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1 **TITLE**

2 # Amplification of the V5 – V8 region of the 16S rRNA gene effectively speciates medically important
3 # genital tract *Lactobacillus* species in the upper female genital tract

4

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24 **<u>Running title</u>**

25 Speciation of genital tract lactobacilli

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31 **ABSTRACT**

32 **Background:** The endometrial cavity is an upper genital tract site largely heralded as sterile, however,
33 advances in culture-independent, next generation sequencing technology have revealed that this site
34 harbours a rich microbial community which includes multiple *Lactobacillus* species. These bacteria are
35 considered to be the most common non-pathogenic genital tract commensals. Next-generation
36 sequencing of the female lower genital tract has revealed significant variation amongst microbial
37 community composition with respect to *Lactobacillus* sp. in samples collected from healthy and
38 diseased women. The aim of this study was to evaluate the ability of the 16S rRNA gene to
39 characterize genital tract lactobacilli to species-level taxonomy.

40 **Methods:** Samples were interrogated for the presence of microbial DNA using two-step next
41 generation sequencing technology to exploit the V5–V8 regions of the 16S rRNA gene and compared
42 to standard speciation using qPCR.

43 **Results:** The V5-V8 region of the 16S rRNA gene has sufficient sequence variation within frequently
44 encountered genital tract lactobacilli to allow accurate determination of relative abundance within
45 the community, and speciation for several key community members without completing additional
46 experimentation.

47 **Conclusions:** Next-generation sequencing of clinical genital tract isolates is an effective method for
48 high throughput identification to species-level of key *Lactobacillus* sp.

49 **KEYWORDS**

50 *Lactobacillus* sp.; genital tract; 16S rRNA; pyrosequencing; qPCR; speciation

51

52 **IMPORTANCE**

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53 Human microbiome experiments, including the low biomass organs such as the upper genital tract,
54 require the development of consensus protocols to ensure accurate comparison between such
55 studies and our data forms an important foundation for future protocols.

56 This paper provides evidence to support the selection of the V5-V8 regions of the 16S rRNA gene
57 improved *Lactobacillus* speciation using next generation sequencing technology. The choice of
58 variable region for broad-range amplification in microbiome studies is important due to preferential
59 primer binding associated with some genera based on nucleotide sequence patterns. By utilising the
60 V5-V8 region, multiple species of *Lactobacillus* can be characterised with relative confidence.

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83 **INTRODUCTION**

84 Molecular microbiology techniques have changed our ability to identify microbial communities,
85 revolutionizing the way we assess female genital tract microbiomes. In cultivation-dependent studies,
86 greater than 95% of the vaginal microbiota in healthy women was classified as lactobacilli. The advent
87 of cultivation-independent technology platforms has provided evidence to suggest that in up to two-
88 thirds of healthy women, the lactobacilli were co-aggregated with a diverse group of microbial
89 community members, and in some cases did not dominate (Fettweis et al., 2012; Klebanoff et al.,
90 1991). Lactobacilli establish niche dominance through co-aggregation, competitive inhibition,
91 production of metabolic acids, hydrogen peroxide, and antimicrobial components including
92 bacteriocins (Amabebe and Anumba, 2018). The discovery that lactobacilli do not dominate the genital
93 tract of all healthy women suggests that: there is redundancy in function and protection based on
94 community membership; and all lactobacilli may not provide the same level of protection in the genital
95 tract environment. This discovery casts doubt over the long-held view that a healthy female genital
96 tract is characterized by a *Lactobacillus* sp.-dominant microbiota. The ability to confidently assign
97 lower order taxonomic classification to lactobacilli is critical in advancing our understanding of the
98 protective role played by the various species within this genus in reproductive health. The objective
99 of this study was to examine the discriminatory power of current molecular microbiology techniques
100 for identification of genital tract lactobacilli.

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109 **METHODS**

110 **Patient cohort, sample collection and genomic DNA preparation**

111 Clinical sample cohorts were constructed as previously described (Pelzer et al., 2018). Genomic DNA
112 was extracted from individual samples prior to pooling using a modified protocol with the Qiagen
113 QiAMP Mini DNA extraction kit (Qiagen, Australia) as previously described (Pelzer et al., 2018).

114 **Details of ethical approval**

115 All patients recruited for this study provided written informed consent. Ethical approval was
116 obtained from the review boards of UnitingCare Health, Human Research Ethics Committee and
117 Queensland University of Technology Human Ethics Committee.

118 **Next-generation sequencing**

119 The 16S rRNA PCR assay was performed using the previously published primers, 803F (5′-TTA GAT ACC
120 CTG GTA GTC -3′) and 1392R (5′-ACG GGC GGT GTG TRC -3′) and PCR cycling conditions (Willner et al.,
121 2014). Fusion primers with 454 adaptor sequences were ligated to the 803F and 1392R primers to
122 amplify the V5 and V8 regions of the 16S rRNA gene (Willner et al., 2014). PCR reactions were
123 performed as previously described (Pelzer et al., 2018). The five frequently encountered genital tract
124 *Lactobacillus* sp. were aligned using the SILVA database to determine the degree of variation within
125 the V5-V8 regions of the 16S rRNA gene. The annealing site of the sequencing primers is marked on
126 the alignment (Figure 1a).

127 ***Lactobacillus* sp.-specific quantitative real-time PCR**

128 Quantitative real-time PCR assays were performed using previously published primer pairs (Table 1)
129 and cycling conditions. A standard curve was generated using *L. gasseri* ATCC strain 19992. Primer

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130 annealing was confirmed using species-specific alignment of the five *Lactobacillus* sp. interrogated in
131 this study (Figure 1b).
132

133 **Taxonomic classification**

134 Sequence clustering and operational taxonomic unit (OTU) selection was performed using a modified
135 version of CD-HIT-OTU-454 which does not remove singleton clusters (Liu et al., 2011). Taxonomy was
136 assigned to representative sequences by comparison to the latest build of the Greengenes database
137 using BLAST, and OTU tables were constructed from the output using a custom Perl script (McDonald
138 et al., 2012).

