1 **Supplementary Figures**

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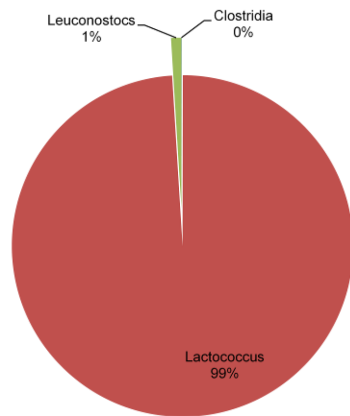

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5 Supplementary Figure 1. Taxonomic composition of the classified 16S
6 rRNA reads in the metagenome of the undefined cheese starter
7 culture.

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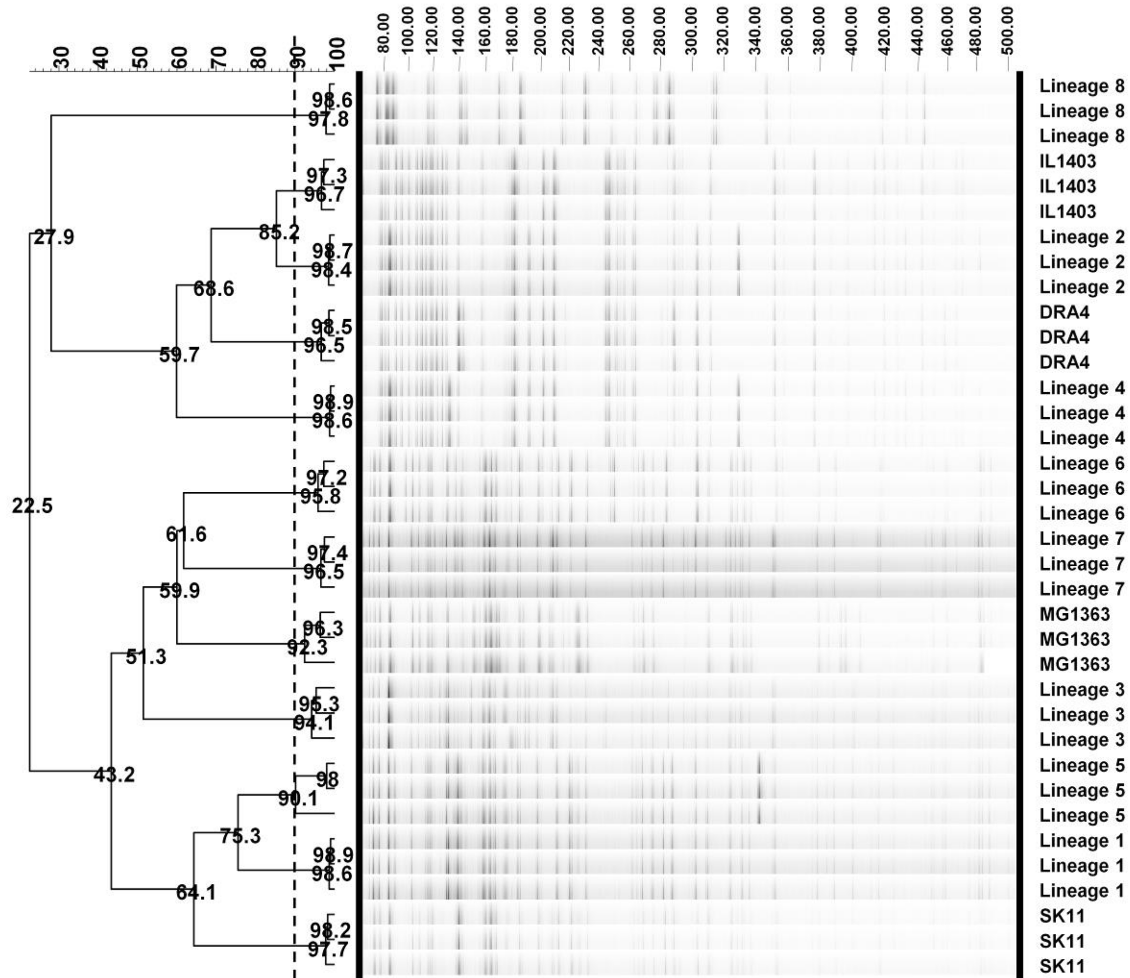

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14 Supplementary Figure 2. Clustering of AFLP profiles of 3 independently
15 picked representatives for the genetic lineages found in starter culture
16 and independent triplicate profiles of the reference *L. Lactis* strains
17 IL1403, MG1363, SK11, and DRA4. Clustering was based on Pearson
18 correlation coefficient on the range of (80-500) base pairs. Similarity
19 of the genetic profiles of less than 90% similarity (dotted line) was
20 considered as cut-off for defining seperate genetic lineages.

