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1 **Identification and expression of *Lactobacillus paracasei* genes for adaptation to**
2 **desiccation and rehydration**

3 **Aurore Palud[a]#, Karima Salem[a,b], Jean-François Cavin[a], Laurent Beney[a] and Hélène**
4 **Licandro[a]#**

5 [a]Université de Bourgogne Franche-Comté, AgroSup Dijon, PAM UMR A 02.102, Dijon,
6 France

7 [a] Ecole polytechnique de Sousse, Sousse, Tunisia

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9 [#] Address correspondence to Aurore Palud, palud.aurore@gmail.com and Hélène Licandro,
10 helene.licandro@u-bourgogne.fr

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 in particular the genetic determinants involved are not clearly understood. To identify the
17 genes involved in adaptation to desiccation, an annotated library of *L. paracasei* random
18 transposon mutants was screened for viability after desiccation (25% relative humidity,
19 25°C). Subsequently, the expression of the identified genes was measured at five stages of the
20 dehydration-rehydration process to formulate the chronology of gene expression. The 24
21 identified genes were related to metabolism and transport, membrane function and structure,
22 regulation of stress response, DNA related enzymes and environmental sensing. They were

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23 classified into four different transcriptomic profiles, in particular genes upregulated during
24 both desiccation and rehydration phases and genes upregulated during the desiccation phase
25 only. Thus, genetic response to hydric fluctuations seems to occur during desiccation and can
26 continue or not during rehydration. The genes identified should contribute to improving the
27 stabilization of lactobacillus starters in dry state.

28

29 **Importance**

30 Since water is the fundamental component of all living organisms, desiccation and
31 rehydration alternation is one of the most prevalent and severe stresses for most
32 microorganisms. Adaptation to this stress occurs via a combination of mechanisms which
33 depend on the genetic background of the microorganism. In *L. paracasei,* we developed a
34 strategy to identify genes involved in the adaptation to hydric fluctuations using random
35 transposon mutagenesis and targeted transcriptomics. Both dehydration and rehydration were
36 studied to decipher the chronology of genetic mechanisms. We found 24 as yet unidentified
37 genes involved in this response. Most of them are linked to either the transport of molecules
38 or to cell wall structure and function. Our screening also identified genes for environment
39 sensing and two alarmones necessary for *L. paracasei* survival. Furthermore, our results show
40 that desiccation is a critical phase for inducing stress response in *L. paracasei.*

41

42 **Keywords** *Lactobacillus paracasei*, transposon mutants, desiccation, rehydration, gene
43 expression

44

45

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Introduction

Water is essential for all living organisms as it contributes to the structure of cells, stabilizes proteins, lipids and nucleic acids, and maintains vital metabolic systems and chemical reactions (1). Desiccation leads to water exit from cells, induces structural modifications, and causes osmotic and oxidative stresses (1–3). In addition, the rehydration phase could lead to membrane alterations (4). The capacity to return to life after hydric fluctuations is not only crucial for bacteria as a function of diurnal and seasonal cycles in natural environments but also during food industry processes such as conventional drying, freeze drying and spray drying (1, 5–7). In many organisms, desiccation survival is correlated with the accumulation of protective molecules, in particular trehalose (8). However, desiccation tolerance involves other mechanisms that are mostly unknown except for extreme desiccation tolerant bacteria (anhydrobiotes) such as such as cyanobacteria (9), yeast (2), resurrection plants (10) and microscopic animals (11).

In the last few years, progress has been made in understanding the mechanisms of bacteria tolerance to partial desiccation using transcriptome analysis, in particular for *Salmonella,* one of the most common foodborne pathogens able to survive for extended periods after desiccation (12–17). The comparison of these studies has revealed differences in identified genes that could be explained by the procedures used to dry bacteria (surface, drying medium, RH, desiccation periods). Several common genes were linked to amino acid transport and metabolism functions. Global transcriptional analyses were also performed for soil-residing bacteria (18–20) and similar responses were identified such as compatible solute and heat shock protein accumulation, reactive oxygen species neutralization, and DNA modification and repair. However, transcriptomics has limitations since genes essential for a function can be constitutively expressed and even inducible genes can be overexpressed during a very short period that does not overlap with that of the experiment. Recently, random transposon

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72 mutagenesis approaches were developed to identify genes for resistance to desiccation in
73 *Listeria monocytogenes* (Hingston et al., 2015) and *Salmonella enterica* (22). For *L.*
74 *monocytogenes* screening, genes were involved in energy production, membrane transport,
75 amino acid metabolism, fatty acid metabolism and oxidative damage control. In *S. enterica*, in
76 comparison to previous transcriptomic studies, several genes were related to amino acid
77 metabolism and more than 20% encoded hypothetical proteins. Recently, changes in
78 proteomic expression were investigated for *S. enterica* during desiccation and rehydration
79 (23). The proteins with higher expression levels in dried samples were mainly ribosomal
80 proteins whereas flagellar proteins, membrane proteins, and export systems as well as stress
81 response proteins were identified in rehydrated samples.