139 ***Lactobacillus* Phylogenetic Trees**

140 Full-length 16S rRNA sequences for *Lactobacillus spp.* (accession numbers: AB680529.1, AB690249.1,
141 AB668940.1.1, AB008203.1.1, AB425941.1.1, AB008206.1, AF243169.1, AF243167.1,
142 CP018809.253324, CP018809.1516019, CP018809.1347636, CP018809.500868, AB547127.1,
143 AB517146.1, AB932527.1, AB008209.1, HZ485829.7, LG085736.7 LF134126.7, LG104504.7),
144 *Pediococcus pentosaceus* (accession numbers: AB018215.1 and AB362987.1), and *Bacillus subtilis*
145 (accession numbers: AP012496.9810 and AP012496.30276) were downloaded from the SILVA
146 database using the web interface (www.arb-silva.de). Sequences were aligned using ClustalW
147 (Thompson et al., 1994) with the default settings. MEGA7 (Kumar et al., 2016) was used to generate
148 the best-known maximum likelihood (ML) tree using a Jukes-Cantor model and 1000 bootstrapping
149 iterations. The ML tree was visualised and edited within FigTree (http://tree.bio.ed.ac.uk ) and Adobe
150 Illustrator (Figure 4).

151 To generate the V5-V8 region phylogenetic tree the same full length 16S rRNA sequences from above
152 were imported into Geneious along with two *Escherichia coli* sequences downloaded from SILVA
153 (accession number: AB045730.1 and AB045731.1). Sequences were aligned using standard Geneious
154 alignment and trimmed to include the variable regions V5-V8 (nucleotides 751 – 1300 for the *E.coli*

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155 sequence). Trimmed sequences were then aligned using ClustalW with default settings and imported
156 into MEGA7 and a tree was constructed and edited same as above (Figure 4).

157 **Hierarchical clustering**

158 A dissimilarity matrix was generated based on the relative abundances of *Lactobacillus spp.* in the
159 pyrosequenced and qPCR analysed samples using the vegdist function in the vegan package in R with
160 the Bray-Curtis dissimilarity metric (J. et al., 2018). Hierarchical clustering was performed using the
161 hclust function in R with 'average' linkage (UPGMA) (Team, 2013). Clustering and relative
162 abundances were visualized in a heatmap with associated dendrogram using the heatmap.2 function
163 from the R package ggplots (Warnes et al., 2005).

164 **RESULTS**

165 **NGS resolution of genital tract *Lactobacillus* sp. OTUs to genus and species**

166 Ten OTUs were attributed to *Lactobacillus* sp. (*Lactobacillus* sp. genus level (n = 2), *L. crispatus* (n = 2),
167 *L. iners* (n = 3), *L. intestinalis* (n = 1), *L. jensenii* (n = 1) and *L. vaginalis* (n = 1)). The majority of
168 *Lactobacillus* sp. OTUs (8/10) were resolved to the genus and species level exploiting the V5-V8 regions
169 of the 16S rRNA gene.

170 ***Lactobacillus* species-specific quantitative real-time PCR assay comparison to NGS output**

171 The species-specific quantitative real-time PCR assays confirmed the identity and relative abundance
172 of *L. crispatus*, *L. jensenii* and *L. iners* in clinical genital tract samples. Two of the species that
173 underwent pyrosequencing identification were not included due to low abundance (*L. intestinalis* and
174 *L. vaginalis*).

175 The abundance of the five commonly encountered species (*L. crispatus, L. gasseri, L. iners, L. jensenii*
176 and *L. acidophilus*) were then compared between the qPCR and the 454 pyrosequencing (Figure 2).
177 *L. crispatus* dominated the *Lactobacillus* community in most samples. All samples displayed similar
178 abundance profiles with enriched lower abundance species including *L. jensenii*, *L. iners* and *L.*

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179 *acidophilus* exposed by qPCR (Table 3, Figure 2). The V5-V8 region of the 16S rRNA gene was not as
180 effective in distinguishing *L. acidophilus* from *L. gasseri* using the primers published by Ma *et al*.
181 (2013) due to high sequence homology between these two species in the V5-V8 variable region.

182 ***Lactobacillus* phylogeny**

183 The phylogenetic tree constructed using the full-length16S rRNA gene sequences of the *Lactobacillus*
184 sp. described in this study indicated that *L. acidophilus* and L. *crispatus* appear more closely related
185 than *L. acidophilus* and *L. gasseri*. When comparing only the V5-V8 region, however, *L. gasseri*
186 clusters closer to *L. acidophilus* (Figure 4). The heat map confirmed that *L. crispatus* and *L. iners*
187 dominated the microbial communities in samples analyzed in this study and samples were more
188 likely to cluster based on *Lactobacillus* community dominance, than the patient history
189 (dysmenorrhea or menorrhagia), the anatomical site of collection (endometrium or cervix), or the
190 analysis technique (qPCR or pyrosequencing).

191 **DISCUSSION**

192 Sequencing of the V5-V8 region of the 16S rRNA gene improves the discriminatory power for
193 speciation of dominant genital tract lactobacilli. This study examined the different bacterial
194 communities within the upper genital tract of women, reporting changes in the bacterial community
195 composition of lactobacilli. Consistent with previous studies, *L. crispatus* and *L. iners* were the most
196 abundant lactobacilli in the samples tested in this study.

197 Sequencing technologies frequently often only report the presence of lactobacilli at genus-level.
198 Studies exploiting some regions of the 16S rRNA gene fail to discriminate lactobacilli beyond higher
199 order taxonomic classification due to limited sequence variation. Therefore, some studies have
200 reported that lactobacilli as a genera are: positively correlated with healthy pregnancy outcomes
201 including successful implantation and delivery at term; and form abundant community members in
202 cases of adverse pregnancy outcomes including recurrent implantation failure and preterm birth
203 (Franasiak et al., 2016; Moreno et al., 2016; Onderdonk et al., 2008; Tao et al., 2017). Our research

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204 design enabled us to overcome the shortfalls commonly associated with genus-level identification.
205 Similar results can be observed in molecular studies characterising the female genital tract when
206 multiple variable regions of the 16S rRNA gene were sequenced (Fettweis et al., 2012; Graspeuntner
207 et al., 2018; Madhivanan et al., 2014; Miles et al., 2017). Van Der Pol *et al*. (Van Der Pol et al., 2019)
208 reported that the choice of 16S rRNA reference sequence database and sample sequence clustering
209 parameters are equally as important as the choice of variable region for amplification characterising
210 microbial community members to lower orders.