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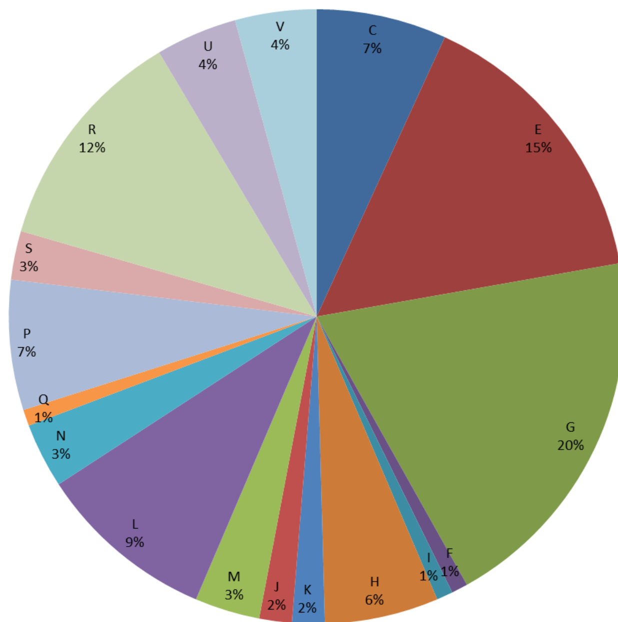

INFORMATION STORAGE AND PROCESSING
[J] Translation, ribosomal structure and biogenesis
[A] RNA processing and modification
[K] Transcription
[L] Replication, recombination and repair
[B] Chromatin structure and dynamics

CELLULAR PROCESSES AND SIGNALING
[D] Cell cycle control, cell division, chromosome partitioning
[Y] Nuclear structure
[V] Defense mechanisms
[T] Signal transduction mechanisms
[M] Cell wall/membrane/envelope biogenesis
[N] Cell motility
[Z] Cytoskeleton
[W] Extracellular structures
[U] Intracellular trafficking, secretion, and vesicular transport
[O] Posttranslational modification, protein turnover, chaperones

METABOLISM
[C] Energy production and conversion
[G] Carbohydrate transport and metabolism
[E] Amino acid transport and metabolism
[F] Nucleotide transport and metabolism
[H] Coenzyme transport and metabolism
[I] Lipid transport and metabolism
[P] Inorganic ion transport and metabolism
[Q] Secondary metabolites biosynthesis, transport and catabolism

