

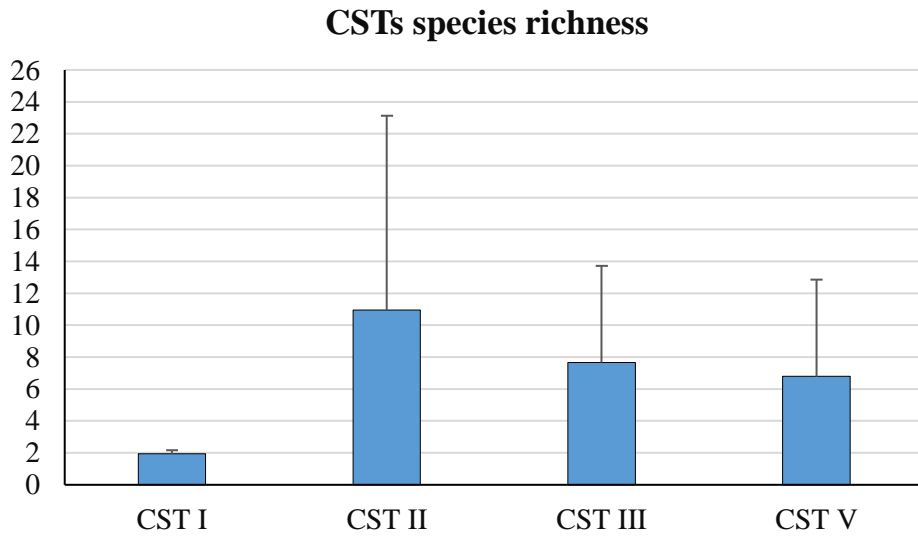
Title	Evaluation of modulatory activities of <i>Lactobacillus crispatus</i> strains in the context of the vaginal microbiota
Authors	Argentini, C.;Fontana, Federico;Alessandri, Giulia;Lugli, Gabriele Andrea;Mancabelli, Leonardo;Ossiprandi, Maria Cristina;van Sinderen, Douwe;Ventura, Marco;Milani, Christian;Turroni, Francesca
Publication date	2022-04
Original Citation	Argentini, C., Fontana, F., Alessandri, G., Lugli, G.A., Mancabelli, L., Ossiprandi, M.C., Van Sinderen, D., Ventura, M., Milani, C. and Turroni, F. (2022) 'Evaluation of modulatory activities of lactobacillus crispatus strains in the context of the vaginal microbiota', <i>Microbiology Spectrum</i> , 10(2), (11pp). doi: 10.1128/spectrum.02733-21
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1128/spectrum.02733-21
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# UCC

**University College Cork, Ireland**  
Coláiste na hOllscoile Corcaigh

a)



b)