211 There is no doubt that sequencing the conserved 16S rRNA gene has improved our understanding of
212 extant biodiversity in human microbial communities and is critical for understanding the impact of
213 low-abundance community members on health and disease. However, there is no consensus best
214 practice for microbiome studies, and significant variability exists between sample collection and
215 storage methods, DNA extraction, universal primer selection, and sequencing platform and data
216 analysis software (Pollock et al., 2018). Characterization of microbial communities using the 16S rRNA
217 gene have been hampered by inherent differences generated in community profiles when sequencing
218 different hypervariable regions, short read lengths, and taxonomic classification difficulties due to
219 limited resolution for closely related species (Poretsky et al., 2014). Sequencing technologies have
220 been used to interrogate the genital tract microbial community in reproductive-aged women but most
221 fail to resolve the isolates to species-level. Consequently, more recent efforts have focused on
222 sequencing multiple variable regions of the gene with amalgamation of all data into a single profile
223 (Fuks et al., 2018). Very current research focuses on removing bias associated with sequencing
224 component variable regions by using full-length gene sequencing (Callahan et al., 2016). The need to
225 characterise the full-length 16S rRNA gene is further required as exhibited by the change in *L. gasseri*
226 clustering when comparing the full-length gene to the V5-V8 region. Within this study, these species
227 were not able to be distinguished from each other using this region alone.

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228 The significance of our research is highlighted by studies confirming that *L. iners* does not protect
229 against preterm birth and is frequently reported as an abundant community member in women with
230 bacterial vaginosis (Madhivanan et al., 2014; Petricevic et al., 2014). Further, significant differences
231 between lactobacilli in term compared to preterm deliveries have not been reported for all studies
232 (Amabebe and Anumba, 2018; Romero et al., 2014). Within our study we are able to identify the
233 bacteria to a species level using pyrosequencing reads with relative confidence. One limitation of this
234 study is the relatively small sample size.

235 Collectively our research confirms what other studies have shown, that health and disease may
236 depend on species and strain-level differences for prominent community members at a given
237 anatomical niche (Kraal et al., 2014). It is clear that additional discriminatory power is required to
238 resolve lower order classifications using current sequencing methods. This current study confirms
239 that speciation of key genital tract *Lactobacillus* sp., capable of modulating reproductive health is
240 possible when the appropriate region of the 16S rRNA gene is interrogated.

241 **CONCLUSION**

242 Studies characterizing microbial communities in the female genital tract report inconsistent results
243 when assessing dysbiosis as a cause of reproductive pathology. Our work provides evidence for the
244 impact of primer selection on evaluating the biological significance of shifts in community taxa. Careful
245 experimental design should include a comparative analysis of microbial community profiling data
246 generated by interrogation of multiple variable regions to the 16S rRNA gene to ensure that species
247 abundance and diversity are accurately reflected.

248 **List of abbreviations**

249 ATCC: American type culture collection; DGC: dysmenorrhea progestin effect endocervix; DGE:
250 dysmenorrhea progestin effect endometrium; DNA: deoxyribonucleic acid; DPC: dysmenorrhea
251 proliferative endocervix; DPE: dysmenorrhea proliferative endometrium; DSC: dysmenorrhea

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252 secretory endocervix; DSE: dysmenorrhea secretory endometrium; HRM: high resolution melt; MGC:
253 menorrhagia progestin effect endocervix; MGE: menorrhagia progestin effect endometrium; MPC:
254 menorrhagia proliferative endocervix; MPE: menorrhagia proliferative endometrium; MSC:
255 menorrhagia secretory endocervix; MSE: menorrhagia secretory endometrium; OTU: operational
256 taxonomic unit; rRNA ribosomal ribonucleic acid; VIC: virgo intacta

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261 samples. We wish to acknowledge the Australian Centre for Ecogenomics for 454 pyrosequencing and
262 Professor Philip Hugenholtz and Dr Fiona May. This work was performed in the John and Wendy
263 Thorsen Women's Health Laboratory.

264 **CONTRIBUTION TO AUTHORSHIP**

265 JOC: designed and completed bioinformatics analyses, contributed to the analysis and interpretation
266 of the data, and contributed to the writing of the manuscript.
267 DW: designed and completed bioinformatics analyses, contributed to the analysis and interpretation
268 of the data, and contributed to the writing of the manuscript.
269 MB: conceived and designed the project, performed collection of clinical specimens and contributed
270 to the writing of the manuscript.
271 FH: designed and completed the qPCR experiments, contributed to the analysis and interpretation of
272 the qPCR data and contributed to the writing of the manuscript.
273 EP: conceived and designed the project, completed tissue processing, DNA extraction and 16S PCR
274 experiments, contributed to the analysis and interpretation of the data, and drafted significant parts
275 of the work.

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370 **TABLE/FIGURE CAPTION LIST**

371 **Table 1.** Sample abbreviations

372 **Table 2.** 16S rRNA *Lactobacillus*-species specific primers (Ma et al., 2013)

373 **Table 3:** Unique to species SNPs from the 16S rRNA gene variable regions.

374

375 **Figure 1a: Annealing site of 454 pyrosequencing 16S rRNA primers to V5-V8 region in the**
376 ***Lactobacillus* species**.