POORLY CHARACTERIZED
[R] General function prediction only

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28 Supplementary Figure 3. Distribution of COG Categories for the 204
29 OGs conserved exclusively on TIFN1-to-7 *L. lactis* core-genome, but
30 not conserved for the reference *L. lactis* strains
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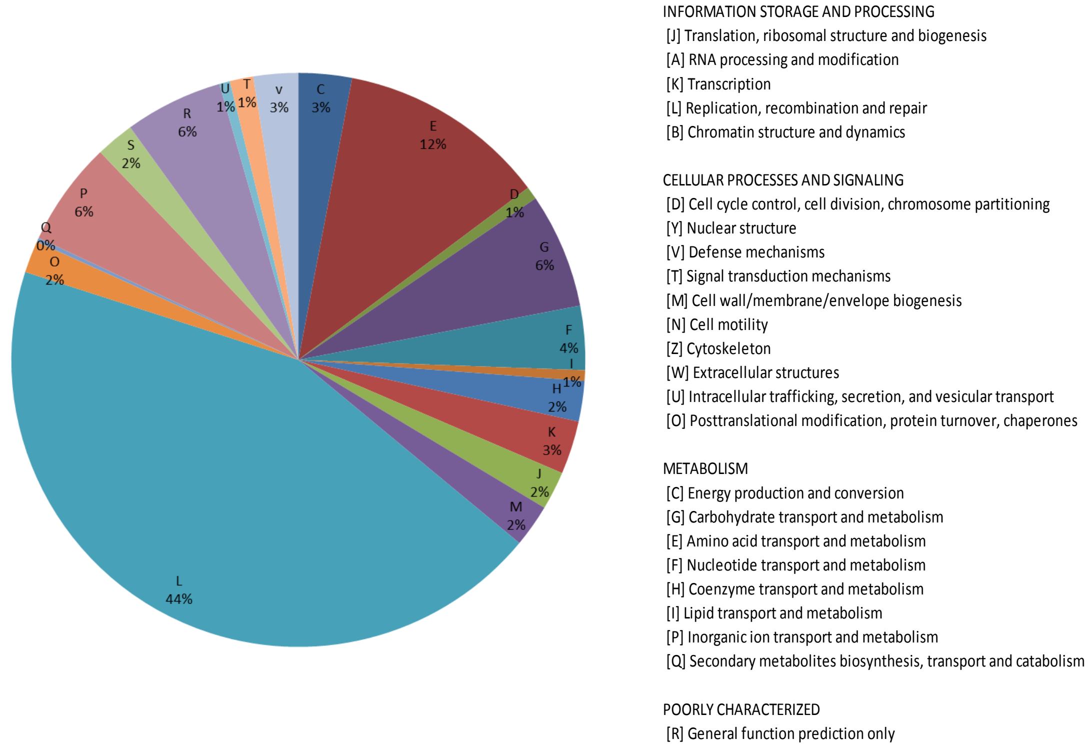
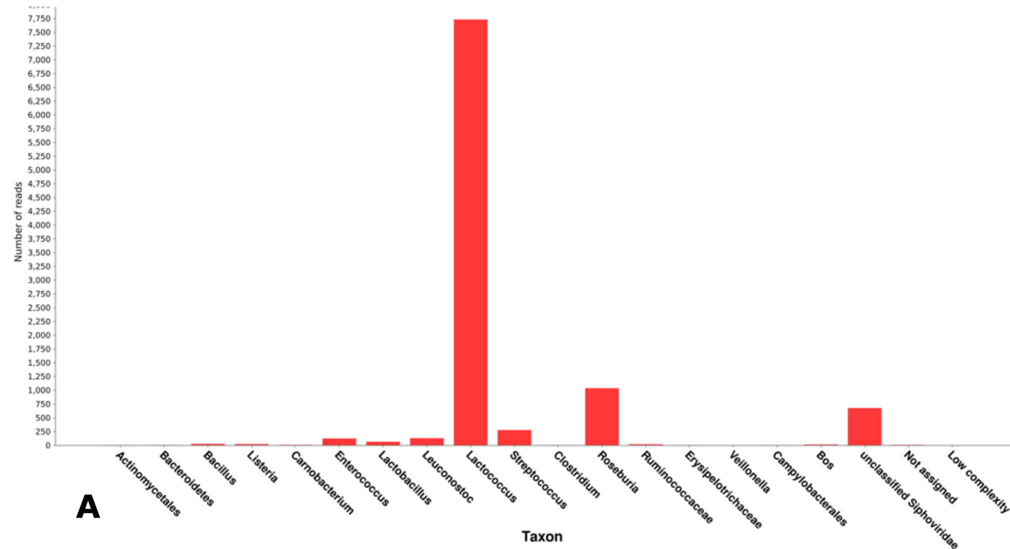
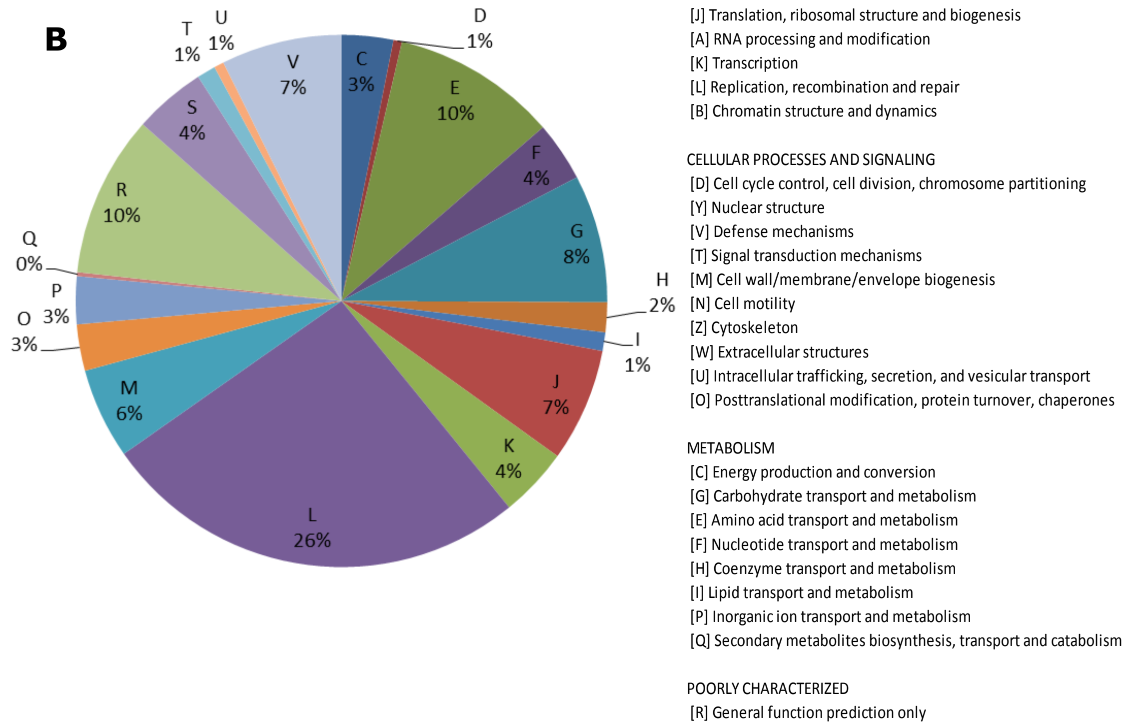


