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1 **Identification and transcriptional profile of *Lactobacillus paracasei* genes involved in the**
2 **response to desiccation and rehydration.**

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11

12 **Abstract**

13 *Lactobacillus paracasei* is able to persist in a variety of natural and technological
14 environments despite physico-chemical perturbations, in particular alternations between
15 desiccation and rehydration. However, the way in which it adapts to hydric fluctuations and
16 the genetic determinants involved are not clearly understood. To identify the genes involved
17 in adaptation to desiccation, an annotated library of *L. paracasei* random transposon mutants
18 was screened for viability after desiccation (25% relative humidity, 25°C). We found 16
19 genes that have not been described as being involved in this response. Most of them are linked
20 to either the transport of molecules or to cell wall structure and function. Our screening also
21 identified genes encoding DNA related enzymes and an alarmone necessary for *L. paracasei*
22 survival. Subsequently, the expression of the identified genes was measured at five stages of

23 the dehydration-rehydration process to decipher the chronology of genetic mechanisms. They
24 were classified into four different transcriptional profiles: genes upregulated during both
25 desiccation and rehydration phases, genes upregulated during the desiccation phase only,
26 genes downregulated during both desiccation and rehydration and genes downregulated only
27 during the rehydration stage. Thus, genetic response to hydric fluctuations seems to occur
28 during desiccation and can continue or not during rehydration. The genes identified should
29 contribute to improve the stabilization of *Lactobacillus* starters in dry state.

30

31 **Keywords** *Lactobacillus paracasei*, transposon mutants, desiccation, rehydration, gene
32 expression

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45 **1. Introduction**

46 Water is essential for all living organisms as it contributes to the structure of cells, stabilizes
47 proteins, lipids and nucleic acids, and maintains vital metabolic systems and chemical
48 reactions (Potts, 1994). Desiccation leads to water exit from cells, induces structural
49 modifications, and causes osmotic and oxidative stresses (Dupont et al., 2014; França et al.,
50 2007; Potts, 1994). In addition, the rehydration phase could lead to membrane alterations
51 (Mille et al., 2003). The capacity to return to life after hydric fluctuations is not only crucial
52 for bacteria as a function of diurnal and seasonal cycles in natural environments but also
53 during food industry processes such as conventional drying, freeze drying and spray drying
54 (Billi and Potts, 2002; Esbelin et al., 2018; Pointing and Belnap, 2012; Potts, 1994). In many
55 organisms, desiccation survival is correlated with the accumulation of protective molecules, in
56 particular trehalose (Crowe et al., 1998). However, desiccation tolerance involves other
57 mechanisms that are mostly unknown except for extreme desiccation tolerant bacteria
58 (anhydrobiotes) such as such as cyanobacteria (Potts, 1999), yeast (Dupont et al., 2014),
59 resurrection plants (Crowe, 2014) and microscopic animals (Welnicz et al., 2011).

60 In the last few years, progress has been made in understanding the mechanisms of bacteria
61 tolerance to partial desiccation using transcriptome analysis, in particular for *Salmonella*, one
62 of the most common foodborne pathogens able to survive for extended periods after
63 desiccation (Chen and Jiang, 2017; Deng et al., 2012; Finn et al., 2013; Gruzdev et al., 2012;
64 Li et al., 2012; Maserati et al., 2017). The comparison of these studies has revealed
65 differences in identified genes that could be explained by the procedures used to dry bacteria
66 (surface, drying medium, RH, desiccation periods). Several common genes were linked to
67 amino acid transport and metabolism functions. Global transcriptional analyses were also
68 performed for soil-residing bacteria (Cytryn et al., 2007; Higo et al., 2007; LeBlanc et al.,
69 2008) and similar responses were identified such as compatible solute and heat shock protein

70 accumulation, reactive oxygen species neutralization, and DNA modification and repair.
71 However, transcriptomics has limitations since genes essential for a function can be
72 constitutively expressed and even inducible genes can be overexpressed during a very short
73 period that does not overlap with that of the experiment. Recently, random transposon
74 mutagenesis approaches were developed to identify genes for resistance to desiccation in
75 *Listeria monocytogenes* (Hingston et al., 2015) and *Salmonella enterica* (Mandal and Kwon,
76 2017). For *L. monocytogenes* screening, genes were involved in energy production, membrane
77 transport, amino acid metabolism, fatty acid metabolism and oxidative damage control. In *S.*
78 *enterica*, in comparison to previous transcriptional studies, several genes were related to
79 amino acid metabolism and more than 20% encoded hypothetical proteins. Recently, changes
80 in proteomic expression were investigated for *S. enterica* during desiccation and rehydration
81 (Maserati et al., 2018). The proteins with higher expression levels in dried samples were
82 mainly ribosomal proteins whereas flagellar proteins, membrane proteins, and export systems
83 as well as stress response proteins were identified in rehydrated samples.

84 Although *L. casei/paracasei* is one of the most emblematic groups of lactic acid bacteria
85 (LAB), the genetic mechanisms involved in desiccation resistance and adaptation are not fully
86 understood, limiting prospects for improving and developing preservation processes.
87 Comparative genomics has demonstrated that LAB are highly adaptable to various niches
88 such as soil, foods (dairy, meat, and vegetable), as well as oral, vaginal and gastrointestinal
89 cavities (Cai et al., 2010; Douillard et al., 2013; Duar et al., 2017). This is correlated with an
90 ability to adapt and persist under diverse environmental stresses (Hosseini Nezhad et al.,
91 2015). Thanks to this capacity, humans have succeeded in selecting and producing certain
92 LAB strains as efficient starters for fermented foods (particularly hard cheese) and as
93 probiotics. The adequate preservation of starter cultures is necessary though it is currently

94 only possible using advanced desiccation technologies and storage in frozen state
95 (Santivarangkna et al., 2007)

96 Considering the importance of the *L.casei/paracasei* group for health and industrial
97 applications, we compiled a non-redundant, annotated transposon mutant library of *L.*
98 *paracasei* (Licandro-Seraut et al., 2014; Scornec et al., 2014) based on the P_{junc}-TpaseIS₁₂₂₃
99 system, a random mutagenesis tool specifically designed for the *Lactobacillus* genus
100 (Licandro-Seraut et al., 2012). Recently, using this approach we determined five
101 *Lactobacillus pentosus* mutants sensitive to olive brine due to multifactorial stress (Perpetuini
102 et al., 2013). In addition, new genetic determinants were identified during the early stage of *L.*
103 *paracasei* establishment in the gut (Licandro-Seraut et al., 2014) and during monofactorial
104 perturbations of mild intensities (Palud et al., 2018). In the present work, the *L. paracasei*
105 transposon mutant library was screened to identify genes involved in the adaptation of this
106 LAB to desiccation and rehydration. The expression of corresponding genes was studied by
107 RT-qPCR during the two-step process to draw a chronological transcriptional profile during
108 hydric fluctuations.

