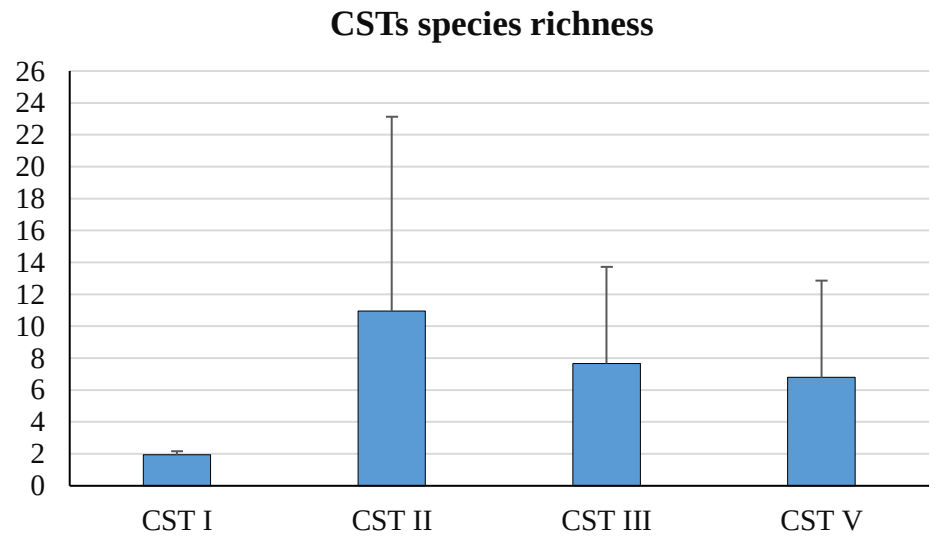
CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

| | |
|---|---|
| Title | Evaluation of modulatory activities of Lactobacillus crispatus 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 | 2022-04-01 |
| 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', Microbiology Spectrum, 10(2), (11pp). doi: 10.1128/spectrum.02733-21 |
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UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

**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)**

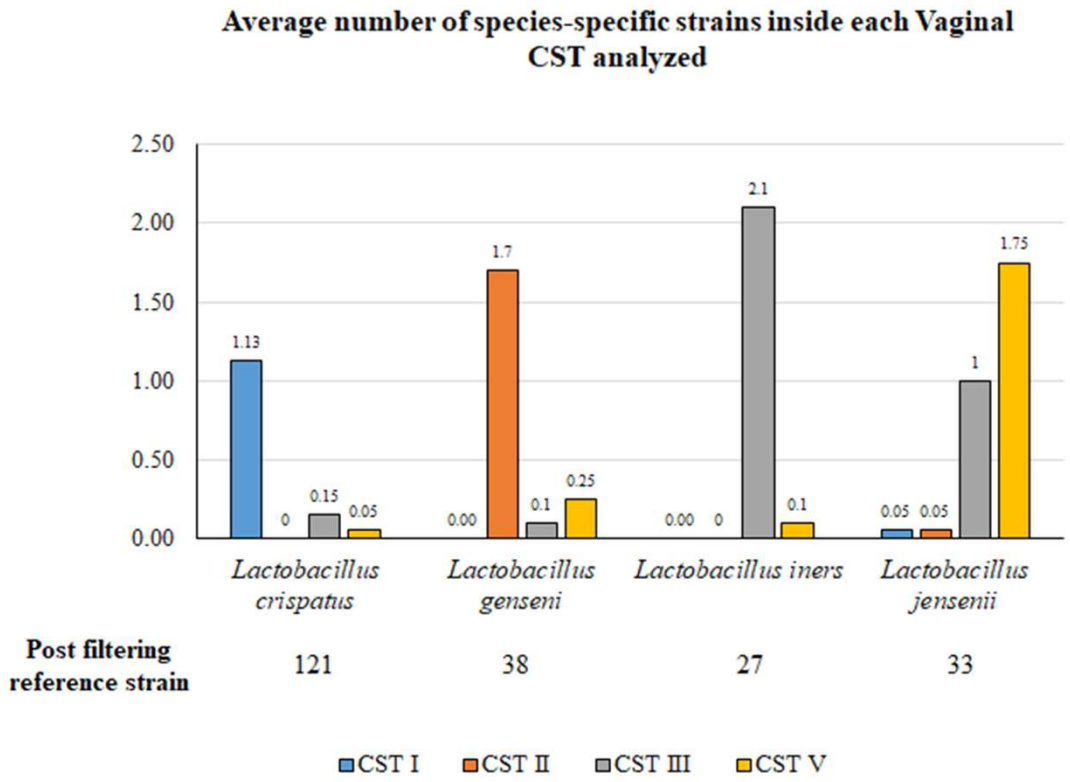
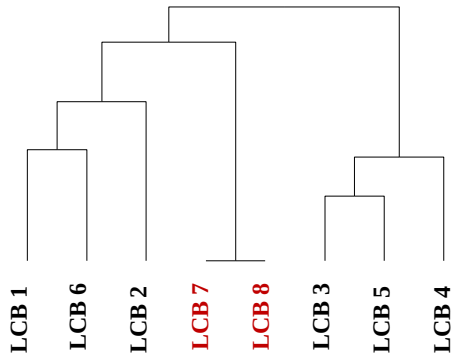