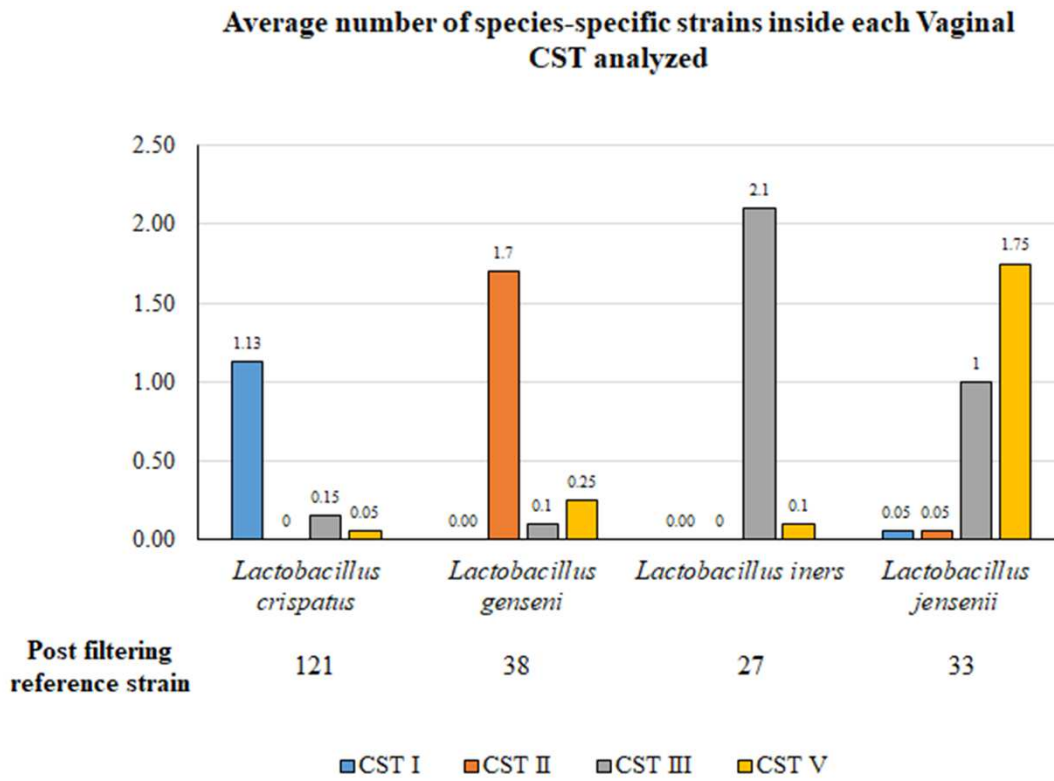
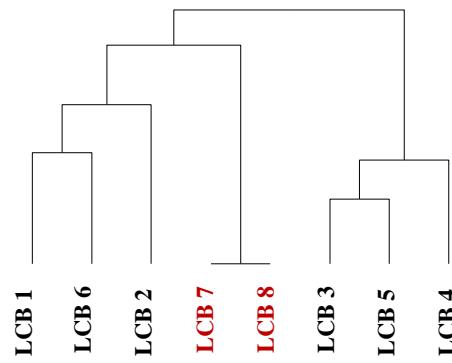


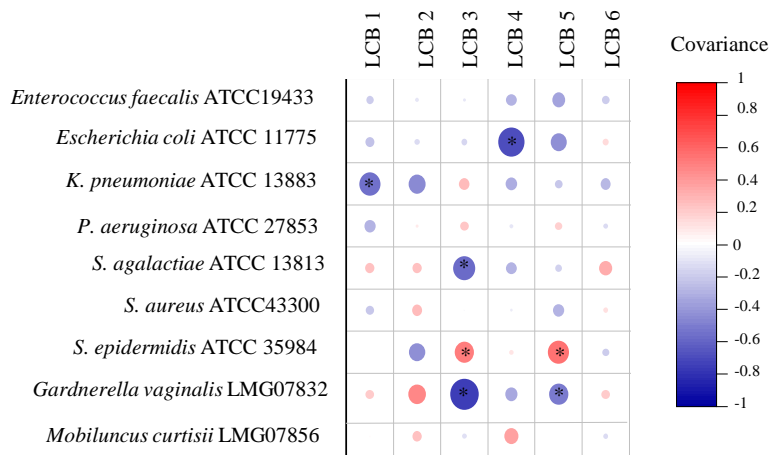
Figure S1

a)