109

110 **2. Materials and Methods**

111 **2.1. Strains and growth conditions**

112 Wild-type (WT) *L. paracasei* ATCC 334 (CIP 107868, Institut Pasteur Collection) and its
113 corresponding mutants obtained by random transposon insertion mutagenesis (Licandro-
114 Seraut et al., 2012) were grown statically at 37°C in MRS (Difco). 5 µg/mL erythromycin
115 (Em) was used to select mutants. The mutants correspond to the 1287 genic and intergenic
116 mutants already described (Palud et al., 2018) (Licandro-Seraut et al., 2014). For each mutant,
117 the putative inactivated function was assigned thanks to the genome annotation of this strain
118 (Cai et al., 2010). The mutant library was grown in 96-well plates (200 µl) for 48 h. Plates

119 were mixed (30 s, 700 rpm, Eppendorf MixMate) and 10 μ L of each well was used to
120 inoculate 190 μ L of MRS in new 96-well plates. Individual mutants were grown in tubes for
121 48h (2 ml) and vortexed to inoculate at a dilution of 1/100 in new tubes. Plates and tubes were
122 incubated for 24h at 37°C to obtain cells in stationary phase (concentration between 1.0×10^9
123 and 2.0×10^9 UFC/mL) because it was the most convenient to obtain the same growth phase
124 for the 1287 mutants, and thus to compare viability after drying.

125 **2.2. Drying and rehydration conditions**

126 Convective drying in a ventilated chamber at room temperature with a relative humidity (RH)
127 of 25% was selected to mimic the severe desiccation conditions that can occur in natural
128 environments during drought in soil, on plants, and on animal skin, for example. This process
129 consisted in passing a flow of dry air over the cell suspension approximately simulating the
130 desiccation conditions that organisms can undergo in the natural environment. The drying
131 chambers were hermetic plastic boxes (20 cm \times 13 cm \times 6 cm) containing 100 mL saturated
132 potassium acetate (Sigma–Aldrich) solution to obtain 25% RH at 25°C. Samples were placed
133 on a rack in the drying chamber to keep them above the salt solution and the atmosphere was
134 maintained using a ventilator (Sunon, Radiospare, France). Temperature and RH were
135 controlled using an EASY Log USB tool (Lascar Electronics). Cells were rehydrated with
136 bromocresol purple (BCP) medium which contains 5 g/L of tryptone, pepsic peptone, yeast
137 extract and sodium acetate, 2 g/L of ammonium citrate and dipotassium phosphate, 1g/L of
138 glucose, 1 ml of tween 80, 0.20 g/L of magnesium sulfate, 0.17 g/L of bromocresol purple and
139 0.05 g/L of manganese sulfate.

140 **2.3. Effect of several sugar solutions on the viability of *L. paracasei* after desiccation**

141 We selected three disaccharides related in the literature to lactic acid bacteria preservation, in
142 particular during freeze drying (Carvalho et al., 2004; Costa et al., 2000; Jofré et al., 2015),
143 and their corresponding monosaccharides. *L. paracasei* cultures in stationary phase (1 mL)
144 were centrifuged (5 min, 4000 g, 25°C) and pellets were suspended by vortexing with one
145 volume of sugar solution diluted in water. After incubation for 15 min, 10 µL of cell
146 suspensions were placed onto a sterile polypropylene (PP) coupon of 15 mm × 10 mm × 2
147 mm (Scientix, Fougères, France). The inoculated coupons were placed in the ventilated
148 chamber in plastic petri dishes. For rehydration, 110 µL of BCP medium was deposited on the
149 dried cells and the latter were resuspended by 15 successive cycles using a micropipette.
150 Colony enumeration by plate counts was averaged and the viability percentage was obtained
151 with the ratio of colony enumeration (in CFU/mL) before desiccation to that after desiccation.
152 Lactose 50 g/L was selected because it corresponds to the composition of milk, thus a
153 condition often encountered by *L. paracasei* in the natural and technological environments of
154 cheese-making, for example.

155 **2.4. Screening of the mutant library for viability after desiccation**

156 A screening method composed of easy and rapid steps to save time and reduce the risk of bias
157 was developed. The whole strategy followed in this work is illustrated in figure 1. The
158 mutant library was subjected to 24-h desiccation (pronounced desiccation) followed by rapid
159 rehydration. Stationary growth phase cultures were mixed (1 min, 1000 rpm) and 50 µL of
160 cells were spotted in a U bottom 96-well microplate and centrifuged (5 min, 4000g, 25°C).
161 Pellets were suspended with 50 µL of 50 g/L lactose diluted in water (1 min, 1700 rpm) and
162 incubated 15 min at 20°C. Then, only 10 µL of suspension were placed in each well and the
163 plates were placed in the ventilated chamber. After 24 h, each well was rehydrated rapidly
164 with 110 µL of BCP medium at 37°C. This specifically designed culture medium with pH
165 indicator turns to yellow when bacteria metabolize sugars after rehydration. Thus, the

166 viability of the 1287 mutants after 24-h desiccation followed by rapid rehydration was
167 evaluated in 96-well microplates by monitoring the acidification kinetics (due to sugar
168 metabolism). Absorbance at 420 nm ($A_{420\text{nm}}$) was measured in a plate reader for 7h at 37°C
169 (Paradigm, Beckman Coulter) and was correlated to the quantity of metabolically active (or
170 viable) bacteria. The viability of the mutants could be compared to that of the WT ($A_{420\text{nm}}$ (7
171 h) = 1.91 ± 0.08). Mutants were considered as potentially sensitive when absorbance at 7 h
172 was lower than 1.75, which is the mean value of the WT minus twice the standard derivation,
173 for the two biological replicates. Mutant phenotypes were subsequently validated by
174 individual drying on PP coupons and by determining viability by the plate count method in
175 order to remove possible false positive mutants (for instance, metabolic deficiency mutants).
176 Colony enumeration by plate counts was averaged and the viability percentage was obtained
177 with the ratio of colony enumeration (in CFU/mL) before desiccation to that after desiccation
178 (3 biological replicates). Mutants were classified into three categories according to their
179 viability after rehydration: + (mean viability at least 1.5-fold less than WT), ++ (mean
180 viability at least 2-fold less than WT) and +++ (mean viability at least 3-fold less than WT).

181 **2.5. Determination of the viability of selected mutants after drying with phosphate** 182 **buffer**

183 *L. paracasei* cultures in stationary phase (1 mL) were centrifuged (5 min, 4000 g, 25°C) and
184 pellets were suspended by vortexing with one volume of drying solution composed of
185 phosphate buffer (10 mM pH 6,5). After incubation for 15 min, 10 μL of cell suspensions
186 were placed onto a sterile PP coupon. Three coupons were prepared for each mutant. The
187 inoculated coupons were placed in the ventilated chamber in plastic petri dishes. For rapid
188 rehydration, 110 μL of BCP medium at 37°C was deposited on the dried cells and the latter
189 were resuspended by 15 successive cycles using a micropipette. Colony enumeration by plate

190 counts was averaged and the viability percentage was obtained with the ratio of colony
191 enumeration (in CFU/mL) before desiccation to that after desiccation. Mutants exhibiting a
192 viability percentage significantly lower than the WT (Student test, $p < 0.05$) and at least 1.5-
193 fold less were determined as sensitive.