82 Although *L. casei/paracasei* is one of the most emblematic groups of lactic acid bacteria
83 (LAB), the genetic mechanisms involved in desiccation resistance and adaptation are not fully
84 understood, limiting prospects for improving and developing preservation processes.
85 Comparative genomics has demonstrated that LAB are highly adaptable to various niches
86 such as soil, foods (dairy, meat, and vegetable), as well as oral, vaginal and gastrointestinal
87 cavities (24–26). This is correlated with an ability to adapt and persist under diverse
88 environmental stresses (27). Thanks to this capacity, humans have succeeded in selecting and
89 producing certain LAB strains as efficient starters for fermented foods (particularly hard
90 cheese) and as probiotics. The adequate preservation of starter cultures is necessary though it
91 is currently only possible using advanced desiccation technologies and storage in frozen state
92 (28)

93 Considering the importance of the *L.casei/paracasei* group for health and industrial
94 applications, we compiled a non-redundant, annotated transposon mutant library of *L.*
95 *paracasei* (29, 30) based on the P_{junc}-TpaseIS$_{1223}$ system, a random mutagenesis tool
96 specifically designed for the *Lactobacillus* genus (31). Recently, using this approach we

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97 determined five *Lactobacillus pentosus* mutants sensitive to olive brine due to multifactorial
98 stress (32). In addition, new genetic determinants were identified during the early stage of *L.*
99 *paracasei* establishment in the gut (29) and during monofactorial perturbations of mild
100 intensities (33). In the present work, the *L. paracasei* transposon mutant library was screened
101 to identify genes involved in the adaptation of this LAB to desiccation and rehydration. The
102 expression of corresponding genes was studied by RT-qPCR during the two-step process to
103 draw a chronological transcriptomic profile during hydric fluctuations.

104

105 **Results**

106 **Development of the screening strategy**

107 A library containing a total number of 1287 *L. paracasei* mutants was screened for their
108 capacity to resist a 24-h desiccation period followed by rapid rehydration in comparison with
109 their parental strain (*L. paracasei* ATCC 334, named WT). Convective drying in a ventilated
110 chamber at room temperature with a relative humidity (RH) of 25% was selected to mimic the
111 severe desiccation conditions that can occur in natural environments during drought in soil, on
112 plants, and on animal skin, for example. This process consisted in passing a flow of dry air
113 over the cell suspension approximately simulating the desiccation conditions that organisms
114 can undergo in the natural environment. To assess the importance of sugars as drying
115 protectors, we selected three disaccharides related in the literature to lactic acid bacteria
116 preservation, in particular during freeze drying (34–36), and their corresponding
117 monosaccharides. The viability of *L. paracasei* WT after 24-h desiccation at 25% RH was
118 determined with and without the protectors (Table 1). Viability was very low in the absence
119 of sugar and with monosaccharides. On the contrary, with disaccharides, viability was
120 between 19 and 45%, which was an adequate range for screening either less or more resistant
121 mutants. Lactose 50 g/L was selected because it corresponds to the composition of milk, thus

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122 a condition often encountered by *L. paracasei* in the natural and technological environments
123 of cheese-making, for example. The whole strategy followed in this work is illustrated in
124 figure 1. A screening method composed of easy and rapid steps to save time and reduce the
125 risk of bias was developed. The viability of the 1287 mutants after 24-h desiccation followed
126 by rapid rehydration was evaluated in 96-well microplates by monitoring the acidification
127 kinetics (due to sugar metabolism) using a medium containing a pH indicator. Absorbance
128 (A_{420nm}) was correlated to the quantity of metabolically active (or viable) bacteria, thus the
129 viability of the mutants could be compared to that of the WT (A_{420nm} (7 h) = 1.91 ± 0.08).
130 Sensitive and resistant mutants were subsequently validated by individual drying on PP
131 coupons and by determining viability by the plate count method in order to remove possible
132 false positive mutants (for instance, metabolic deficiency mutants).

