T	T	T	G	G	G	G
Hours	**PRL2021**	**LB56**	**LB61**	**LB57**	**LB58**	**LB59**	**LB63**	**LB62**
0	5.766805	5.89645	5.666625	5.93095	5.66703	5.866285	5.76248	5.75008
1	5.70809	5.73268	5.605935	5.751005	5.525155	5.743165	5.634885	5.67624
2	5.6583	5.6745	5.53725	5.649115	5.43512	5.67178	5.558415	5.618385
3	5.57904	5.57261	5.42669	5.51574	5.2899	5.56089	5.42215	5.533295
4	5.44218	5.40904	5.24976	5.2872	5.085535	5.386605	5.19885	5.396715
5	5.22695	5.18953	5.00504	4.96311	4.854435	5.149875	4.919025	5.204105
6	4.949475	4.95217	4.732485	4.63109	4.63129	4.87417	4.628135	4.974515
Ph Excursion	0.81733	0.94428	0.93414	1.29986	1.03574	0.992115	1.134345	0.775565
7	4.67057	4.73466	4.483935	4.36832	4.43747	4.62313	4.386115	4.739225
8	4.435645	4.54851	4.29602	4.18756	4.28159	4.41868	4.205605	4.520535
9	4.26137	4.39263	4.16063	4.06571	4.161185	4.262245	4.082175	4.337365
10	4.13414	4.26818	4.061655	3.980915	4.06775	4.14523	3.994295	4.19322
11	4.039435	4.17148	3.98761	3.91935	3.995935	4.057	3.92962	4.07945
12	3.96656	4.09486	3.92936	3.8712	3.940075	3.988735	3.87749	3.989475
Ph Excursion	1.800245	1.80159	1.737265	2.05975	1.726955	1.87755	1.88499	1.760605
13	3.90805	4.03474	3.8812	3.832235	3.896345	3.93558	3.835365	3.91764
14	3.860305	3.98509	3.84037	3.79926	3.859285	3.893585	3.797925	3.858235
15	3.819135	3.94451	3.80632	3.77141	3.829005	3.85991	3.771725	3.80925
16	3.784045	3.90874	3.77663	3.75026	3.804535	3.83261	3.75259	3.768415
17	3.75297	3.87842	3.75409	3.738365	3.785305	3.8101	3.74119	3.73382
18	3.725335	3.85147	3.742845	3.741965	3.77208	3.79125	3.737195	3.703765
19	3.702165	3.82818	3.738295	3.746865	3.767125	3.775195	3.736845	3.680825
20	3.68938	3.80656	3.73455	3.751995	3.763135	3.762155	3.736985	3.673875
21	3.687655	3.78752	3.731435	3.756	3.766565	3.75178	3.737775	3.67404
22	3.68785	3.77014	3.728865	3.760015	3.772115	3.74309	3.738765	3.67457
23	3.68693	3.75365	3.728865	3.76109	3.77556	3.73659	3.739235	3.67576
24	3.68556	3.73903	3.72942	3.76165	3.776195	3.73418	3.740185	3.676345
Ph Excursion	2.081245	2.15742	1.93776	2.192585	1.903895	2.132105	2.025635	2.076205

Figure S4

a) **LB57 Cluster** (17679 bps)



Genes involved in glycogen metabolism

HYQ49_2120 – hypothetical protein
HYQ49_2119 – beta-phosphoglucomutase
HYQ49_2118 – alpha-glycosidase
HYQ49_2117 – maltose phosphorylase
HYQ49_2116 – beta-phosphoglucomutase
HYQ49_2115 – sugar ABC transporter ATP-binding protein
HYQ49_2114 – sugar ABC transporter sugar-binding protein
HYQ49_2113 – sugar ABC transporter permease
HYQ49_2112 – sugar ABC transporter permease