194 **2.6. Determination of the viability of selected mutants after progressive and rapid** 195 **rehydration**

196 *L. paracasei* cultures in stationary phase (1 mL) were centrifuged (5 min, 4000 g, 25°C) and
197 pellets were suspended by vortexing with one volume of drying solution composed of lactose
198 50 g/L. After incubation for 15 min, 10 μ L of cell suspensions were placed onto a sterile PP
199 coupon. Three coupons were prepared for each mutant. The inoculated coupons were placed
200 in the ventilated chamber in plastic petri dishes. For rapid rehydration, 110 μ L of BCP
201 medium was deposited on the dried cells and the latter were resuspended by 15 successive
202 cycles using a micropipette. For progressive rehydration, dried cells were introduced into a
203 hermetic chamber at 99% RH for 2 h at 25°C. Then, 110 μ L of BCP medium was deposited
204 on the wet bacteria cells that were recovered by 15 successive cycles using a micropipette.
205 Colony enumeration by plate counts was averaged and the viability percentage was obtained
206 with the ratio of colony enumeration (in CFU/mL) before desiccation to that after desiccation.
207 Mutants exhibiting a viability percentage significantly lower than the WT (Student test, $p <$
208 0.05) and at least 1.5-fold less were determined as sensitive.

209 **2.7. Bioinformatics**

210 The putative operon organization of corresponding genes was established using the Biocyc
211 website. Then, genes were aligned using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>)
212 against all bacteria to determine their specificity.

213 **2.8.RT-qPCR**

214 The transcriptional analysis was performed using mid-exponential growth phase cells (OD₆₀₀
215 between 0.5 and 0.6) which are the most metabolically active and which generally present the
216 strongest and the fastest gene regulation. Cells were centrifuged and concentrated to an OD₆₀₀
217 of 20. Cell pellets were suspended with phosphate buffer (10 mM pH 6.5) supplemented with
218 50 g/L lactose except for control. The suspension was incubated for 15 min at 25°C and 1 mL
219 was deposited on a 0.22 µm polyvinylidene membrane in a glass Petri dish to prevent cell
220 adhesion. Cells were dried in ventilated chambers and RNA extractions were performed at
221 two different stages of desiccation: partial desiccation D1 (76% of water evaporated after 1 h)
222 and pronounced desiccation D2 (96% of water evaporated after 2 h) which corresponded to
223 completely dried cells (Figure 2). As rehydration kinetics is decisive for bacterial survival,
224 rapid and progressive rehydration were applied for RNA extractions after 2 h. Rehydrated
225 cells were subsequently detached from the membrane using a cell lifter. Total RNA isolation,
226 cDNA synthesis and qPCR were performed as previously described (Licandro-Seraut et al.,
227 2008) using TRI Reagent (Sigma Aldrich), DNase I (Roche), iScript™ Reverse Transcription
228 Supermix (Bio-Rad) and SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad).
229 Primers were designed by using Primer3Plus (Untergasser et al., 2007) (Table 1). Quantitative
230 PCR were performed using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad)
231 in triplicate, in a 20 µL-reaction mixture. Cq (threshold value) calculation was determined by
232 a regression model of the CFX Manager™ Software. The relative transcript levels of genes
233 were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). In order to select
234 appropriate reference genes, 10 potential housekeeping genes (*fusA*, *ileS*, *lepA*, *leuS*, *mutL*,
235 *pcrA*, *pyrG*, *recA*, *recG* and *rpoB*) (Landete et al., 2010) were tested with all the experimental
236 conditions and analyzed using the CFX Manager™ Software. The genes *fusA*, *lepA* and *rpoB*
237 were selected as the references because they displayed the lowest M values (0.22) and

238 coefficients of variation (0.09), meaning that they have the most stable expression in the
239 tested conditions.

240

241

242

243 **3. Results**

244 **3.1. Selection of a protective drying solution for the screening**

245 A library containing a total number of 1287 *L. paracasei* mutants was screened for their
246 capacity to resist a 24-h desiccation period followed by rapid rehydration in comparison with
247 their parental strain (*L. paracasei* ATCC 334, named WT). The viability of *L. paracasei* WT
248 after 24-h desiccation at 25% RH was determined with and without the protectors (Table 2).
249 Viability was very low in the absence of sugar and with monosaccharides. On the contrary,
250 with disaccharides, viability was between 19 and 45%, which was an adequate range for
251 screening sensitive mutants.

252 **3.2. Identification of genes involved in resistance to pronounced desiccation followed** 253 **by rapid rehydration.**

254 Screening of the 1287 transposon mutants resulted in the detection of 47 sensitive mutants
255 (A_{420nm} at 7 h < 1.75 for 2 biological replicates). After the validation step by individual drying
256 of the 47 mutants, 16 mutants displayed a significant decrease of survival at least 1.5-fold less
257 compared to the WT ($p < 0.05$) (Table 3). Identified genes were mostly involved in metabolism
258 and transport, cell wall structure and function, regulation of stress response and DNA related
259 enzymes. Moreover, three sensitive mutants containing transposon in a gene encoding a
260 hypothetical protein were identified (LSEI_0040, LSEI_1045 and LSEI_1316). The majority
261 of mutants (12) were categorized as +. The two most sensitive mutants (+++) were disrupted

262 for a putative ribonucleotide diphosphate reductase (LSEI_1468) and for a hypothetical
263 protein (LSEI_1316). Two mutants disrupted for a putative PTS (LSEI_0178) and for a
264 putative alpha/beta fold family hydrolase (LSEI_0756) were categorized as ++. Nine genes
265 out of the 16 belonged to putative operons (Table 4). Seven genes were specific to *L.*
266 *paracasei* or related species, four to *Lactobacillus* genus and five were well conserved among
267 Gram (+) bacteria. Two genes among the three encoding hypothetical proteins presented a
268 transmembrane domain (LSEI_0040 and LSEI_1045). All hypothetical protein genes were
269 specific to the *L. paracasei* group.

270 **3.3. Analysis of selected mutant after pronounced desiccation with phosphate buffer**

271 We chose to omit the protector in order to strictly analyze the impact of the drying process.
272 The WT and the 16 sensitive mutants identified previously (viability reduction > 1.5 in
273 comparison of the WT) were subjected to a desiccation for 24 h without protector. In these
274 conditions, the viability of the WT was 0.059 ± 0.006 %, which is considerably lower than
275 that obtained for the pronounced desiccation with lactose 50 g/L. Among the 16 sensitive
276 mutants identified, nine were also more sensitive to a drying without protector than the WT
277 (Table 5). They are disrupted for the following genes: a putative diadenosine tetraphosphatase
278 (LSEI_0167), a PTS system (LSEI_0178), a Pyridoxine 5'-phosphate oxidase (LSEI_0363),
279 an NAD (FAD)-dependent dehydrogenase (LSEI_0397), a ribonucleotide reductase
280 (LSEI_1468), a sorbitol operon transcription regulator (LSEI_2725) and hypothetical protein
281 (LSEI_0040, LSEI_1045 and LSEI_1316). As observed for drying with lactose, the most
282 sensitive mutant was disrupted for LSEI_1468.