Average number of species-specific strains inside each Vaginal CST analyzed
2.50
2.00
1.50
1.00
0.50
0.00
1.13
0
0.15
0.05
0.00
1.7
0.1
0.25
0.00
0
2.1
0.1
0.05
0.05
1
1.75
Lactobacillus crispatus
Lactobacillus genseni
Lactobacillus iners
Lactobacillus jensenii
Post filtering reference strain
121
38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

**Figure S1**

**a)**




| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **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)**

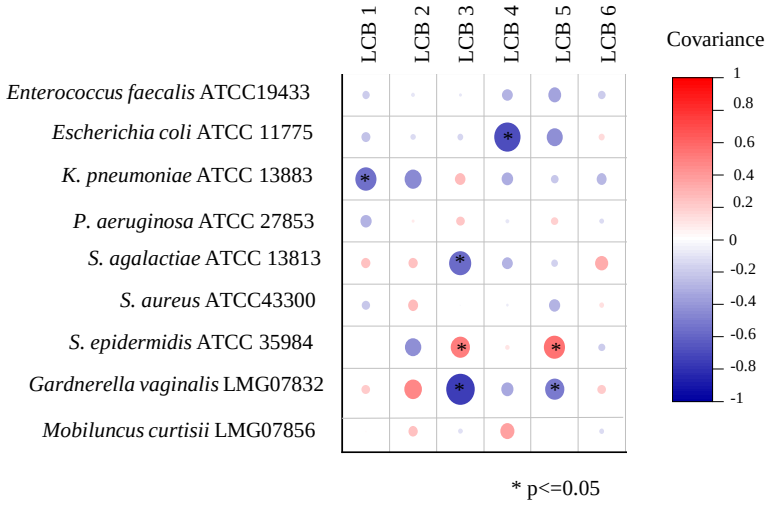
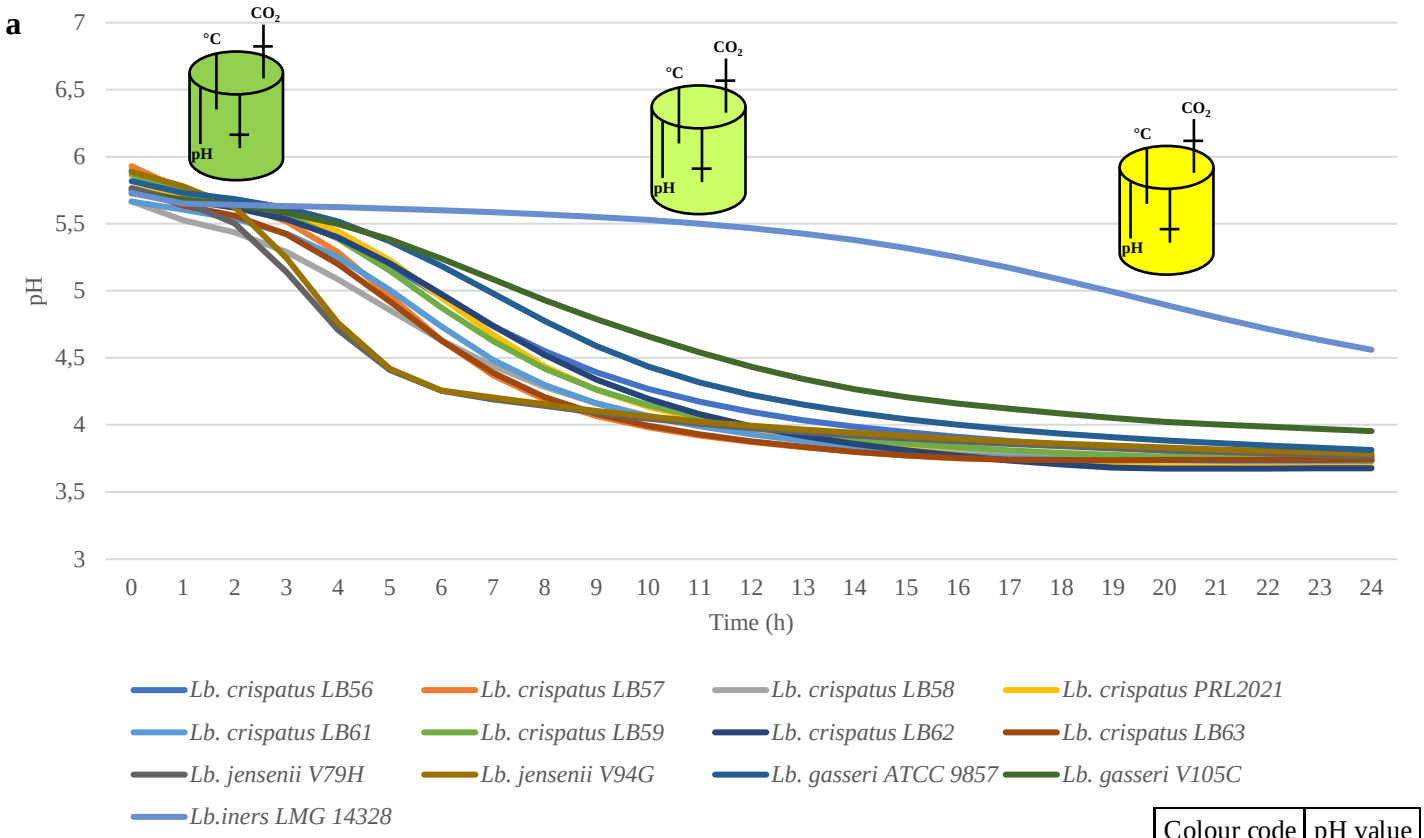
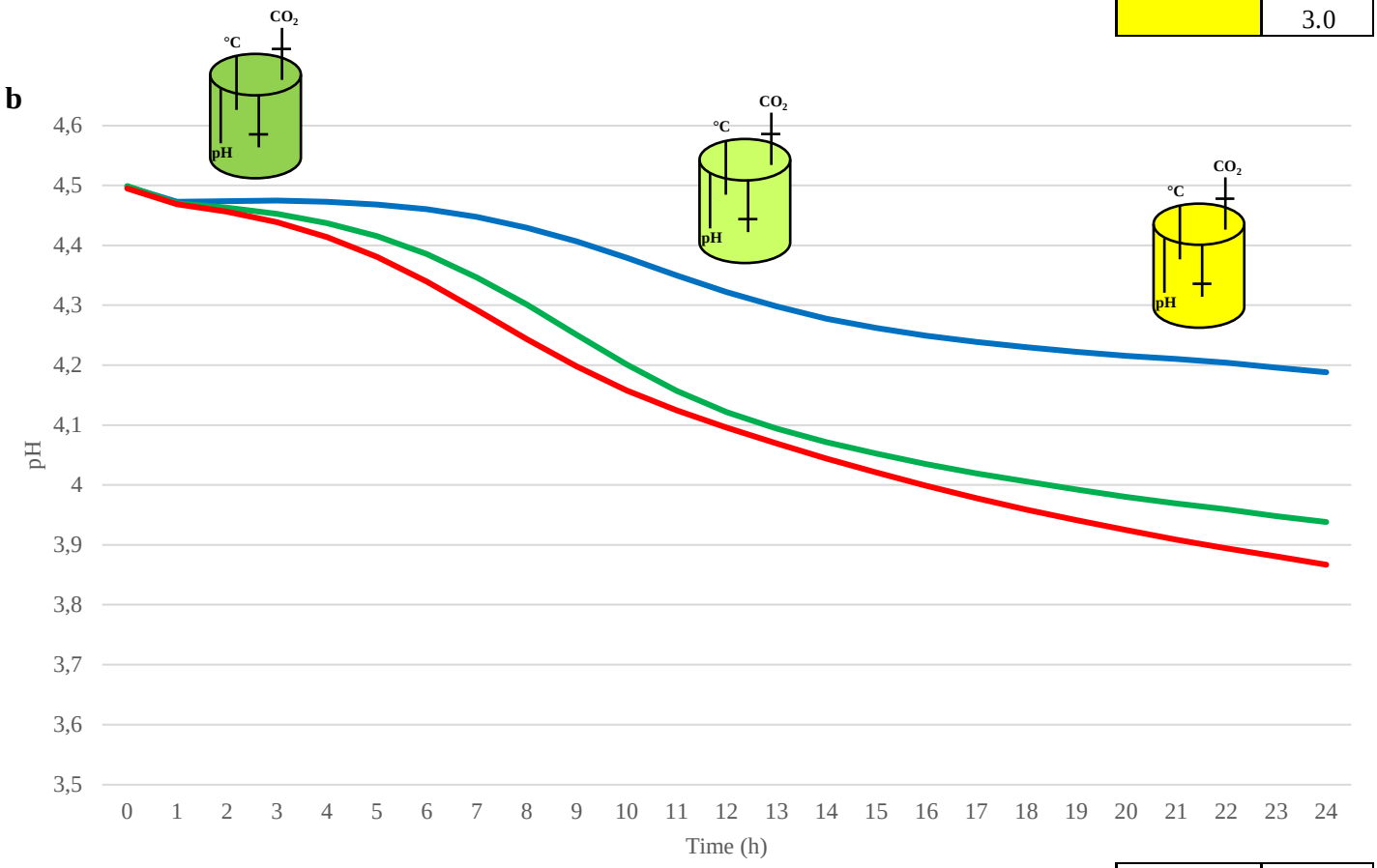