377

378 **Figure 1b: Annealing site of species specific primers (Table 2) in the *Lactobacillus* species which**
379 **underwent qPCR.**

380

381 **Figure 2: Comparison of relative microbial abundance all sites and phases between 454**
382 **pyrosequencing of the V5 - V8 region and species specific Lactobacillus qPCR.**

383

384 **Figure 3: Hierarchical clustering/distance ordination quantifying similarities between qPCR and**
385 **PYRO pairs**

386 *Lactobacillus* relative abundance distribution and hierarchical clustering in pyrosequenced and qPCR
387 analysed samples. The heatmap shows relative abundances of detected *Lactobacillus* species in each
388 sample, with columns organised by relative positions in the dendrogram. Some subsets of paired
389 pyrosequenced and qPCR samples have been coloured to highlight clustering.

390 **Figure 4a: Full length and region specific 16S rRNA gene phylogeny for key genital tract lactobacilli**

391 ML tree derived from full-length and trimmed (V5-V8 region) 16S rRNA sequences of the multiple
392 species of *Lactobacillus* identified by pyrosequencing and 16S qPCR. Accession numbers for reference
393 sequences are provided in the methods. *Pediococcus pentasaecus* and *Bacillus subtilis* were included
394 as outgroups. Branch support values are based on 1000 bootstrap repetitions.

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398 **Table 1.** Sample abbreviations

| **Sample Abbreviations** | **Full Sample Name** |
|---|---|
| DGC | dysmenorrhea progestin effect endocervix |
| DSC | dysmenorrhea secretory endometrium |
| MGC | menorrhagia progestin effect endocervix |
| DPC | dysmenorrhea proliferative endocervix |
| DGE | dysmenorrhea progestin effect endometrium; |
| DPE | dysmenorrhea proliferative endometrium |
| MGE | menorrhagia progestin effect endometrium |
| MSE | menorrhagia secretory endometrium |
| MSC | menorrhagia secretory endocervix |
| MPE | menorrhagia proliferative endometrium |
| MPC | menorrhagia proliferative endocervix |
| VIC | virgo intacta |

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400 **Table 2. 16S rRNA *Lactobacillus*-species specific primers** (Ma et al., 2013)

| *Lactobacillus* species | Primer name | Primer sequence |
|---|---|---|
| *L. acidophilus* | LAA-1 | 5'CATCCAGTGCAAACCTAAGAG3' |
| | LAA-2 | 5'GATCCGCTTGCCTTCGCA3' |
| *L. crispatus* | LcrisF | 5'AGCGAGCGGAACTAACAGATTTAC3' |
| | LcrisR | 5'AGCTGATCATGCGATCTGCTT3' |
| *L. gasseri* | LgassF | 5'AGCGAGCTTGCCTAGATGAATTTG3' |
| | LgassR | 5'TCTTTTAAACTCTAGACATGCGTC3' |
| *L. jensenii* | LjensF | 5'AAGTCGAGCGAGCTTGCCTATAGA3' |
| | LjensR | 5'CTTCTTTCATGCGAAAGTAGC3' |
| *L. iners* | InersF | 5'GTCTGCCTTGAAGATCGG3' |
| | InersR | 5'ACAGTTGATAGGCATCATC3' |

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409 **Table 3.** Unique to species SNPs from the 16S rRNA gene variable regions.

| | 16S rRNA gene variable Region | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Species** | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8** | **V9** |
| *L. iners* | 5 | 17 | 0 | 2 | 0 | 0 | 1 | 2 | 4 |
| *L. gasseri* | 4 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 1 |
| *L. acidophilus* | 2 | 4 | 0 | 3 | 2 | 0 | 0 | 2 | 0 |
| *L. crispatus* | 2 | 1 | 0 | 1 | 3 | 2 | 0 | 0 | 0 |
| *L. jensenii* | 6 | 6 | 0 | 1 | 1 | 5 | 1 | 0 | 5 |

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429 **Figure 1a :** Annealing site of 454 pyrosequencing 16S rRNA primers to V5-V8 region in the

430 *Lactobacillus* species.

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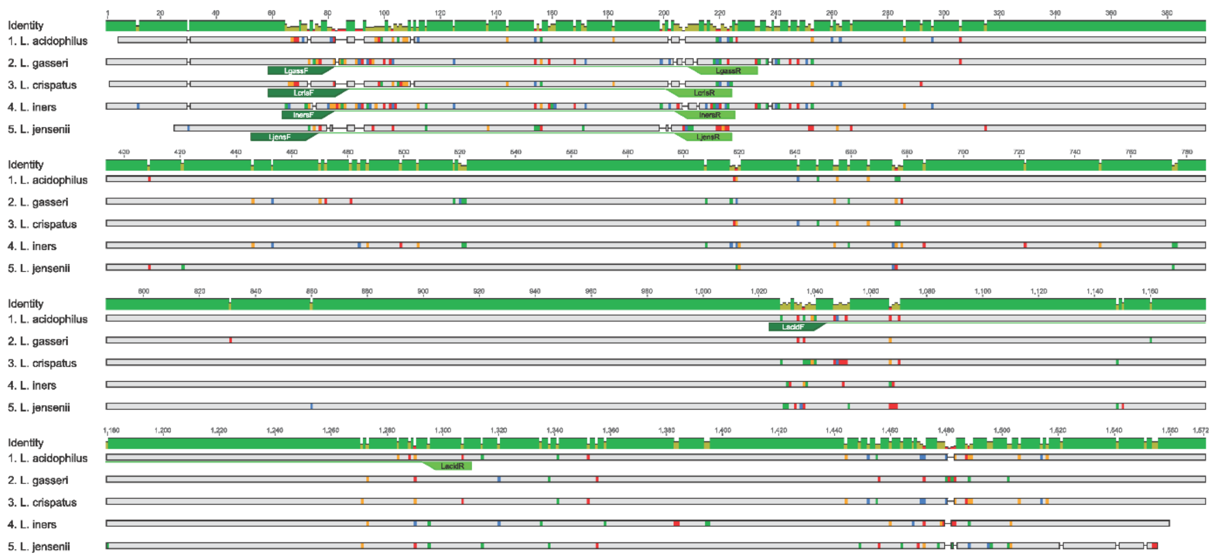


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433 **Figure 1b :** Annealing site of species specific primers (Table 2) for the *Lactobacillus* species which

434 underwent qPCR.