283 **3.4. Analysis of selected mutant after pronounced desiccation followed by** 284 **progressive rehydration**

285 To assess if the identified genes were involved during dehydration whatever the rehydration
286 process, the WT and the 16 sensitive mutants identified previously (viability reduction > 1.5

287 in comparison of the WT) were subjected to desiccation for 24-h followed by progressive
288 rehydration (for 2 h) instead of rapid rehydration. In these conditions, the viability of the WT
289 was $98 \pm 3\%$, which is considerably higher than obtained with a rapid rehydration. Among the
290 16 sensitive mutants identified previously, only four were also sensitive after desiccation and
291 progressive rehydration (Table 6). They encode a putative diadenosine tetraphosphatase
292 (LSEI_0167), an NAD (FAD)-dependent dehydrogenase (LSEI_0397), a cation transport
293 ATPase (LSEI_0749) and a ribonucleotide reductase (LSEI_1468). As observed for
294 desiccation and rapid rehydration, the most sensitive mutant was ribonucleotide diphosphate
295 reductase.

296 **3.5. Transcriptional analysis of the identified genes during a desiccation –** 297 **rehydration cycle**

298 Mutant library screening allowed the identification of 16 genes involved in the global
299 perturbation which consisted in desiccation followed by rehydration. Thus, it was not possible
300 to determine during which phase these genes were involved since rehydration was necessary
301 to analyze mutant phenotypes. To determine the chronology of the genetic mechanisms during
302 humidity fluctuations, transcriptional analysis of the identified genes was carried out on the
303 WT.

304 As lactose was required to ensure good survival of bacteria during the desiccation/rehydration
305 process it was reasonable to expect that some candidate genes could be differentially
306 expressed in the presence of lactose. However, for the 16 genes, expressions after 15-min
307 incubation in lactose were comparable to that after a 15-min phosphate buffer incubation
308 (Figure 3). Differentially expressed genes were classified to obtain the most upregulated
309 (Table 7, values in dark red) and downregulated (Table 7, values in dark blue) genes, with a
310 mean expression value higher or lower than 2.0. Among the 16 genes studied, 13 were
311 differentially expressed ($p < 0.05$) for at least one condition (Table 7). The three genes

312 involved in hydric changes but not differentially expressed encoded a putative PTS
313 transporter (LSEI_0178), a reductase (LSEI_0758), and hypothetical proteins (LSEI_1045).
314 Four genes were upregulated for all the hydric fluctuations tested. These genes encoded a
315 putative diadenosine tetraphosphatase (LSEI_0167), a polysaccharide transporter
316 (LSEI_0238), a pyridoxine 5'-phosphate oxidase (LSEI_0363), and hypothetical proteins
317 (LSEI_0040). Also, LSEI_0397 encoding a putative NAD dependent dehydrogenase was
318 upregulated during desiccation and progressive rehydration. Conversely, LSEI_0749
319 encoding a putative cation transport ATPase was upregulated during desiccation and rapid
320 rehydration. Interestingly, these genes were the most upregulated (> 2-fold change) for at
321 least one condition. One gene, LSEI_1754, encoding a putative SAICAR synthase was the
322 most downregulated gene in these experiments (mean expression value < 2.0 for all the drying
323 and rehydration conditions).

324 One gene, LSEI_2725 (a putative sorbitol operon transcription regulator) was upregulated
325 after only 2 h of desiccation and represent potential dry state biomarkers. LSEI_1316
326 encoding a putative hypothetical protein was upregulated after 1 h and 2 h of desiccation.
327 Interestingly, no gene was specifically upregulated during subsequent rehydration.
328 Conversely, four genes were downregulated specifically during rehydration: LSEI_0756 (a
329 putative hydrolase) LSEI_1009 (a putative spermidine/putrescine-binding protein),
330 LSEI_1468 (a putative ribonucleotide reductase) and LSEI_1659 (a putative glucokinase).

331

332 **4. Discussion**

333 Since water is the fundamental component of all living organisms, desiccation and
334 rehydration alternation is one of the most prevalent and severe stress for most
335 microorganisms. Adaptation to this stress occurs via a combination of mechanisms which
336 depend on the genetic background of the microorganism. In *L. paracasei*, we developed a

337 strategy to identify genes involved in the adaptation to hydric fluctuations using random
338 transposon mutagenesis and targeted transcriptional analysis.

339 Sixteen desiccation-sensitive mutants were identified from the *L. paracasei* mutant library
340 screening after desiccation followed by rapid rehydration. Contrary to our recent screening of
341 this library for mild stress sensitivity (Palud et al., 2018), no mutants for putative promoters
342 were identified as sensitive to desiccation. Seven of the identified genes were specific of the
343 *L. casei/paracasei/rhamnosus* group. On the contrary, five genes were well conserved among
344 Gram-positive bacteria. Interestingly, these genes, although present in *Listeria monocytogenes*
345 genome, were not identified by the screening of *L. monocytogenes* random mutants for
346 desiccation resistance (Hingston et al., 2015). Otherwise, this study of *L. monocytogenes*
347 desiccation resistance shows the involvement of a gene encoding a putative glutathione
348 peroxidase, known to prevent oxidative damages. This gene is present in the *L. paracasei*
349 mutant library but was not identified as sensitive. These differences may result from the
350 drying conditions applied such as the surface used for desiccation, the relative humidity, the
351 drying medium or the rehydration conditions.

352 **4.1. Metabolism and transport**

353 Two transporters are required for survival to hydric fluctuation including a putative
354 phosphotransferase system (PTS, LSEI_0178) and one cation transporter (LSEI_0749).
355 Several authors have reported a link between PTS systems and stress response for
356 *Lactobacillus* genus. *L. plantarum* mutants with an impaired expression of the mannose PTS
357 operon exhibited increased sensitivity to peroxide, probably due to a diminution of glucose
358 capture and energy production (Stevens et al., 2010).

359 Next, we identified a membrane associated protein (LSEI_1009) predicted to bind polyamines
360 before their transport into the cell by the ABC transporters encoded by other genes of the
361 LSEI_1005-1009 operon. Polyamines have various important physiological roles during stress

362 response, including the modulation of gene expression, signal transduction, oxidative defense
363 mechanism, and cell-to-cell communication (Miller-Fleming et al., 2015; Shah and Swiatlo,
364 2008).

365 One identified gene encoded a glucokinase (LSEI_1659), a key enzyme of glucose
366 metabolism. In *S. aureus*, glucokinase is also involved in pathogenicity (biofilm formation,
367 virulence factors, cell wall synthesis) (Kumar et al., 2014). In our case, glucokinase as well as
368 PTS interruption could lead to energy diminution and then sensitivity to hydric fluctuations.
369 Another gene, LSEI_0363, encodes a putative enzyme that catalyzes the oxidation of
370 pyridoxamine-5-P (PMP) and pyridoxine-5-P (PNP) to pyridoxal-5-P (PLP) in the vitamin B6
371 pathway. Vitamin B6, an antioxidant molecule, has been implicated in defense against cellular
372 oxidative stress in *Saccharomyces cerevisiae* (Chumnantana et al., 2005).