**Figure S2**




| Colour code | pH value |
|---|---|
| | 5.0 |
| | 4.0 |
| | 3.0 |




| Colour code | pH value |
|---|---|
| | 4.5 |
| | 4.3 |
| | 4.1 |

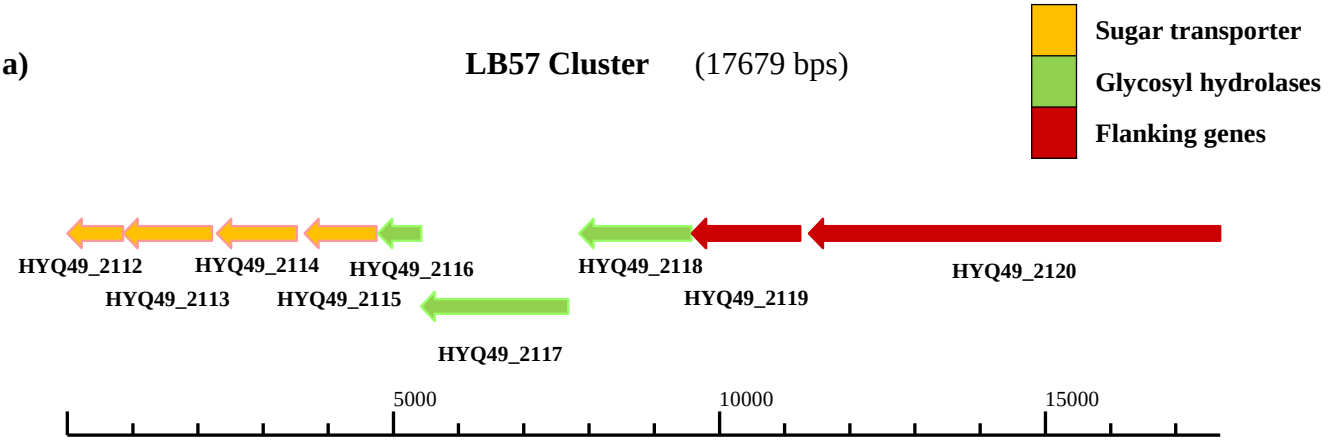
**Figure S3**

| 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 |

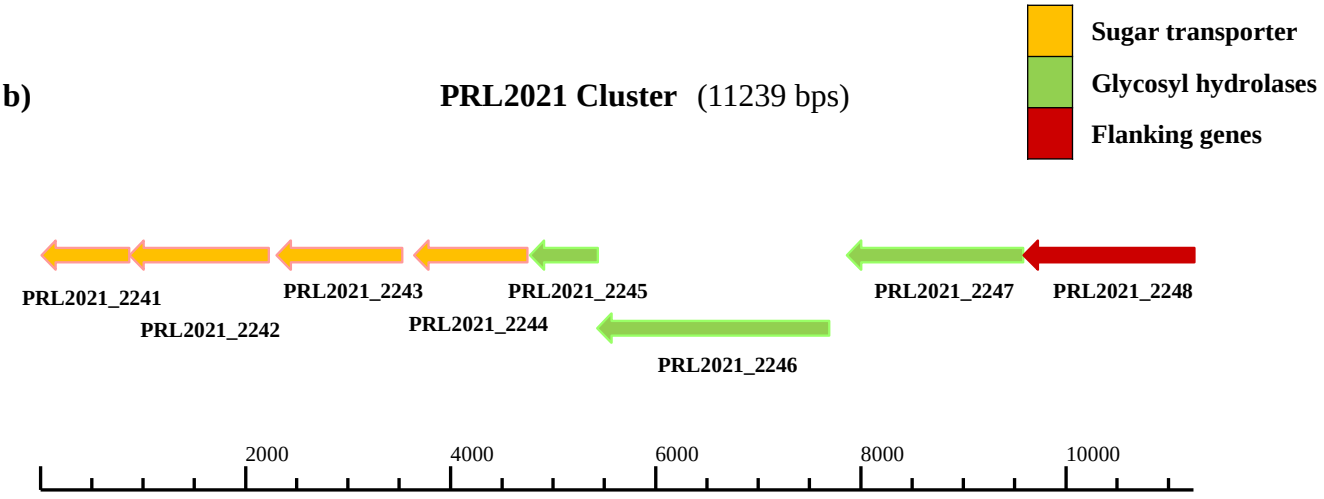
**HMMER analysis**

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.