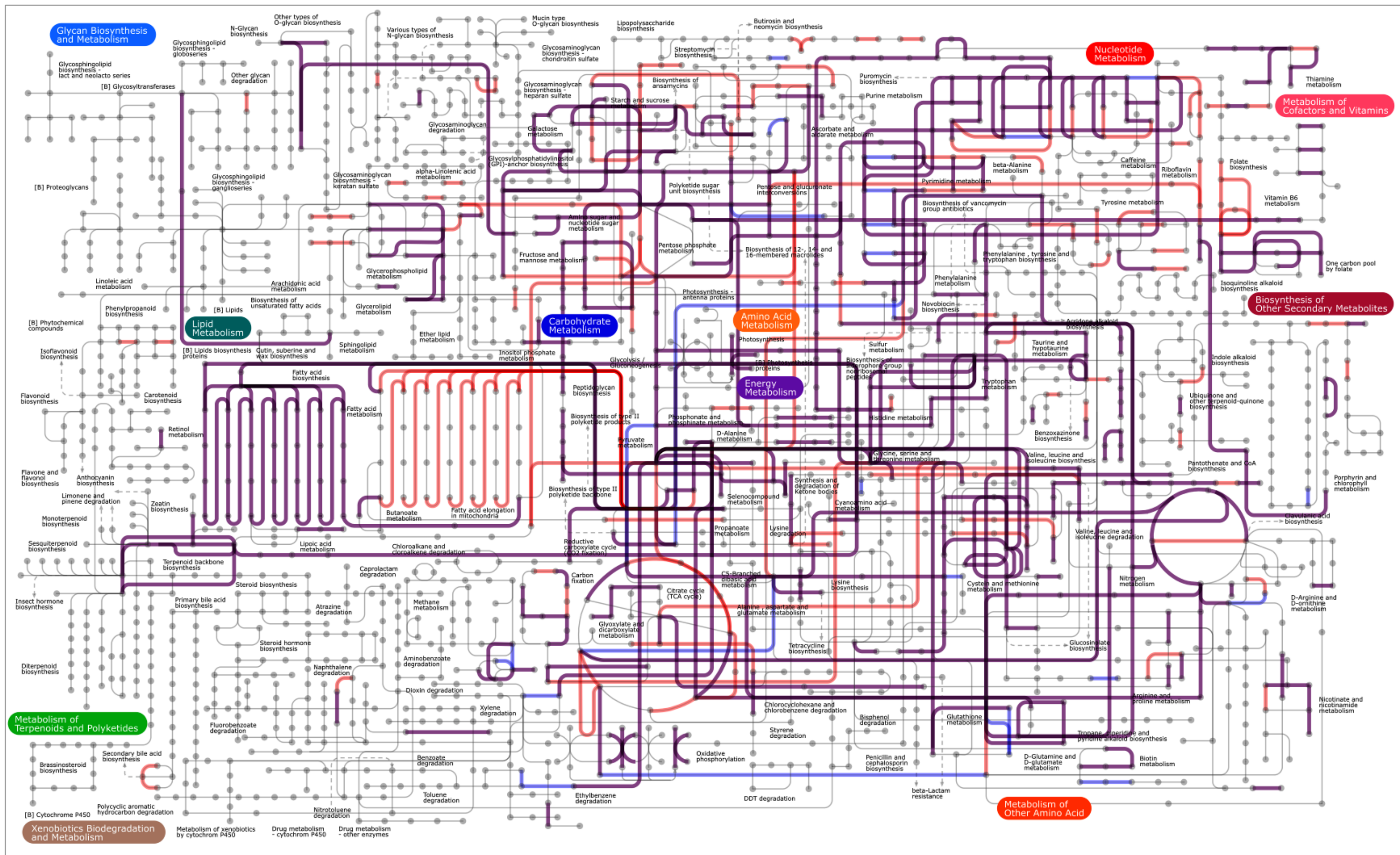
Supplementary Figure 4. Distribution of COG Categories for the 2860 OGs contributed by TIFN1-to-7 *L. lactis* genomes to the pan-genome of *L. lactis* reference strains

Supplementary Figure 5. Clustering of TIFN 1-to-8 *L. lactis*, *Lc. mesenteroides* and the 4 reference *L. lactis* strains. The dendrogram was constructed based on the concatenated variable nucleotides of the core OGs that are present as a single copy on all the genomes included to the clustering.