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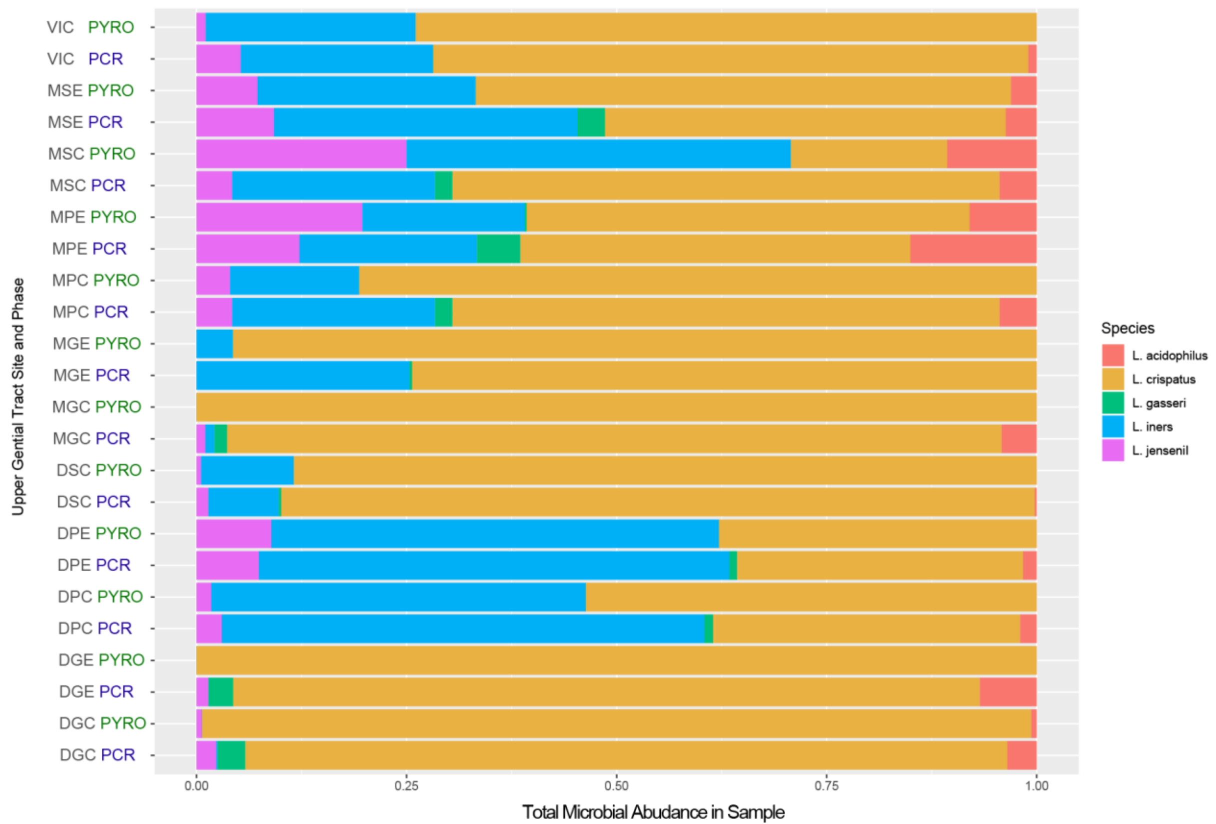


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438 **Figure 2**: Comparison of relative microbial abundance at all sites and phases between 454
439 pyrosequencing of the V5 - V8 region and species specific *Lactobacillus* qPCR.
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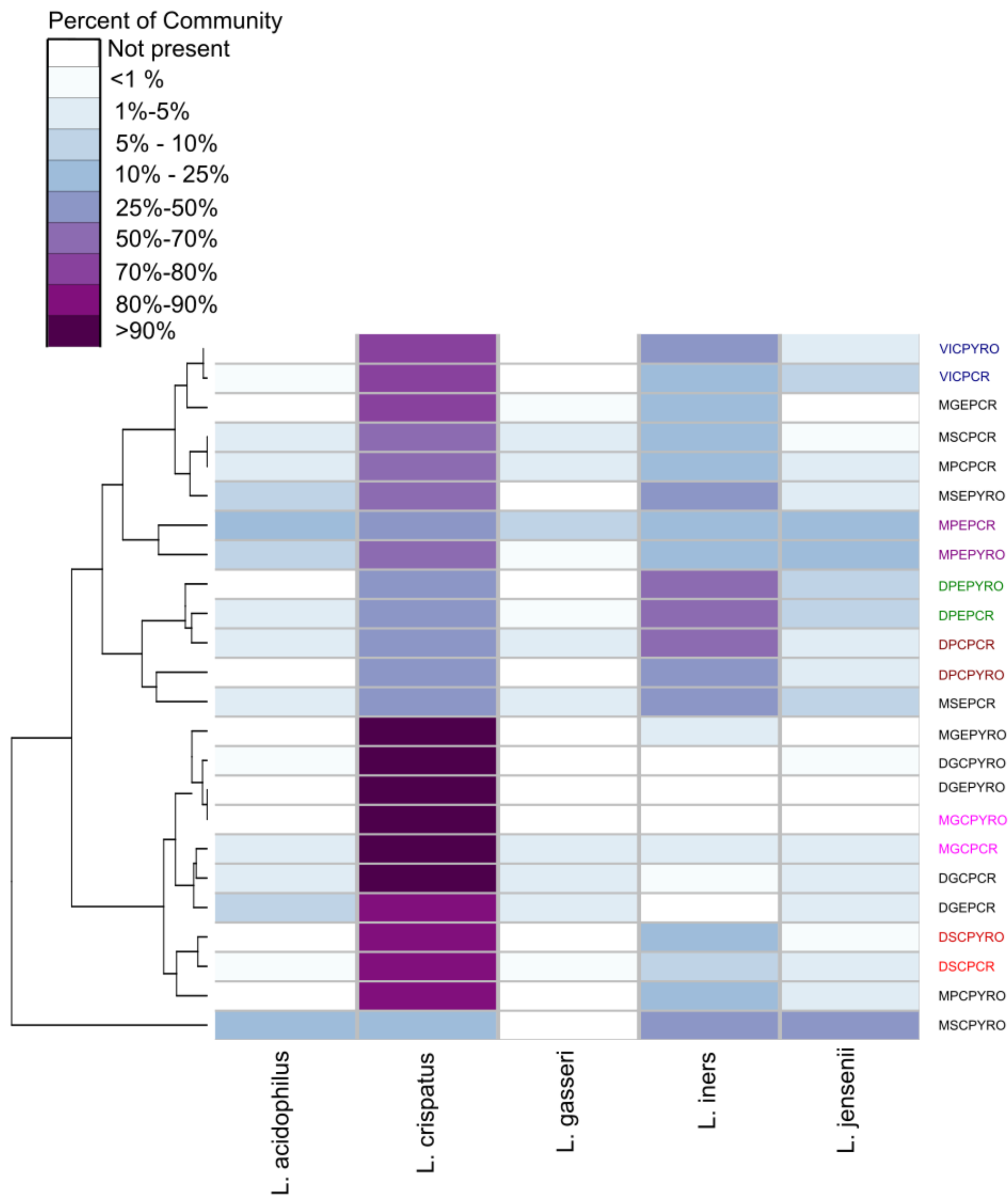