373 **4.2. Cell wall function and structure**

374 Modification at the cell wall level appears to be essential for surviving hydric fluctuation,
375 considering the three genes related to the cell wall and identified as essential for hydric
376 fluctuation survival. The gene LSEI_0238 encodes a polysaccharide transporter involved in
377 the export of lipoteichoic acid (LTA), a major constituent of the Gram-positive cell wall (Cai
378 et al., 2010). Interestingly, this gene was also needed for *L. paracasei* gut establishment
379 (Licandro-Seraut et al., 2014). The mutant for LSEI_2546, another polysaccharide transporter
380 involved in LTA export, was not identified as sensitive.

381 Finally, two hypothetical proteins exhibited a transmembrane domain (LSEI_0040 and
382 LSEI_1045). Interestingly LSEI_0040 was involved in the adaptation of *L. paracasei* to
383 thermal, ethanol and oxidative stresses (Palud et al., 2018). The present and previous results
384 show that membrane proteins are important for *L. paracasei* adaptation to various
385 environments and involved in general stress response.

4.3. Regulation of stress response

386
387 One gene involved in alarmone degradation is needed for *L. paracasei* survival during hydric
388 fluctuations. Alarmones are putative chemical messengers produced during environmental
389 changes. LSEI_0167 encodes a putative diadenosine tetraphosphatase, an enzyme that
390 hydrolyzes diadenosine tetraphosphate (Ap4A) into two molecules of adenosine diphosphate
391 (ADP). Ap4A has been reported to be involved in heat stress response in *E. coli* (Lee et al.,
392 1983). Moreover *apaH* (diadenosine tetraphosphatase) mutation causes Ap4A accumulation
393 and sensitivity to thermal stress (Johnstone and Farr, 1991). Nevertheless, Despotović and
394 collaborators wondered whether Ap4A was an alarmone or a damage metabolite because they
395 observed that no signaling cascade was triggered by Ap4A and that its accumulation at high
396 level was toxic for *E. coli* (Despotović et al., 2017).

397 LSEI_2725 (*gutR*) has been identified as a determinant for surviving hydric fluctuations.
398 Although it is annotated as a sorbitol operon transcription regulator, its predicted function
399 should be reconsidered. Alcantara *et al.*, 2008 reported that in *L. paracasei* ATCC334, *gutR* is
400 split into 3 ORFs (LSEI_2728, LSEI_2726 and LSEI_2725) as the result of transposition
401 events, and consequently this strain is unable to use sorbitol, contrary to *L. casei* BL23. In
402 addition, the LSEI_2724 (*gutM*) gene, encoding a sorbitol operon activator with *gutR*, is
403 dispensable for desiccation resistance. In conclusion, we assume that LSEI_2725 functions as
404 a regulator but independently of the sorbitol operon.

4.4. DNA related enzymes

405
406 It was widely reported that desiccation induces DNA damage (Fredrickson et al., 2008; Potts,
407 1994). Indeed, the mutant library screening identified two genes involved in nucleotide
408 metabolism, LSEI_1468 (a ribonucleotide reductase, RNR) and LSEI_1754 (a SAICAR
409 synthase). The RNR mutant was particularly sensitive (mean viability 10-fold less than WT).
410 RNR is an essential enzyme since it mediates the synthesis of deoxyribonucleotides, the

411 precursors of DNA synthesis (Torrents, 2014), and we have previously reported that the RNR
412 gene was involved in general response to mild stresses in *L. paracasei* (Palud et al., 2018).
413 SAICAR synthase is involved in de novo purine biosynthesis (Nelson et al., 2005). These two
414 genes could be involved in DNA repair. Recently, García-Fontana *et al.* (2016) reported that
415 the DNA molecule was overproduced and acted as a protein protector in desiccation tolerant
416 bacteria. This phenomenon could occur in *L. paracasei*.

417 **4.5. Taking into account desiccation and rehydration kinetics**

418 The results indicate that rapid rehydration of WT strain cells was more detrimental than
419 progressive rehydration. It has been reported that progressive rehydration could reduce the
420 stress applied to bacteria as it promotes membrane integrity recovery (Lang et al., 2017; Zoz
421 et al., 2016). We found nine common sensitive mutants after drying with or without protector
422 and only four sensitive mutants whatever their rehydration kinetics. Identified genes were
423 generally specific to the screening strategy applied for the mutant library. It is very likely that
424 the modifications of drying conditions lead to the identification of different genes. Studies on
425 *Salmonella* species exposed to desiccation stress showed a response specificity in function of
426 methodologies used (Finn et al., 2013)

427 **4.6. Transcriptional profiles**

428 Our findings are schematized in Figure 3. Among the genes essential for survival, three genes
429 were expressed at the same level as the control whatever the hydration phase. This result
430 again shows the strength of global reverse genetics, since some genes may be essential for a
431 function, although expressed constitutively. Four transcriptional profiles were observed
432 during hydric fluctuations: six genes were upregulated during both desiccation and
433 rehydration, two genes were upregulated only during the desiccation stage, one gene was
434 downregulated during both desiccation and rehydration, four genes were downregulated only
435 during the rehydration stage. We assume that adaption to hydric fluctuations occurs during

436 dehydration and continues or not during rehydration. Up regulation of genes was particularly
437 observed after 2 hours of drying (96% of water evaporated). These results suggest that this
438 step could be the most stressful for bacteria. Our data highlighted the importance of cell wall
439 structure as well as diadenosine tetraphosphate degradation since LSEI_0238 and LSEI_0167
440 were up regulated for all dehydration and rehydration phases.

441

442 In conclusion, this work identified 16 genetic determinants for the resistance of *L. paracasei*
443 to desiccation. Transcriptional analysis of the corresponding genes highlighted that six genes
444 were upregulated during both desiccation and rehydration and two during desiccation only.
445 Thus, our results show that desiccation is a critical phase for inducing stress response in *L.*
446 *paracasei*. This analysis provides clues for developing genetic biomarkers to monitor the
447 intensity of desiccation during industrial processes since we identified two genes
448 overexpressed only when the cells are dried during 2 h at 25% RH (dry state). However, it
449 was reported that drying conditions (surface used for desiccation, the drying medium, the
450 rehydration conditions) may modify the genes involved in the desiccation stress. Indeed, gene
451 expression of these two genes should be studied during industrial drying process before
452 stating that they constitute genetic biomarkers. Some genes identified in this study have
453 higher potential since they are not limited to *L. paracasei* genomes. From an applied point of
454 view, these genes will be preferred targets for obtaining more efficient *Lactobacillus* starters.
455 Transcriptional studies during freeze drying could bring complementary information. In a
456 fundamental point of view, these findings provide novel insights into the genetic mechanisms
457 involved in desiccation and rehydration adaptation in *L. paracasei*.

458 **Conflict of Interest Statement**

459 The authors declare that the research was conducted in the absence of any commercial or
460 financial relationships that could be construed as a potential conflict of interest.

461 **Author Contributions**

462 Study conception and design: AP, KS, HL, LB and JFC. Acquisition of data: AP, KS.
463 Analysis and interpretation of data: AP, HL, LB and JFC. Drafting of manuscript: AP. Critical
464 revision: AP, HL, LB and JFC. All authors read and approved the final manuscript.