Supplementary Figure 6. Taxonomy-based binning of metagenome reads that do not match to TIFN 1-to-8 genomes. Panel A: Taxa identified for the sequences of the metagenome that do not match to TIFN genomes. Panel B: Distribution of COG Categories for the reads in Panel A.



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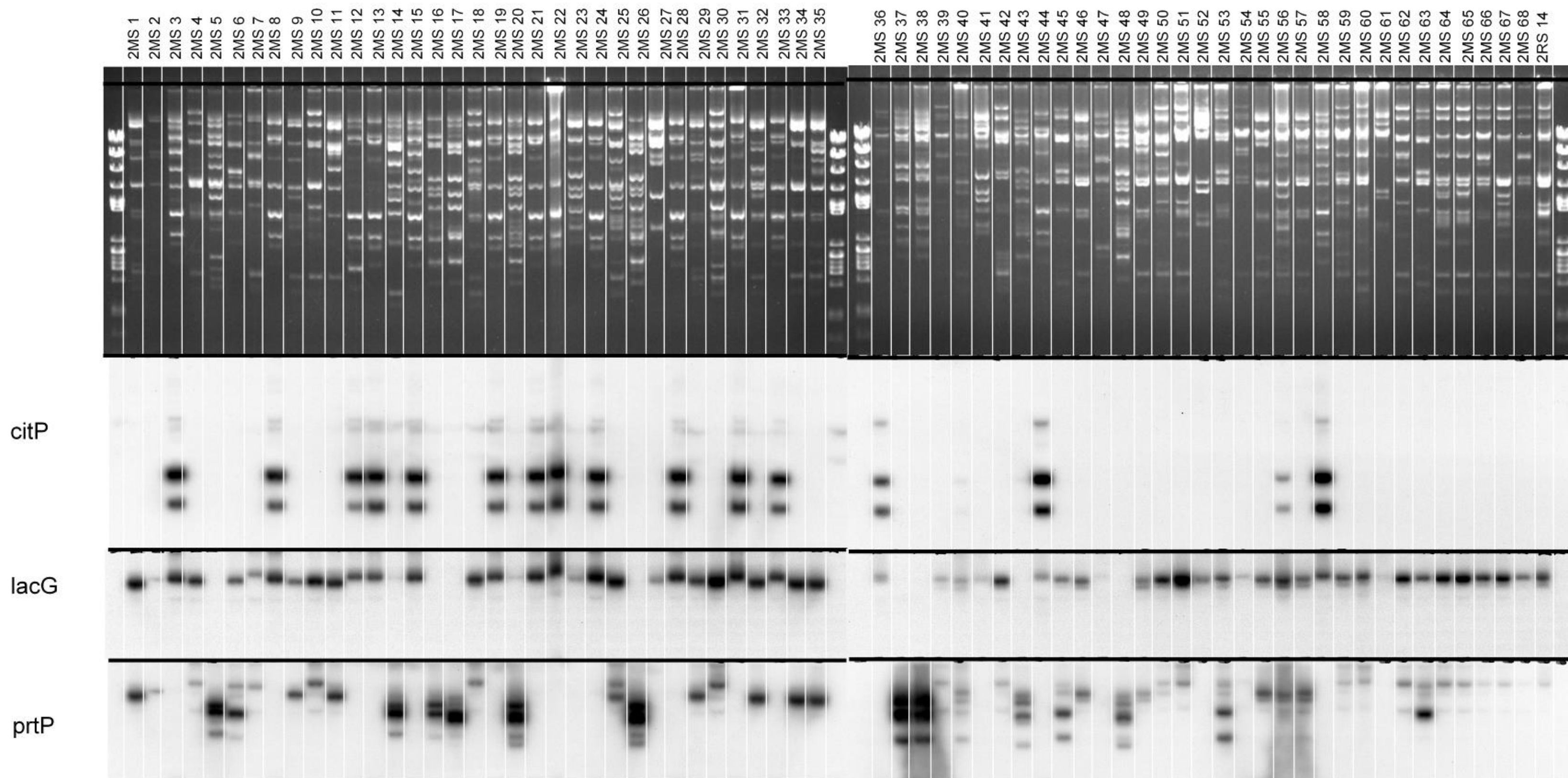
104 Supplementary Figure 7. Global metabolic maps of *L. lactis* pangenome (red lines) and TIFN8 *Lc.*

105 *mesenteroides* genome (blue lines). The shared pathways are represented with purple lines.