133 **Identification of genes involved in resistance to pronounced desiccation followed by**
134 **rapid rehydration.**

135 The mutant library was subjected to 24-h desiccation (pronounced desiccation) followed by
136 rapid rehydration. Screening of the 1287 transposon mutants resulted in the detection of 47
137 sensitive mutants (A_{420nm} at 7 h < 1.75 for 2 biological replicates) but no resistant mutants
138 (A_{420nm} at 7 h > 2.07 for 2 biological replicates). After the validation step by individual drying
139 of the 47 mutants, 24 mutants displayed a significant decrease of survival compared to the
140 WT ($p<0.05$) (Table 2). Identified genes were mostly involved in transport, regulation and
141 membrane binding proteins. Moreover, five sensitive mutants containing transposon in a gene
142 encoding a hypothetical protein were identified (LSEI_0040, LSEI_0733, LSEI_0806,
143 LSEI_1045 and LSEI_1316). Mutants were classified into three categories according to their
144 viability: + (mean viability at least 1.2-fold less), ++ (mean viability at least 2-fold less) and
145 +++ (mean viability at least 3-fold less than WT). The majority of mutants (20) were
146 categorized as +. The two most sensitive mutants (+++) were disrupted for a putative

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147 ribonucleotide diphosphate reductase (LSEI_1468) and for a hypothetical protein (LSEI_
148 1316). Two mutants disrupted for a putative PTS (LSEI_0178) and for a putative alpha/beta
149 fold family hydrolase (LSEI_0756) were categorized as ++. Fifteen genes out of the 24
150 belonged to putative operons (Table 3). Eleven genes were specific to *L. paracasei* or related
151 species, five to *Lactobacillus* genus and eight were well conserved among Gram (+) bacteria.
152 Two genes among the five encoding hypothetical proteins presented a transmembrane domain
153 (LSEI_0040 and LSEI_1045). All hypothetical protein genes, except LSEI_0733, were
154 specific to the *L. paracasei* group.

155 **Analysis of mutant sensitivity after pronounced desiccation followed by progressive**
156 **rehydration**

157 To assess if the identified genes were involved during dehydration whatever the rehydration
158 process, the WT and the 24 sensitive mutants identified previously were subjected to
159 desiccation for 24-h followed by progressive rehydration (for 2 h) instead of rapid
160 rehydration. In these conditions, the viability of the WT was 99 ± 3%, which is considerably
161 higher than obtained with a rapid rehydration. Among the 24 sensitive mutants identified
162 previously, only seven were also sensitive after desiccation and progressive rehydration
163 (Table 4). They encode a putative diadenosine tetraphosphatase (LSEI_0167), a PTS system
164 (LSEI_0178), an NAD (FAD)-dependent dehydrogenase (LSEI_0397), a cation transport
165 ATPase (LSEI_0749), a ribonucleotide reductase (LSEI_1468), a SAICAR synthase
166 (LSEI_1754) and a hypothetical protein (LSEI_0040). As observed for desiccation and rapid
167 rehydration, the most sensitive mutant was ribonucleotide diphosphate reductase.

168 **Transcriptomic analysis of the identified genes during a desiccation – rehydration cycle**

169 Mutant library screening allowed the identification of 24 genes involved in the global
170 perturbation which consisted in desiccation followed by rehydration. Thus, it was not possible
171 to determine during which phase these genes were involved since rehydration was necessary

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172 to analyze mutant phenotypes. To determine the chronology of the genetic mechanisms during
173 humidity fluctuations, transcriptomic analysis of the identified genes was carried out on the
174 WT. RNA extractions were performed at two different stages of desiccation: partial
175 desiccation D1 (76% of water evaporated after 1 h) and pronounced desiccation D2 (96% of
176 water evaporated after 2 h) which corresponded to completely dried cells (Figure 2). As
177 rehydration kinetics is decisive for bacterial survival, rapid and progressive rehydration were
178 applied for RNA extractions after 2 h.