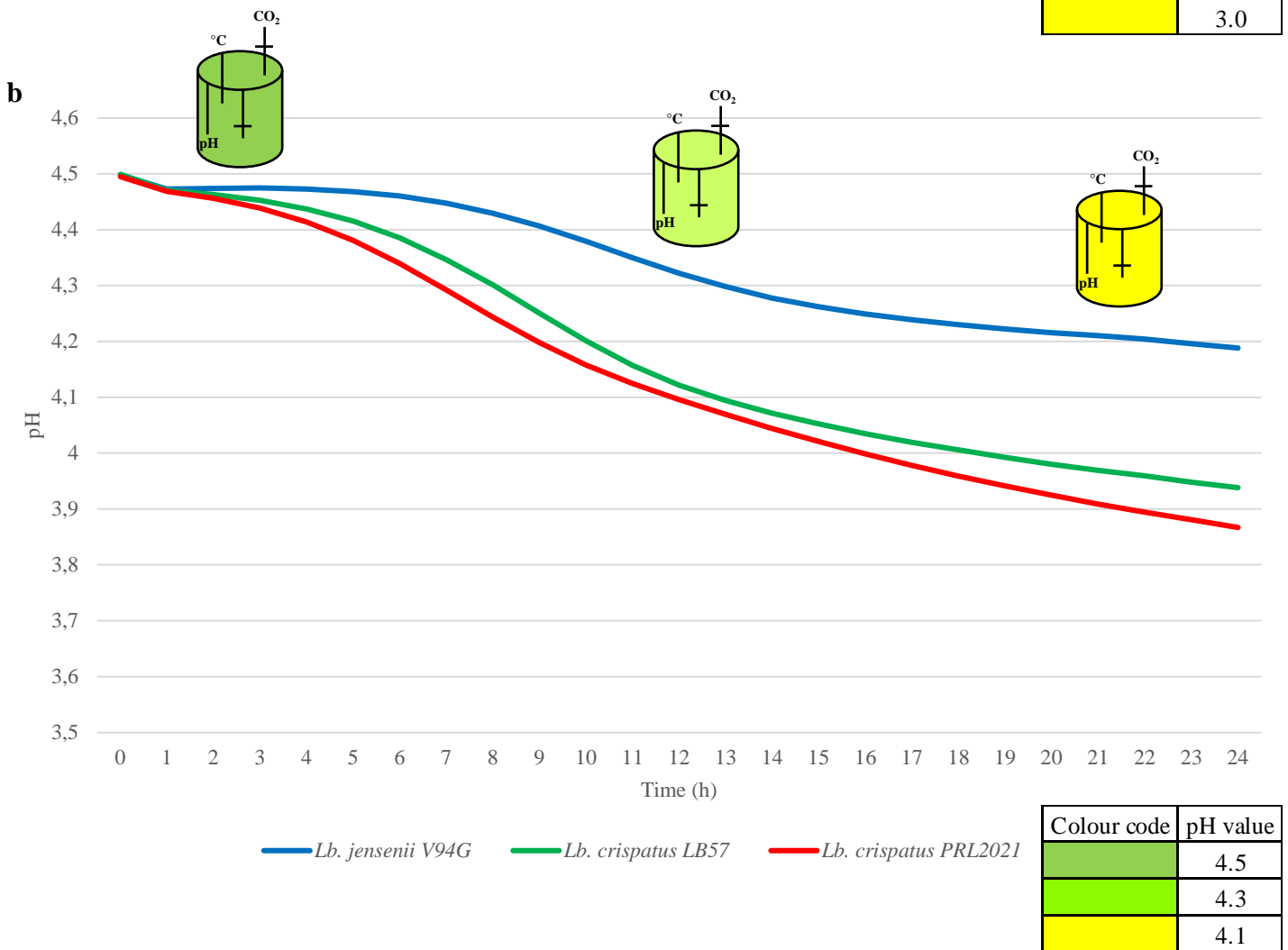