Supplementary Figure 8: Agarose gel electrophoresis of plasmid preparations, and southern blot hybridization patterns for *citP*[1], *lacG*[2], and *prtP*[3] genes. The codes of the isolates that the plasmid profiles belong to were given at the top of the figure.

Supplementary Information for Methods

Lab scale mini-cheese making

2L of bovine milk was standardized for fat (3.5 %), protein (3.4 %) and lactose (4.5 %) content, and heat treated at 72.5 °C for 9 s. The starter culture was propagated at 20 °C for 20 h in sterile skimmed milk two times prior to cheese making. The cheese milk was warmed to 32 °C, and supplemented with 230 μL of renneting enzyme (Kalase, 150 IMCU, CSK Food Enrichment, Ede, The Netherlands), 400 μL of a 33 % (wt/vol) $CaCl_2$ solution, 400 μL of $NaNO_3$ solution, and 1 % (v/v) of propagated starter culture per liter of milk. After curdling at 30.5 °C for 35-45 min, the curd was cut for 10 min using a custom-made stirring device. 800 mL of whey was removed and replaced with 700 mL of sterile deionized water at 45 °C. The curd was incubated at 36 °C for 45 min with gentle stirring. Whey was removed from curd under 1kg weight pressure for 1,5 h at 30°C. The cheese was incubated at 30 °C until the pH was reduced to the range of 5.4-5.6. Subsequently, it was brined with a sterile 19 % (wt/vol) NaCl solution for 4 h at 13 °C to obtain 3 % salt in dry matter in the center of the cheese. The cheese with the final moisture content in the range of (42-44) % and pH in the range of (5.4-5.2) was vacuum-packed and ripened at 13 °C for 6 weeks.

Total genomic DNA isolation from the starter culture

2,5 gr of propagated starter culture was mixed with 2 % (wt/vol) sodium citrate solution at 45 °C in 25 ml final volume, and homogenized for 5 min with stomacher. The homogenate was centrifuged at 13.750 g for 10 min at room temperature (Beckman Avanti J-20, CA, USA). The supernatant and fat layers were removed

139 and the cells were re-suspended in 1 ml of the same solution at 45 °C
140 for washing. The cell suspension was centrifuged at 10.750 g for 5
141 min (Eppendorf 5417R Microcentrifuge, Hamburg, Germany), and the
142 washing step was repeated for 2-3 times until all fat was removed. The
143 DNA from the washed cell pellet was further isolated with Quiagen
144 DNeasy Blood and Tissue Isolation Kit with the following modifications.
145 The cells were lysed in 1 ml of lysis buffer for 1 h at 37 °C. All kit
146 ingredients were adjusted to 1 ml lysis volume. The lysed cell
147 suspension was protease treated for 1 h at 56 °C and RNase treated
148 for 30 min at room temperature subsequently. Final DNA solution was
149 applied to a single spin column, and washing steps were repeated 2
150 times to get rid of rest of the milk culture impurities. DNA was eluted
151 with 300 μl of nuclease free water in three steps (100 μl in each step),
152 the concentrations were determined by Nanodrop (Coleman
153 technologies Inc., Glen Mills, PA, USA) and 0.8 % (wt/vol) agarose
154 gels.
155
156 **Quantitative PCR**
157 QPCR reactions were performed in 20 μl of final volume, combining 10
158 μl of SYBR® Green PCR master mix (Applied Biosystem, Warrington,
159 UK) with 0,2 μl of each primer (10 μM), and 2 μl of DNA template. PCR
160 amplification (7500 Fast System, Applied Biosystem, Warrington, UK)
161 was initiated with 10 min of initial denaturation at 95 °C, followed with
162 40 cycles of 15 s of denaturation at 95 °C and 1 min of annealing and
163 extension at 60 °C.
164
165 **Determination of community dynamics during cheese**
166 **manufacturing and ripening**

167 For the time series analysis, 10 g of samples were collected at 8 points
168 during cheese manufacturing: from initial starter culture just before
169 milk inoculation, starter culture inoculated milk (zero point), pressed
170 cheese, 24-h-incubated cheese, brined cheese, 2-weeks-ripened
171 cheese and 6-weeks-ripened cheese. The samples were homogenized
172 with 2 % (wt/vol) sodium citrate solution at 45 °C in 100 ml final
173 volume, diluted from 10^{-1} to 10^{-8} with peptone water, and plated on
174 selective media. M17 agar[4] supplemented with 0.5 % (wt/vol) lactose
175 and Reddy agar[5] were used for the isolation of lactococci at 30 °C and
176 MRS agar (Merck, Darmstadt, Germany) supplemented with
177 vancomycine (20 μg/ml) for the isolation of *Leuconostoc* at 25 °C. The
178 dilutions plates that has between 10 and 150 colonies were considered
179 relevant for isolation, and all the colonies present on the relevant
180 dilution plate were picked to obtain an unbiased lineage distribution.
181 The isolated colonies were activated in the liquid culture of the same
182 isolation medium, and stored in glycerol stocks (30 % glycerol, 70 %
183 liquid culture) at -80 °C.