179 As lactose was required to ensure good survival of bacteria during the desiccation/rehydration
180 process it was reasonable to expect that some candidate genes could be differentially
181 expressed in the presence of lactose. However, for the 24 genes, expressions after 15-min
182 incubation in lactose were comparable to that after a 15-min phosphate buffer incubation
183 (Figure 3). Differentially expressed genes were classified to obtain the most upregulated
184 (Table 5, values in dark red) and downregulated (Table 5, values in dark blue) genes, with a
185 mean expression value higher or lower than 2.0. Among the 24 genes studied, 18 were
186 differentially expressed ($p < 0.05$) for at least one condition (Table 5). The six genes involved
187 in hydric changes but not differentially expressed encoded a putative PTS transporter
188 (LSEI_0178), a reductase (LSEI_0758), a ppGpp alormone (LSEI_1539), a DNA polymerase
189 (LSEI_1709) and hypothetical proteins (LSEI_0733 and LSEI_1045). Six genes were
190 upregulated for all the hydric fluctuations tested. These genes encoded a putative diadenosine
191 tetraphosphatase (LSEI_0167), a polysaccharide transporter (LSEI_0238), a pyridoxine 5'-
192 phosphate oxidase (LSEI_0363), a PTS transporter (LSEI_A05) and hypothetical proteins
193 (LSEI_0040 and LSEI_0806). Also, LSEI_0397 encoding a putative NAD dependent
194 dehydrogenase was upregulated during desiccation and progressive rehydration. Conversely,
195 LSEI_0749 encoding a putative cation transport ATPase was upregulated during desiccation
196 and rapid rehydration. Interestingly, these genes were the most upregulated (> 2-fold change)

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197 for at least one condition. LSEI_A05 (putative lactose specific PTS system) was the most
198 upregulated gene during desiccation (D1 and D2). One gene, LSEI_1754, encoding a putative
199 SAICAR synthase was the most downregulated gene in these experiments (mean expression
200 value < 2.0 for all the drying and rehydration conditions).

201 Three genes, LSEI_0934 (a putative DNA binding response regulator), LSEI_2505 (a putative
202 phosphate starvation inducible protein) and LSEI_2725 (a putative sorbitol operon
203 transcription regulator) were upregulated after only 2 h of desiccation and represent potential
204 dry state biomarkers. LSEI_1316 encoding a putative hypothetical protein was upregulated
205 after 1 h and 2 h of desiccation. Interestingly, no gene was specifically upregulated during
206 subsequent rehydration. Conversely, five genes were downregulated specifically during
207 rehydration: LSEI_0756 (a putative hydrolase) LSEI_1009 (a putative spermidine/putrescine-
208 binding protein), LSEI_1468 (a putative ribonucleotide reductase), LSEI_1324 (a putative
209 membrane metal-binding protein) and LSEI_1659 (a putative glucokinase).

210

211 **Discussion**

212 Twenty-four desiccation-sensitive mutants were identified from the *L. paracasei* mutant
213 library screening after desiccation followed by rapid rehydration. Contrary to our recent
214 screening of this library for mild stress sensitivity (33), no mutants for putative promoters
215 were identified as sensitive to desiccation. Half of the identified genes were specific of the *L.*
216 *casei/paracasei/rhamnosus* group. On the contrary, eight genes were well conserved among
217 Gram-positive bacteria. Interestingly, these genes, although present in *Listeria monocytogenes*
218 genome, were not identified by the screening of *L. monocytogenes* random mutants for
219 desiccation resistance (Hingston et al., 2015). Otherwise, this study of *L. monocytogenes*
220 desiccation resistance shows the involvement of a gene encoding a putative glutathione
221 peroxidase, known to prevent oxidative damages. This gene is present in the *L. paracasei*

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222 mutant library but was not identified as sensitive. These differences may result from the
223 drying conditions applied such as the surface used for desiccation, the relative humidity, the
224 drying medium or the rehydration conditions.

225 **Metabolism and transport**

226 Three transporters are required for survival to hydric fluctuation including two putative
227 phosphotransferase systems (LSEI_0178 and LSEI_A05) and one cation transporter
228 (LSEI_0749). Whereas the function of LSEI_0178 is obvious, the LSEI_A05 function as a
229 lactose transporter is uncertain because the corresponding operon is plasmidic and incomplete
230 in this strain. However, this strain is able to metabolize lactose, suggesting that this sugar can
231 enter the cell via another transporter (37). Several authors have reported a link between PTS
232 systems and stress response for *Lactobacillus* genus. *L. plantarum* mutants with an impaired
233 expression of the mannose PTS operon exhibited increased sensitivity to peroxide, probably
234 due to a diminution of glucose capture and energy production (38).

235 Next, we identified a membrane associated protein (LSEI_1009) predicted to bind polyamines
236 before their transport into the cell by the ABC transporters encoded by other genes of the
237 LSEI_1005-1009 operon. Polyamines have various important physiological roles during stress
238 response, including the modulation of gene expression, signal transduction, oxidative defense
239 mechanism, and cell-to-cell communication (39, 40).