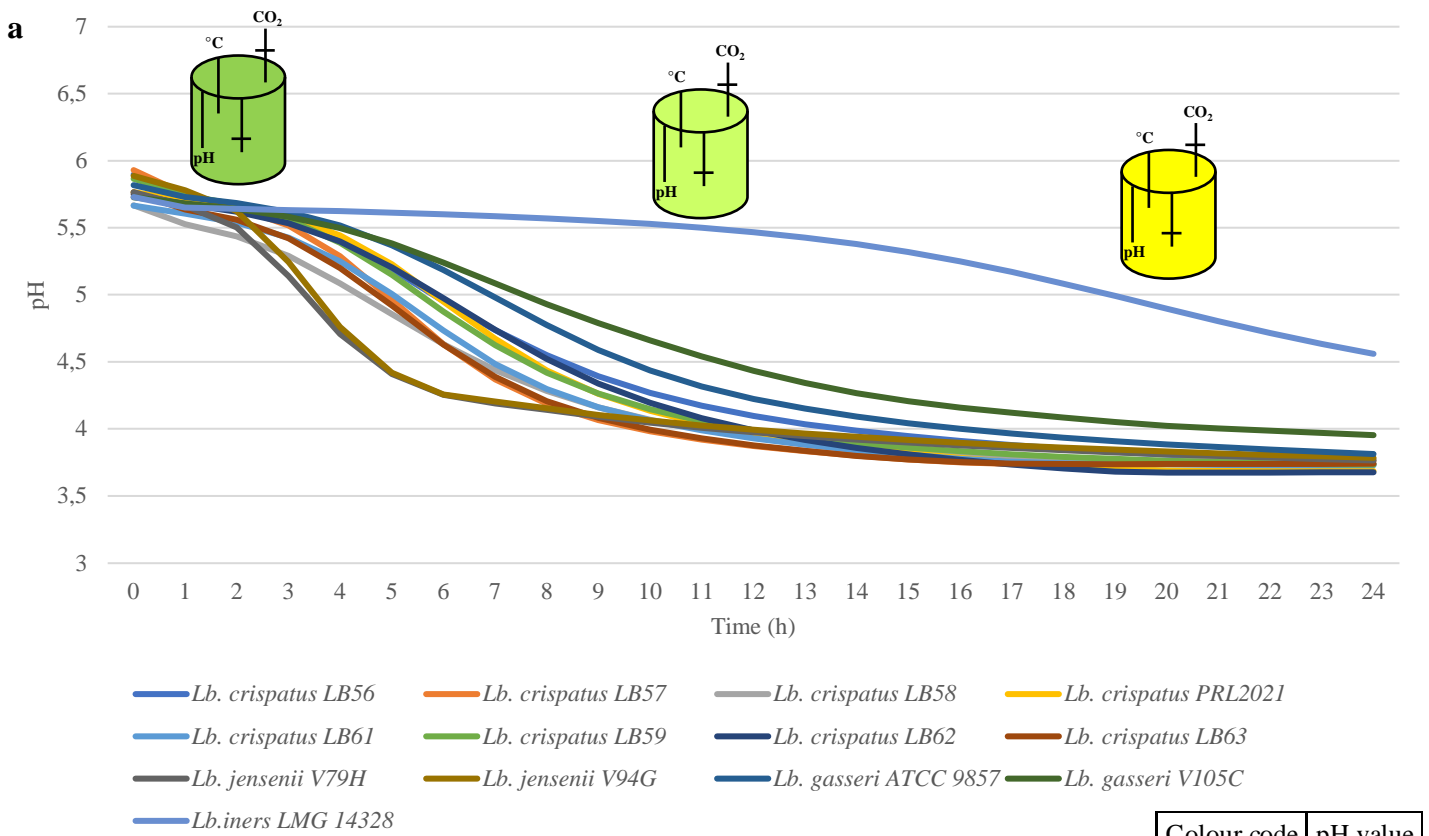