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457 **Figure 3: Hierarchical clustering/distance ordination quantifying similarities between qPCR and**
458 **PYRO pairs**

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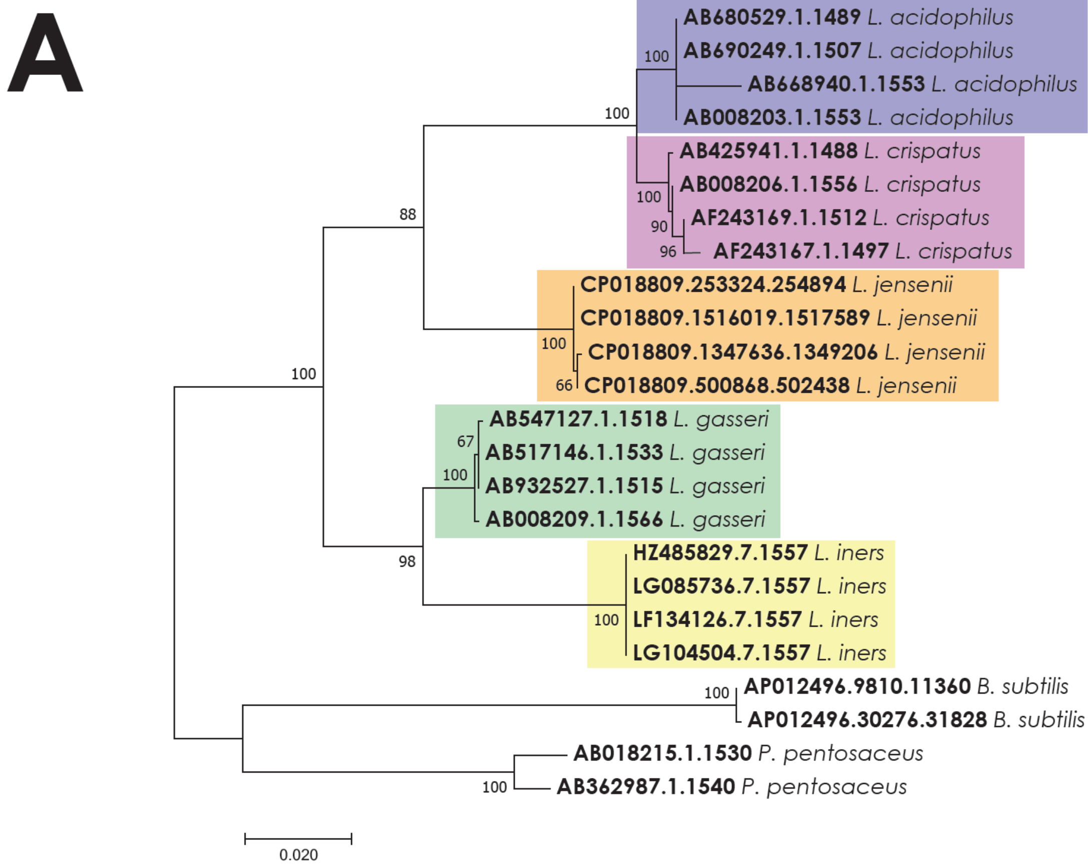