240 One identified gene encoded a glucokinase (LSEI_1659), a key enzyme of glucose
241 metabolism. In *S. aureus*, glucokinase is also involved in pathogenicity (biofilm formation,
242 virulence factors, cell wall synthesis) (41). In our case, glucokinase as well as PTS
243 interruption could lead to energy diminution and then sensitivity to hydric fluctuations.
244 Another gene, LSEI_0363, encodes a putative enzyme that catalyzes the oxidation of
245 pyridoxamine-5-P (PMP) and pyridoxine-5-P (PNP) to pyridoxal-5-P (PLP) in the vitamin B6

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246 pathway. Vitamin B6, an antioxidant molecule, has been implicated in defense against cellular
247 oxidative stress in *Saccharomyces cerevisiae* (42).

248 **Cell wall function and structure**

249 Modification at the cell wall level appears to be essential for surviving hydric fluctuation,
250 considering the 6 genes related to the cell wall and identified as essential for hydric
251 fluctuation survival. The gene LSEI_0238 encodes a polysaccharide transporter involved in
252 the export of lipoteichoic acid (LTA), a major constituent of the Gram-positive cell wall (24).
253 Interestingly, this gene was also needed for *L. paracasei* gut establishment (29). The mutant
254 for LSEI_2546, another polysaccharide transporter involved in LTA export, was not identified
255 as sensitive.

256 LSEI_2505 encodes a putative membrane protein involved in response to phosphate
257 starvation. Although the regulation of this family of genes has been deciphered in *Bacillus*
258 *subtilis*, their exact role remains unclear (43). Then, LSEI_1324 encodes a putative
259 membrane metal binding protein. Finally, three hypothetical proteins exhibited a
260 transmembrane domain (LSEI_0040, LSEI_0806 and LSEI_1316). Interestingly LSEI_0040
261 was involved in the adaptation of *L. paracasei* to thermal, ethanol and oxidative stresses (33)
262 and LSEI_0806 to oxidative stress. Both the latter gene and LSEI_1316 were also required for
263 gut establishment (29). The present and previous results show that membrane proteins are
264 important for *L. paracasei* adaptation to various environments and involved in general stress
265 response.

266 **Regulation of stress response**

267 Two genes involved in alarmone synthesis or degradation are needed for *L. paracasei* survival
268 during hydric fluctuations. Alarmones are putative chemical messengers produced during
269 environmental changes. LSEI_0167 encodes a putative diadenosine tetraphosphatase, an

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270 enzyme that hydrolyzes diadenosine tetraphosphate (Ap4A) into two molecules of adenosine
271 diphosphate (ADP). Ap4A has been reported to be involved in heat stress response in *E.coli*
272 (44). Moreover *apaH* (diadenosine tetraphosphatase) mutation causes Ap4A accumulation
273 and sensitivity to thermal stress (45). Nevertheless, Despotović and collaborators wondered
274 whether Ap4A was an alarmone or a damage metabolite because they observed that no
275 signaling cascade was triggered by Ap4A and that its accumulation at high level was toxic for
276 *E. coli*.(46). The second gene (LSEI_1539, *relA*) encoded a putative ppGpp synthetase. The
277 second ppGpp synthetase (LSEI_0901, RelQ) was not present in the mutant library. ppGpp
278 functions as a chemical messenger for metabolism, growth, stress tolerance and virulence in
279 bacteria (47). It is well known that ppGpp interacts with RNA polymerase to affect gene
280 repression or induction. Broadbent et al., (2010) suggested that ppGpp is a signal for acid
281 tolerance response in *L. paracasei*. The LSEI_1539 mutant was also necessary for gut
282 establishment (29).

283 LSEI_2725 (*gutR*) has been identified as a determinant for surviving hydric fluctuations.
284 Although it is annotated as a sorbitol operon transcription regulator, its predicted function
285 should be reconsidered. Alcantara *et al.,* 2008 reported that in *L. paracasei ATCC334, gutR* is
286 split into 3 ORFs (LSEI_2728, LSEI_2726 and LSEI_2725) as the result of transposition
287 events, and consequently this strain is unable to use sorbitol, contrary to *L. casei* BL23. In
288 addition, the LSEI_ 2724 (*gutM*) gene, encoding a sorbitol operon activator with *gutR*, is
289 dispensable for desiccation resistance. In conclusion, we assume that LSEI_2725 functions as
290 a regulator but independently of the sorbitol operon.