184

185 Phenotypic differentiation of *Lactococcus* subspecies and bio-varieties
186 was achieved using previously established phenotypic analysis[6].
187 Protease activities were determined with glycerophosphate milk agar[7]
188 screening, and fingerprinted with the AFLP protocol optimized for *L.*
189 *lactis*[8]. For the quantification of genetic lineages during cheese
190 manufacturing, the highest viable count for a particular genetic lineage
191 among all selective media was taken as the closest approximation of
192 the contribution for that genetic lineage to the community.

193

194 **Plasmid isolation, Southern blot hybridizations, and high-**
195 **throughput plasmid profiling**

196 The overnight grown cultures in M17 were diluted 10 times with the
197 same medium, incubated further to OD $_{600nm}$ of 0,5. 5 ml of this culture
198 was centrifuged at 1560 g for 10 min (Heraeus Megafuge 1R, Heraeus
199 Instruments, Hanau, Germany), washed with 1 ml of nuclease free
200 water and centrifuged again at 20.800 g for 2 min (Eppendorf 5417R
201 Microcentrifuge, Hamburg, Germany). The pellet was re-suspended in
202 380 µl of lysis buffer (6.7 % sucrose, 500 mM Tris.HCl, 1 mM EDTA pH
203 8.0, 10 µg/ml RNase) and incubated for 2 min at 37 °C.
204 Subsequently, 96 µl of lysozyme (10 mg/ml in 25mM Tris.HCl pH 8.0)
205 was added to lysis solution and incubated further for 7 min at 37 °C.
206 250 µl of Tris-EDTA solution (50 mM EDTA, 50 mM Tris pH 8.0) and 28
207 µl of SDS solution (20 % SDS in 50 mM Tris-HCl, 20 mM EDTA pH
208 8.0) was added sequentially with gentle mixing in the end of lysozyme
209 treatment. The lysis was completed with 10 min incubation at 37 °C,
210 and 10 s full speed vortexing. Before the DNA extraction, the lysate
211 was treated with NaOH (addition of 28 µl of NaOH (3 M), 10 min
212 incubation on ice), with Tris.HCl (addition of 50 µl of Tris.HCl (2 M, pH
213 7.0), 5 min incubation at 25 °C), and with NaCl (addition of 72 µl of 5
214 M NaCl, 30 min incubation on ice) sequentially. The cell debris was
215 separated by 10 min centrifugation at 20.800 g (Eppendorf 5417R
216 Microcentrifuge, Hamburg, Germany), and the plasmid containing
217 supernatant was taken for phenol-chloroform extraction (addition of
218 700 µl cold phenol saturated with 3 % NaCl, centrifugation at 20.800 g
219 (Eppendorf 5417R Microcentrifuge, Hamburg, Germany) for 5 min,
220 transferring the water phase into 700 µl chloroform, centrifugation at
221 20.800 g (Eppendorf 5417R Microcentrifuge, Hamburg, Germany) for 2
222 min. The DNA in the water phase was precipitated with an equal
223 volume of isopropanol, and by centrifugation at 20.800 g (Eppendorf
224 5417R Microcentrifuge, Hamburg, Germany) for 5 min. The pellet was

225 washed with 500 µl of ethanol (70 %), dried for 20 min at room
226 temperature and dissolved in 50 µl TE buffer. Finally 10 µl was loaded
227 on agarose gel (0.7 % agarose, 0.5 µg/ml EtBr) and run at 70 mA for
228 4 h for profiling. Southern Blot Hybridizations were performed as
229 described before[9].
230
231 High-throughput Plasmid Profiling was based on fluorophore labeling of
232 digested plasmid DNA fragments. FAM6 fluorophore labeled adapters
233 were prepared from linker I ([6FAM] GAC GAT GAG TCC TGA G) and
234 linker II (TAC TCA GGA CTC AT) in adapter mix (50 µM linker I, 50 µM
235 linker II, 4.5 mM Tris, 0.45 mM EDTA) by heating for 5 min at 95 °C
236 and cooling down to 5 °C in 30 min. 10 µl of plasmid DNA was
237 digested with 0.2 Unit *MseI* restriction enzyme (New England Biolabs,
238 Beverly, MA, USA) in 10 µl of restriction buffer for 2 h at 37 °C. 5 µl of
239 digested plasmid DNA was ligated with adapters in ligation mix (1 X
240 ligation buffer, 2.5 µM adapter, 0.2 Unit T4 DNA ligase) at 37 °C for 3
241 h. Ligated fragments were purified with MSB HTS PCRapace DNA
242 fragment purification kit (Invitek GmbH, Berlin, Germany) according to
243 the manufacturer's instruction. For capillary electrophoresis, 8 volumes
244 of ligation product were mixed with 1.75 volume of PCR grade water
245 and 0.25 volume of MegaBACE™ ET550-R size standard (GE
246 Healthcare, Little Chalfont Buckinghamshire, UK). All samples were
247 analyzed with MegaBACE 500 48-capillary electrophoresis system (GE
248 Healthcare, Diegem, Belgium) using FAM6 sensitive detector according
249 to manufacturer`s protocol.
250