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472

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Tables

Table 1. Primers designed for RT-qPCR.

Primers	Sequence (5' → 3')	Primers	Sequence (5' → 3')
0040-F	CCACAAATGAGTCAAACCATCT	0040-R	GGTCGTTATCGCACCTAATGA
0167-F	ATGCCGACTGCTGTTGAGTT	0167-R	CGGACCCACATTCGATTA
0178-F	TAGGCAACATTCGCAGTCAA	0178-R	ATAATCGGCAACGGCTTTC
0238-F	CGGATCGCTTCCCTTATTCT	0238-R	CCAACATCTGTGAGCCAGAC
0363-F	GGTCCAAGGGTTCACTACA	0363-R	GCCAAATGGTTGTTGAGAG
0397-F	GGCACGACGCAGGATTAT	0397-R	CCACGCATCAAGAAGACATT
0749-F	ATGACTGGTGACGGTGTGAA	0749-R	AACGAAATCCGCAATAGAGC
0756-F	CACTGACAGCCACGATCAAA	0756-R	CTTGCTGCCGTGAATAAACA
0758-F	TGATTCAGGCACAACGATTT	0758-R	GCGTCACGTCGGTTTCTTTA
1009-F	CAGGTGAAGCCAGTGAGATG	1009-R	CCGTCTTAGGGATGACCAAGT
1045-F	CAATACACAGACCGCCAGTG	1045-R	ATCGTTGGCTTTGTGCTCTT
1316-F	CGCCGCATGAAGTATGTTTA	1316-R	GAGGGCTCAACACGTTTAACA
1468-F	CAATGGTTCGTGCTTTGACTT	1468-R	TGCTGTGAGCCTGGTGATTA
1659-F	TGGATGAAGGCAGTCACATC	1659-R	CCCATACCGATACCGACAAA
1754-F	CCGCCAATCCACGAATACTA	1754-R	GCACTTGCTTGAGTTGTTTCAG
2725-F	CAGATCGACCATTGACACACA	2725-R	GCCGATGCCCTTACCTTAGT
fusA-F	CCTGAAACTGGCGAAACATT	fusA-R	CCAACCTTAGCAGCAACCTT
ileS-F	GCAACGGTTGACTCTTCCTC	ileS-R	GCTACCATAAACCGCATCGT
lepA-F	AAGAGCAGCACGAAGGGATA	lepA-R	AGGATCGCCATTAAGCAAGA
leuS-F	GCTGGATGCTGGTATTGCTT	leuS-R	ATCACGCAGTTTGCCTTCAT
mutL-F	CCTGCCAGTGTTGTCAAAGA	mutL-R	CGTTATCGCTAACCCGAATC
pcrA-F	GCAACACAAGCGTTGAGAAG	pcrA-R	ACCAGCAATCGGACTGAGTT
pyrG-F	GAAGCGTGATGTTGGTTCG	pyrG-R	TGTGCTGAGTTGGTTTCGTC
recA-F	TTGGCAACCGATAAAGACAA	recA-R	TGAGATGCGTTGACAAGTCC
recG-F	CGTAGCGTGATTCTGGTGAC	recG-R	TTCCAAAGATTGCTGCTTCA
rpoB-F	AATACAAGGCAGCCCATGAC	rpoB-R	ATCCAAGGCACCATCTTCAC

Table 2. *L. paracasei* ATCC 334 viability after a pronounced desiccation period followed by rapid rehydration with or without saccharides used as protectors.

Condition of drying	Concentration (g/L)	Viability percentage after desiccation/rehydration
No protector (distilled water)		0.001 ± 0.000
Monosaccharides		
Glucose	25	0.13 ± 0.09
	50	0.01 ± 0.01
Fructose	25	0.01 ± 0.01
	50	0.04 ± 0.02
Galactose	25	0.05 ± 0.02
	50	0.09 ± 0.04
Disaccharides		
Lactose	25	21 ± 2
	50	35 ± 7
Trehalose	25	41 ± 9
	50	19 ± 7
Sucrose	25	27 ± 9
	50	45 ± 0

Bacterial cells in stationary growth phase suspended in various protective solutions were air dried for 24 h at 25% RH and 25°C on PP coupons. Survival was determined after suspending dried cells in BCP medium. Viability was measured by plate count (CFU/ml).

Table 3. Viability of *L. paracasei* sensitive mutants after a pronounced desiccation period followed by rapid rehydration

Disrupted gene	Predictive function	A _{420nm} at 7 h	Viability %	Sensitivity level
WT		1.91 ± 0.08	41 ± 2	
LSEI_0040	Hypothetical protein	1.56 / 1.69	23 ± 4**	+
LSEI_0167	Diadenosine tetraphosphatase	1.71 / 1.64	22 ± 2**	+
LSEI_0178	PTS	1.73 / 1.59	18 ± 9*	++
LSEI_0238	PST transporter	1.63 / 1.66	23 ± 7**	+
LSEI_0363	Pyridoxine 5'-phosphate oxidase	1.74 / 1.75	26 ± 6*	+
LSEI_0397	NAD (FAD)-dependent dehydrogenase	1.34 / 1.40	25 ± 5**	+
LSEI_0749	Cation transport ATPase	1.74 / 1.75	23 ± 3**	+
LSEI_0756	Alpha/beta fold family hydrolase	1.62 / 1.75	18 ± 1**	++
LSEI_0758	Aldo/keto reductase related enzyme	1.45 / 1.48	22 ± 3**	+
LSEI_1009	Spermidine/putrescine-binding protein	1.63 / 1.45	26 ± 1**	+
LSEI_1045	Hypothetical protein	1.72 / 1.69	23 ± 4**	+
LSEI_1316	Hypothetical protein	1.30 / 1.27	14 ± 5**	+++
LSEI_1468	Ribonucleotide diphosphate reductase	1.10 / 1.11	4 ± 1**	+++
LSEI_1659	Glucokinase	1.65 / 1.66	25 ± 8*	+
LSEI_1754	SAICAR synthase	1.75 / 1.46	23 ± 4**	+
LSEI_2725	Sorbitol transcription regulator	1.74 / 1.70	27 ± 5*	+

Cells in stationary growth phase were air dried for 24 h at 25% RH and 25°C with 50 g/L lactose solution in microplates or on PP coupons. Sensitive mutants were determined by monitoring the A_{420nm} of dried cells suspended in BCP medium (2 biological replicates) and confirmed by individual drying on PP coupons and plate count (3 biological replicates). Mutants were classified into three categories according to their viability: + (mean viability at least 1.5-fold less), ++ (mean viability at least 2-fold less) and +++ (mean viability at least 3-fold less than WT).

*, significant changes in viability (p<0.05) compared to the WT strain (3 biological replicates).

**, significant changes in viability (p<0.01) compared to the WT strain (3 biological replicates).

Table 4. Operon organization and specificity of the 16 genes involved in adaptation to pronounced desiccation followed by rapid rehydration.