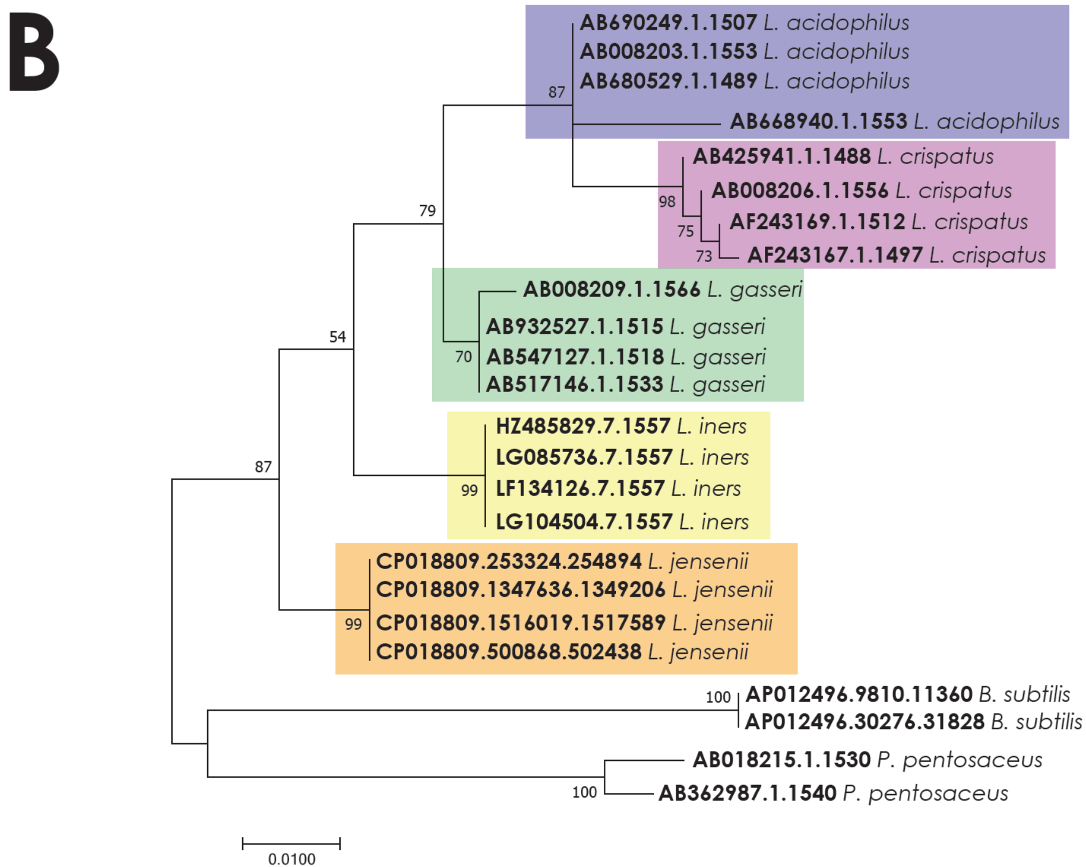


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461 **Figure 4: 16S rRNA gene phylogeny for key genital tract lactobacilli**

462 **(A) Full length**

463 **(B) V5-V8 region**

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495 **CONFLICT OF INTEREST STATEMENT**

496 The authors declare that there are no conflicts of interest.

497 **FUNDING STATEMENT**

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499 funding body played no role in conducting the research or preparing the manuscript.

500

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Consensus        GGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCG
                   PyroFORWARD
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1. L. acidophilus GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTAGTGCAATCCGTAGAGATACGGAGTTCCCTTCGGGGACACTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
2. L. gasseri     GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCAGTGCAAACCTAAGAGATTAGGTGTTCCCTTCGGGGACGCTGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
3. L. crispatus   GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTAGTGCCATTTGTAGAGATACAAAGTTCCCTTCGGGGACGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
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5. L. jensenii    GTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACCTAAGAGATTAGGTTTTCCCTTCGGGGACAAAGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG

Consensus        CAACGAGCGCAACCCTTGTCATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGTACAACGAGAAG
Identity
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2. L. gasseri     CAACGAGCGCAACCCTTGTCATTAGTTGCCATCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGAAG
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5. L. jensenii    CAACGAGCGCAACCCTTGTTAATAGTTGCCAGCATTAAGTTGGGCACTCTATTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCAGTACAACGAGAAG

Consensus        CGARCCTGCGAAGGCAAGCGGATCTCTGAAAGCTGTTCTCAGTTCGGACTGCAGGCTGCAACTCGCCTGCACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
                                                                                                                                                         PyroREVERSE
Identity
1. L. acidophilus CAAGCCTGCGAAGGCAAGCGAATCTCTTAAAGCTGTTCTCAGTTCGGACTGCAGTCTGCAACTCGACTGCACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
2. L. gasseri     CGAACCTGCGAAGGCAAGCGGATCTCTGAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
3. L. crispatus   CGAGCCTGCGAAGGCAAGCGAATCTCTGAAAGCTGTTCTCAGTTCGGACTGCAGTCTGCAACTCGACTGCACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
4. L. iners       CGACCCTGTGAAGGCAAGCGGATCTCTGAAAGCCGTTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCTGGAATCGCTAGTAATCGCAAATCAGCACGTTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
5. L. jensenii    CGAACCTGTGAAGGCAAGCGGATCTCTTAAAGCTGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
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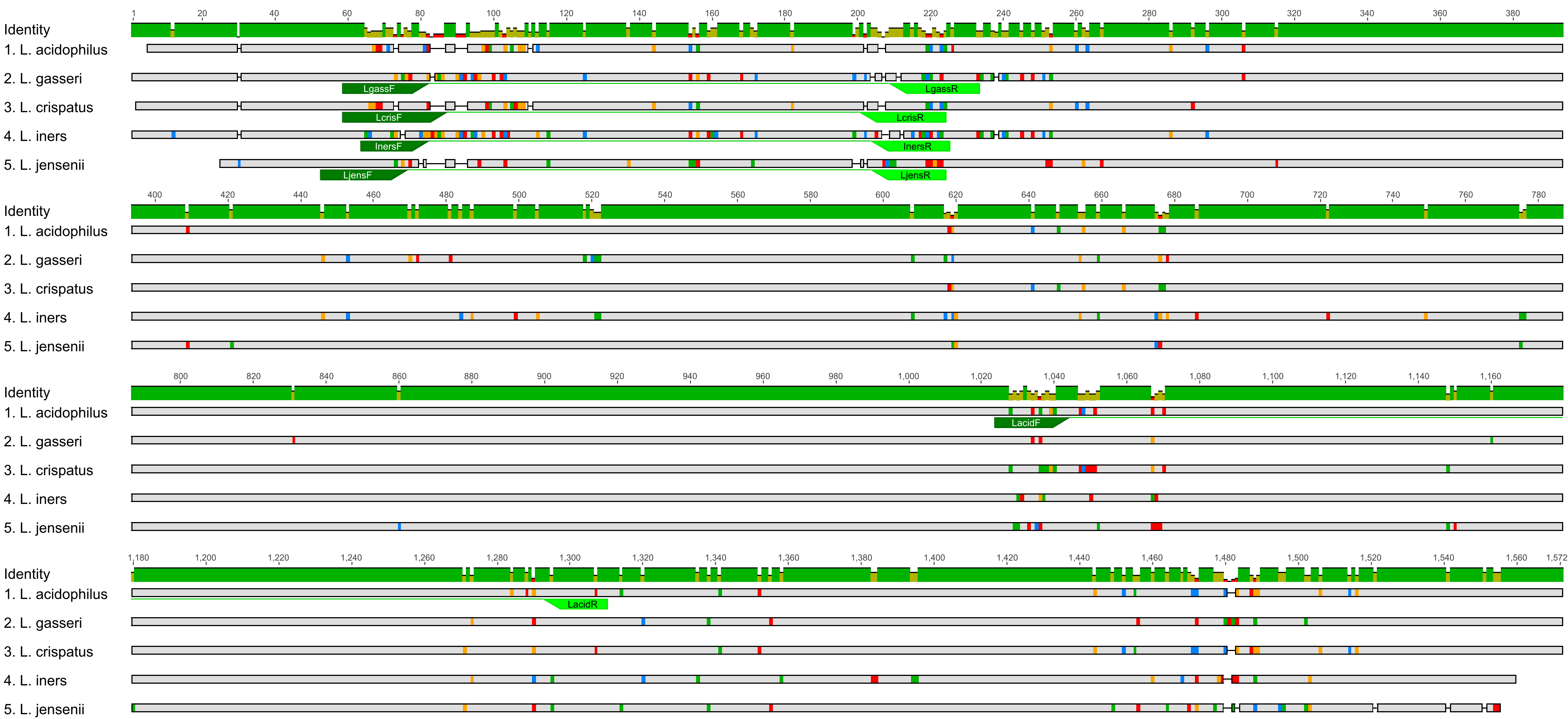