251 **Phage isolations and sensitivity testing**

252 Starter culture was propagated (1 % inoculated) two times in sterile
253 skimmed milk for 20 h at 20 °C. 10 ml of culture was centrifuged at

3000 g for 10 min (Heraeus Megafuge 1R, Heraeus Instruments, Hanau, Germany), and the supernatant was used as a phage solution. TIFN1-7 strains were used as host to test the presence of phages in the supernatant. 100 µl of a full grown strain was mixed with 100 µl of phage solution (in dilution series) and incubated for 10 min at 37 °C. 3 ml LM17 soft agar with 10 mM $CaCl_2$ (46 °C) was added to phage-bacteria mix and the mixture was plated on M17 plates (1 % lactose and 10 mM $CaCl_2$). The plates were incubated at 30 °C, and the phage titer was determined after 20 h. Single plaques were isolated with a Pasteur pipette from agar plates with the host strains TIFN1, TIFN5 and TIFN7, and kept in 1 ml of 50 mM Tris.HCl phage buffer (pH 7.5, 100 mM NaCl, 5 mM $CalCl_2$, 1 mM $MgSO_4$, 0.01 % gelatin). The phage solution was stored at 4 °C.

High density phage solutions were prepared by cell culture lysis for sensitivity testing. Phage solutions from previous isolations were mixed with over-night grown cells in 5:1 volume ratio, followed by 10 min adsorption time at 37 °C and diluted 10 times in M17 broth (1 % lactose, 10 mM $CalCl_2$). Lysis of the culture occurred after 6 h of incubation at 30 °C. Cell debris was removed by centrifugation, the supernatant was filter sterilized and the phage titer was determined by plating as described before. If the phage titer was low, the phage isolation was repeated either by another plate lysis or cell culture lysis. Four isolates of each genetic lineage (including the TIFN strains) were tested for their phage sensitivity, using the high density phage preparations of ϕtifn1, ϕtifn5, ϕtifn7 and Ur-supernatant with dilutions from 10^{10} to 10^{2} pfu/ml.

UR Back-Slopping and Phage Titer Determinations

283 The starter culture was cultivated in skimmed milk (0 % fat, UHT, 284 Friesland Campina, Ede, The Netherlands) at 30 °C overnight. In back-285 slopping regime, the culture was further sequentially propagated by 286 inoculation of the 5 ml skimmed milk with 50 µl (1 %) of the previous 287 day culture and incubated at 30 °C for 24 hours. The back-slopping 288 was performed in duplicate. Indicator strain TIFN1 was grown in M17 289 broth media (Oxoid, Hampshire, UK) with 1 % of lactose (w/v) at 30 290 °C overnight. Bacteriophage titers were determined as follow; 10 ml of 291 culture was centrifuged at 3000 g for 10 min (Heraeus Megafuge 1R, 292 Heraeus Instruments, Hanau, Germany) and the supernatant was used 293 as phage solution. Serial dilutions of phage solution were prepared 294 with 10 times dilutions in phage buffer (pH 7.5, 50 mM Tris.HCl, 100 295 mM NaCl, 5 mM $CalCl_2$, 1 mM $MgSO_4$, 0.01 % gelatin). 100 µl of Ur 296 culture supernatant (or phage dilutions) were added to 100 µl of the 297 overnight grown indicator strain, kept at 37 °C for 10 min, and 298 subsequently mixed with 3 ml of M17 agar (1 % lactose, 10 mM of 299 $CalCl_2$, 0.75 % agar) to be used as top agar. Finally, the top agar 300 preparations were poured on M17 bottom agar plates (1 % lactose, 10 301 mM of $CaCl_2$, 1.5 % agar) and incubated at 30°C for 24 h in duplicate. 302 Plaque forming units were counted and expressed as log pfu/ml.

303

304 REFERENCES FOR SUPPLEMENTARY FIGURES AND NOTE

305

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