Gene	Predictive function	Putative operon	DNA strand	Specificity
LSEI_0040	Hypothetical protein	No	(+)	<i>L.casei/paracasei/rhamnosus</i>
LSEI_0167	Diadenosine tetraphosphatase	No	(-)	<i>L.casei/paracasei/rhamnosus</i>
LSEI_0178	PTS	0178-0180	(+)	<i>L.casei/paracasei/rhamnosus</i>
LSEI_0238	PST transporter	0238-0240	(+)	<i>L.casei/paracasei/rhamnosus</i>
LSEI_0363	Pyridoxine 5'-phosphate oxidase	No	(-)	<i>Lactobacillus</i>
LSEI_0397	NAD (FAD)- dehydrogenase	No	(-)	<i>Lactobacillus</i>
LSEI_0749	Cation transport ATPase	No	(-)	<i>Lactobacillus</i>
LSEI_0756	Alpha/beta fold family hydrolase	No	(+)	<i>Lactobacillus</i>
LSEI_0758	Aldo/keto reductase related enzyme	0757-0758	(-)	Gram +
LSEI_1009	Spermidine/putrescine-binding protein	1005-1009	(+)	Gram +
LSEI_1045	Hypothetical protein	No	(-)	<i>L.casei/paracasei/rhamnosus</i>
LSEI_1316	Hypothetical protein	1314-1319	(+)	<i>L.casei/paracasei/rhamnosus</i>
LSEI_1468	Ribonucleotide reductase	1467-1470	(+)	Gram +
LSEI_1659	Glucokinase	1658-1661	(-)	Gram +
LSEI_1754	SAICAR synthase	1746-1756	(-)	Gram +
LSEI_2725	Sorbitol transcription regulator	2720-2726	(-)	<i>L.casei/paracasei/rhamnosus</i>

Table 5. Viability of selected *L. paracasei* mutants after a pronounced desiccation without drying protector

Disrupted gene	Predictive function	Viability %
WT		0,059 ± 0,006
LSEI_0040	Hypothetical protein	0.034 ± 0,002**
LSEI_0167	Diadenosine tetraphosphatase	0.032 ± 0.003**
LSEI_0178	PTS	0.037 ± 0.003**
LSEI_0363	Pyridoxine 5'-phosphate oxidase	0,035 ± 0,005**
LSEI_0397	NAD (FAD)-dependent dehydrogenase	0,034 ± 0,000**
LSEI_1045	Hypothetical protein	0,017 ± 0,001**
LSEI_1316	Hypothetical protein	0,026 ± 0,002**
LSEI_1468	Ribonucleotide diphosphate reductase	0,002 ± 0,000**
LSEI_2725	Sorbitol operon transcription regulator	0,021 ± 0,003**

Cells in stationary growth phase were air dried for 24 h at 25% RH and 25°C with phosphate buffer on PP coupons. Viability percentages were determined after rapid rehydration by suspending dried cells with 110 µL of BCP medium at 37°C (15 successive cycles).

*, significant changes in viability ($p < 0.05$) compared to the WT strain (3 biological replicates).

**, significant changes in viability ($p < 0.01$) compared to the WT strain (3 biological replicates).

Table 6. Viability of *L. paracasei* selected mutants after a pronounced desiccation followed by a progressive rehydration

Disrupted gene	Predictive function	Viability %
WT		98 ± 3
LSEI_0167	Diadenosine tetraphosphatase	60 ± 0**
LSEI_0397	NAD (FAD)-dependent dehydrogenase	41 ± 6**
LSEI_0749	Cation transport ATPase	57 ± 4**
LSEI_1468	Ribonucleotide diphosphate reductase	14 ± 2**

Cells in stationary growth phase were air dried for 24 h at 25% RH and 25°C with 50 g/L lactose solution on PP coupons. Progressive rehydration was performed in a closed chamber with RH adjusted at 99% for 2 h. Viability percentages were determined by suspending dried cells with 110 µL of BCP medium at 37°C (15 successive cycles).

*, significant changes in viability ($p < 0.05$) compared to the WT strain (3 biological replicates).

**, significant changes in viability ($p < 0.01$) compared to the WT strain (3 biological replicates).

Table 7. Relative transcript level of *L. paracasei* genes after desiccation (1 h and 2 h) and rehydration (rapid or progressive) for the 16 genes identified after library mutant screening.

Gene	Predictive function	LAC	D1	D2	RR	PR
LSEI_0040	Hypothetical protein	1.0 ± 0.1	1.7 ± 0.6*	2.6 ± 0.3*	2.1 ± 0.2*	1.9 ± 0.4
LSEI_0167	Diadenosine tetraphosphatase	1.0 ± 0.1	1.8 ± 0.4*	2.3 ± 0.3*	1.6 ± 0.2	2.1 ± 0.4*
LSEI_0178	PTS	1.0 ± 0.2	-1.1 ± 0.2	-1.1 ± 0.1	-1.3 ± 0.2	-1.3 ± 0.3
LSEI_0238	PST family polysaccharide transporter	1.0 ± 0.1	1.3 ± 0.5*	2.2 ± 0.3*	1.6 ± 0.1*	1.9 ± 0.3*
LSEI_0363	Pyridoxine 5'-phosphate oxidase	1.0 ± 0.1	1.4 ± 0.4*	1.9 ± 0.2*	1.6 ± 0.2*	1.9 ± 0.3*
LSEI_0397	NAD(FAD)-dependent dehydrogenase	1.0 ± 0.2	1.6 ± 0.4*	1.8 ± 0.2*	1.7 ± 0.2*	-1.3 ± 0.2
LSEI_0749	Cation transport ATPase	1.1 ± 0.1	2.1 ± 0.2*	2.1 ± 0.2*	1.3 ± 0.3	1.6 ± 0.1*
LSEI_0756	Alpha/beta fold family hydrolase	1.0 ± 0.2	1.2 ± 0.1	1.1 ± 0.1	-1.4 ± 0.2*	-1.7 ± 0.3*
LSEI_0758	Aldo/keto reductase	-1.1 ± 0.2	-1.4 ± 0.4	-1.1 ± 0.1	-1.1 ± 0.1	1.0 ± 0.2
LSEI_1009	Spermidine/putrescine-binding protein	-1.1 ± 0.1	-1.3 ± 0.2	-1.4 ± 0.1	-2.0 ± 0.4*	-2.0 ± 0.4*
LSEI_1045	Hypothetical protein	-1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.2	-1.3 ± 0.2	-1.4 ± 0.2
LSEI_1316	Hypothetical protein	-1.1 ± 0.1	1.7 ± 0.2*	1.6 ± 0.2*	1.0 ± 0.1	-1.1 ± 0.2
LSEI_1468	Ribonucleotide diphosphate reductase	1.0 ± 0.2	1.5 ± 0.1	1.2 ± 0.1	-1.3 ± 0.2*	-1.7 ± 0.3*
LSEI_1659	Glucokinase	1.1 ± 0.1	-1.4 ± 0.4	-1.3 ± 0.2	-1.4 ± 0.2*	-1.4 ± 0.2
LSEI_1754	SAICAR synthase	-1.1 ± 0.1	-2.5 ± 0.6*	-2.5 ± 0.6*	-2.5 ± 0.6*	-2.5 ± 0.6*
LSEI_2725	Sorbitol operon transcription regulator	-1.1 ± 0.1	1.3 ± 0.3	1.5 ± 0.2*	1.2 ± 0.1	1.4 ± 0.3
Total of genes differentially expressed		0	10	13	13	11

LAC (cells incubated 15 min with lactose), D1 (cells dehydrated for 1 h), D2 (cells dehydrated for 2 h), RR (rapid rehydration with 1 mL of distilled water), PR (progressive rehydration in a closed chamber with RH adjusted at 99% for 2 h). RTL were calculated using $2^{-\Delta\Delta C_t}$ method. For the phosphate buffer control condition, a gene expression value of 1.0 was attributed and RTL of genes in stress condition were calculated as a function of this value. Positive values (> 1.0) represent upregulation and negative values (< 1.0) represent downregulation. *, significant changes in gene expression (p<0.05) compared to the phosphate buffer condition (4 biological replicates). Values in light red correspond to

upregulation and in dark red to upregulations > 2.0 ; values in light blue correspond to downregulation and in dark blue to down regulation < -2.0 .