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LcrisF
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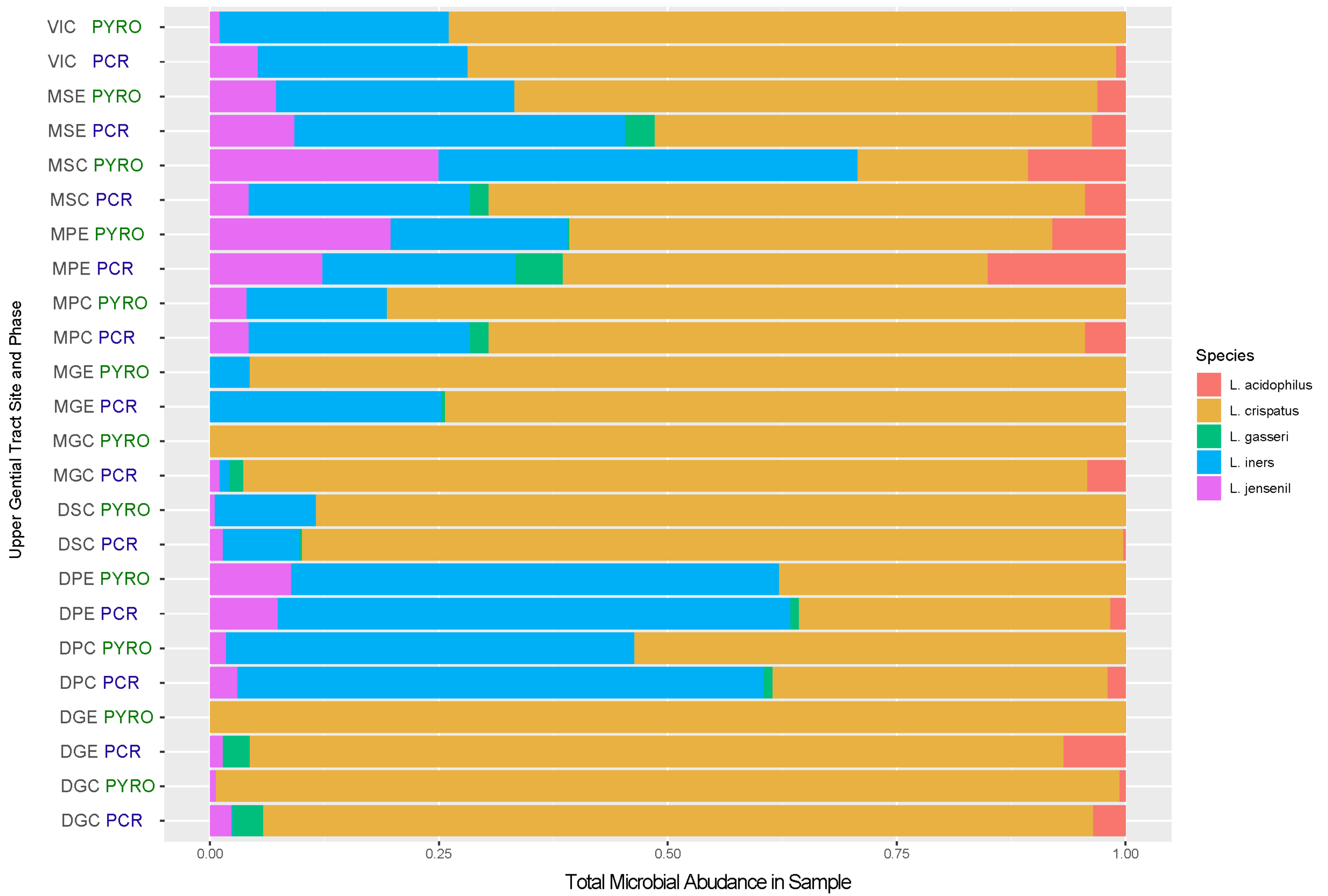
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L. acidophilus
L. crispatus
L. gasseri
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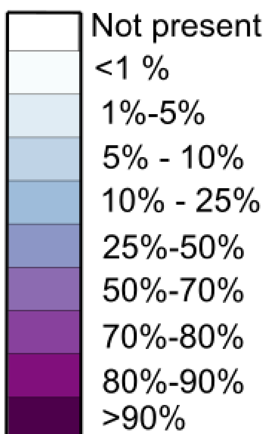
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L. acidophilus
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