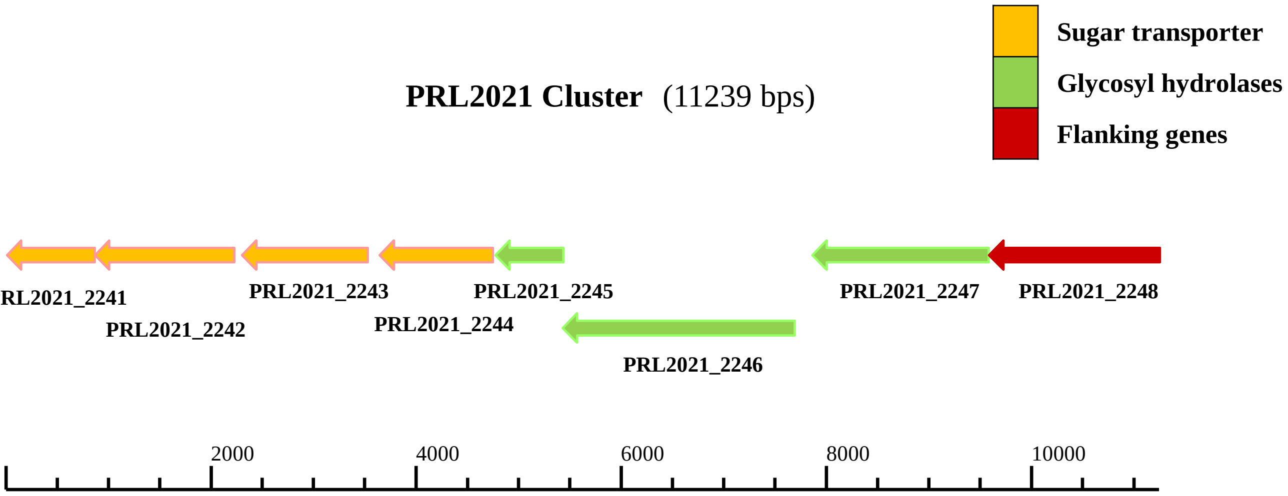
HMMER analysis

Alpha amylase,
catalytic domain

Domain E-values

Ind.	Cond.
3.6e-76	3.7e-80

b) **PRL2021 Cluster** (11239 bps)



Genes involved in glycogen metabolism

PRL2021_2248 – alpha,alpha-phosphotrehalase
PRL2021_2247 – alpha-glycosidase
PRL2021_2246 – maltose phosphorylase
PRL2021_2245 – beta-phosphoglucomutase
PRL2021_2244 – sugar ABC transporter ATP-binding protein
PRL2021_2243 – maltose ABC superfamily ATP-binding substrate-binding protein
PRL2021_2242 – sugar ABC transporter permease
PRL2021_2241 – sugar ABC transporter permease

FigureS5

Supplementary figure legends

Figure S1. Microbial biodiversity in the vaginal microbiota.

Panel a reports the species richness analysis obtained through METAnnotatorX2 profiling of the 80 vaginal samples. Panel b shows the strain tracking result for the 4 *Lactobacillus* species analyzed in 80 vaginal samples, divided by CST I, II, III and V, through the use of strainGST software.

Figure S2. Analysis of bacteriocin gene distribution and correlation to inhibition halo.

Panel a reports an HCl analysis relative to the distribution of the six bacteriocin clusters (LCB) between the analyzed strains of *Lb. crispatus*, while panel b) shows correlation of the presence of the six different LCB and the inhibition halo of *Lb. crispatus* grown with the nine species used as test.

Figure S3. Evaluation of lactic acid production by vaginal lactobacilli.

Panel a displays the pH lowering through fermentation process in MRS. The variation of pH as a function of time is reported. Panel b) shows the evaluation of lactic acid production through fermentation process in Simulated Vaginal Fluid (SVF). The variation of pH as a function of time is reported.

Figure S4. SNP analysis in DH genes.

The figure reports all five SNPs identified in the DH1 gene and the single SNP identified in the DH3 gene, with the relative NT substitution. Additionally, for the SNP at position 655 in the DH1 gene, is reported also the relative AA subsitution, correlated to pH profile of each *Lb. crispatus* genome isolated from human vagina.

Figure S5. Cluster of genes for glycogen metabolism in *Lactobacillus crispatus*.

The figure shows the cluster of genes involved in glycogen metabolism conserved within the *Lactobacillus crispatus* strains used in the growth assays. Panels a) and b) show the genes present within the conserved cluster for glycogen metabolism both in LB57 and PRL2021 strains.