Figure legends

Figure 1. Organizational chart of the strategy developed in this work to identify genetic determinants of *L. paracasei* resistance to desiccation and rehydration. PPC, polypropylene coupons.

Figure 2. Evolution of the cell suspension mass during desiccation (orange curve) and rehydration (blue curve) on a polyvinylidene membrane. Sampling times for RNA extractions are represented by the grey boxes: PB (1 mL of cells incubated 15 min with phosphate buffer control), LAC (1 mL of cells incubated 15 min with phosphate buffer supplemented with 50 g/L lactose), D1 (cells dried 1 h, partial desiccation), D2 (cells dried 2 h, pronounced desiccation), RR (rapid rehydration with 1 mL of distilled water), PR (progressive rehydration in a closed chamber with RH adjusted at 99% for 2 h and addition of water to obtain the mass before drying).

Figure 3. Schematic representation of genes differentially transcribed in *L. paracasei* during desiccation and rehydration. Gene expressions are represented by color boxes in light red for upregulation, dark red for upregulations > 2.0 , light blue for downregulation, dark blue for downregulation < -2.0 and in white for constitutive regulation. For each gene, boxes from left to right correspond to: lactose incubation (LT), desiccation for 1h (D1), desiccation for 2h (D2), rapid rehydration (RR) and progressive rehydration (PR).

Mutant Library – 1287 mutants
(1110 genic mutants + 177 intergenic mutants)

24 h air drying in 96 well plates
25 % RH – 25°C – lactose 50 g/L

Absorbance at 420 nm in 96 well plates
Rehydration with BCP medium – 37°C

Sensitive mutants
 A_{420} at 7h < 0.75
(2 biological replicates)

WT absorbance
 A_{420} at 7h
= 0.91 ± 0.08

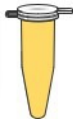
Validation of mutants on PPC
Percentage viability ($p < 0.05$)
(3 biological replicates)

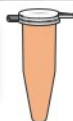
WT survival on PPC
Percentage survival
= $41 \pm 2 \%$

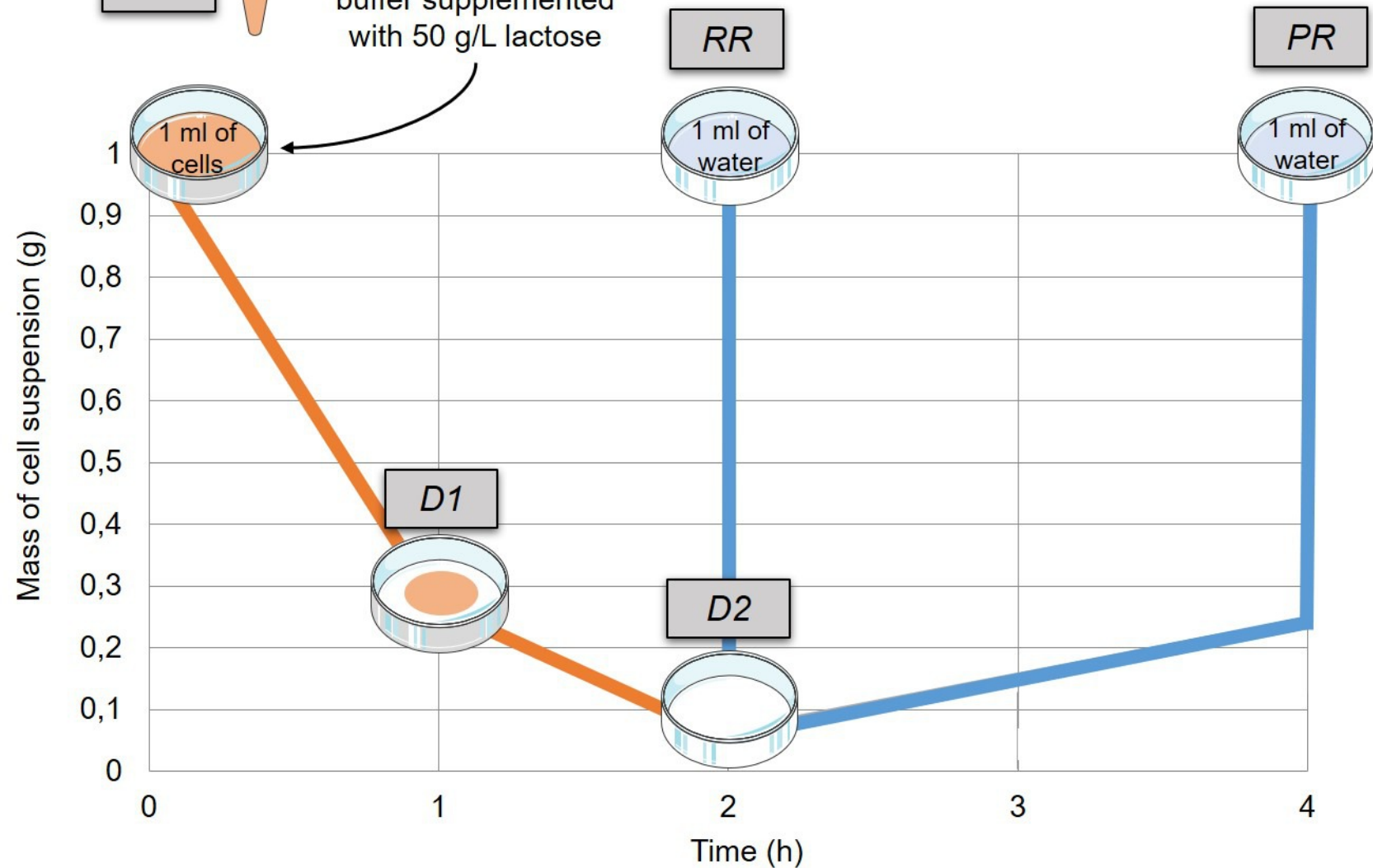
Identification of genes
Involved in dehydration and
rehydration phases

WT
RNA extraction in the
different phases

RT-qPCR
Expression of genes in the
different phases

PB  15 min in phosphate buffer control

LAC  15 min in phosphate buffer supplemented with 50 g/L lactose



LT | D1 | D2 | RR | PR