Probiotic	M247/LB60	2	1	1	0	0	6	1	0
Vaginal	PRL2021	1	1	1	0	0	6	0	0
Vaginal	LB56	1	0	1	0	0	6	0	0
Vaginal	LB57	2	1	2	0	0	6	1	1
Vaginal	LB58	2	1	1	0	0	6	1	0
Vaginal	LB59	2	0	1	0	0	6	1	1
Vaginal	LB61	2	1	1	0	0	5	1	0
Vaginal	LB62	2	1	1	0	0	6	1	1
Vaginal	LB63	2	1	1	0	0	6	1	1
Chicken	LB64	2	1	2	0	0	0	0	0
Chicken	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



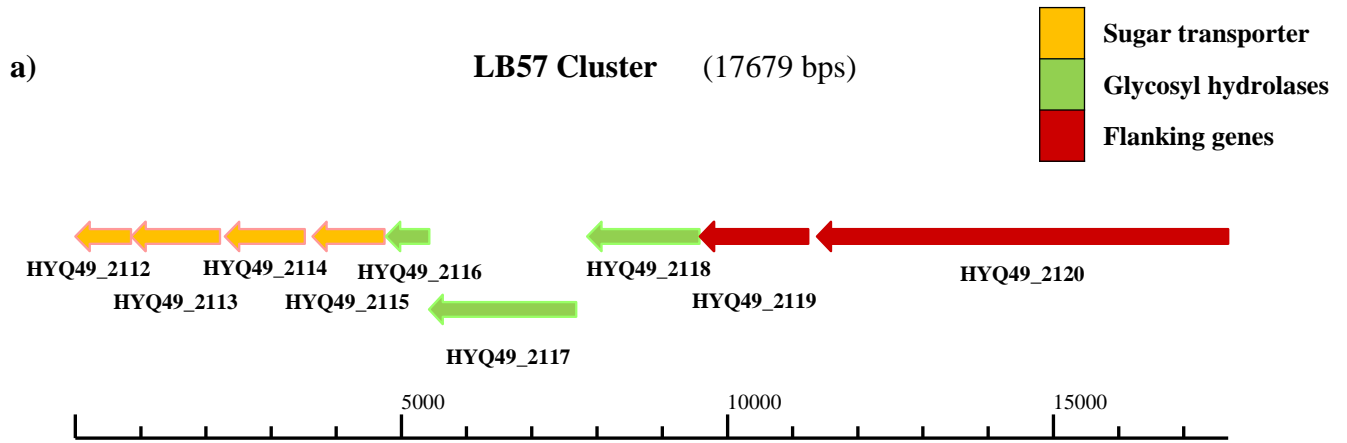
**Figure S3**

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

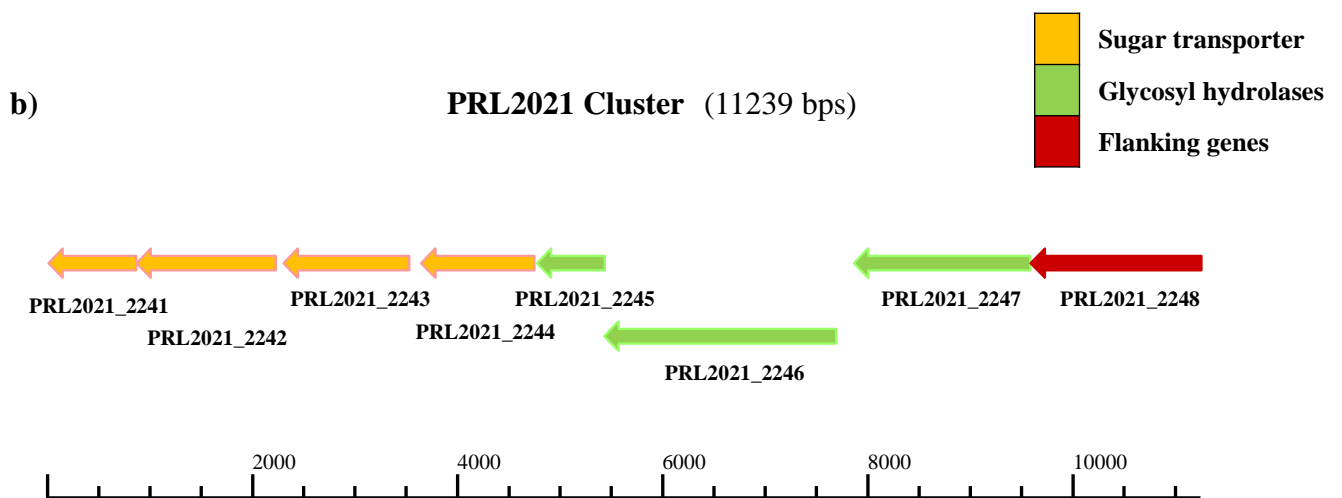
Figure S4



Genes involved in glycogen metabolism

Gene ID	Description	HMMER analysis	Ind.	Cond.
HYQ49_2120	hypothetical protein			
HYQ49_2119	beta-phosphoglucomutase			
HYQ49_2118	alpha-glycosidase	Alpha amylase, catalytic domain		
HYQ49_2117	maltose phosphorylase			
HYQ49_2116	beta-phosphoglucomutase			
HYQ49_2115	sugar ABC transporter ATP-binding protein			
HYQ49_2114	sugar ABC transporter sugar-binding protein		Ind.	Cond.
HYQ49_2113	sugar ABC transporter permease		3.6e-76	3.7e-80
HYQ49_2112	sugar ABC transporter permease			

Domain E-values



Genes involved in glycogen metabolism

Gene ID	Description
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 substitution, 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 DH 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.