291 **DNA related enzymes**

292 It was widely reported that desiccation induces DNA damage (1, 50). Indeed, the mutant
293 library screening identified two genes involved in nucleotide metabolism, LSEI_1468 (a
294 ribonucleotide reductase, RNR) and LSEI_1754 (a SAICAR synthase). The RNR mutant was

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295 particularly sensitive (mean viability 10-fold less than WT). RNR is an essential enzyme since
296 its mediates the synthesis of deoxyribonucleotides, the precursors of DNA synthesis (51), and
297 we have previously reported that the RNR gene was involved in general response to mild
298 stresses in *L. paracasei* (33). SAICAR synthase is involved in de novo purine biosynthesis
299 (52). These two genes could be involved in DNA repair. Recently, García-Fontana *et al.*
300 (2016) reported that the DNA molecule was overproduced and acted as a protein protector in
301 desiccation tolerant bacteria. This phenomenon could occur in *L. paracasei*. This assumption
302 is supported by the identification of a mutant in a DNA polymerase gene (LSEI_1709).

303

304 **Environment sensing**

305 A DNA binding response regulator (RR, LSEI_0934) was required for *L. paracasei* survival
306 of desiccation. LSEI_0934 forms a two-component system (TCS) with histidine kinase (HK,
307 LSEI_0935). The involvement of TCS in *Lactobacillus* response to acid and bile stresses has
308 been reported (54, 55) and the *L. paracasei* ATCC334 genome contains 17 putative TCS
309 (56). LSEI_0934-0935 TCS is organized in a putative operon with five genes encoding
310 phosphate ABC transporters, probably involved in phosphate assimilation. Intracellular poly-
311 P accumulation synthetized from Pi by polyphosphate kinase (Ppk) is a characteristic of some
312 *Lactobacillus* strains to adapt to stress (57). This could be the case during desiccation stress in
313 combination with the phosphate starvation protein gene (LSEI_2505). This assumption should
314 be modulated because mutants for the HK (LSEI_0935) and a phosphate ABC transporter
315 permease (LSEI_0938) are available in the library but were not identified as sensitive to
316 hydric fluctuations.

317 **Taking into account desiccation and rehydration kinetics**

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318 The results indicate that rapid rehydration of WT strain cells was more detrimental than
319 progressive rehydration. It has been reported that progressive rehydration could reduce the
320 stress applied to bacteria as it promotes membrane integrity recovery (58, 59). We found only
321 seven sensitive mutants whatever their rehydration kinetics (Table 4).

322 Among the genes essential for survival, five genes were expressed at the same level as the
323 control whatever the hydration phase (Table 5). This result again shows the strength of global
324 reverse genetics, since some genes may be essential for a function, although expressed
325 constitutively. Four transcriptomic profiles were observed during hydric fluctuations (Table
326 5): seven genes were upregulated during both desiccation and rehydration, four genes were
327 upregulated only during the desiccation stage, one gene was downregulated during both
328 desiccation and rehydration, five genes were downregulated only during the rehydration stage.
329 We assume that adaption to hydric fluctuations occurs during dehydration and continues or
330 not during rehydration.

331 In conclusion, this work identified 24 genetic determinants for the resistance of *L. paracasei*
332 to desiccation. Transcriptomic analysis of the corresponding genes highlighted that seven
333 genes were upregulated during both desiccation and rehydration and four during desiccation
334 only. This analysis provides clues for developing genetic biomarkers to monitor the intensity
335 of desiccation. These findings provide novel insights into the genetic mechanisms involved in
336 desiccation and rehydration adaptation in *L. paracasei*.

337

338 **Materials and Methods**

339 **Strains and growth conditions.** Wild-type (WT) *L. paracasei* ATCC 334 (CIP 107868,
340 Institut Pasteur Collection) and its corresponding mutants obtained by random transposon
341 insertion mutagenesis (31) were grown statically at 37°C in MRS (Difco). 5 µg/mL

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342 erythromycin (Em) was used to select mutants. The mutants correspond to the 1287 genic and
343 intergenic mutants already described (33) (29). For each mutant, the putative inactivated
344 function was assigned thanks to the genome annotation of this strain (24). The mutant library
345 was grown in 96-well plates (200 µl) for 48 h. Plates were mixed (30 s, 700 rpm, Eppendorf
346 MixMate) and 10 µL of each well was used to inoculate 190 µL of MRS in new 96-well
347 plates. Individual mutants were grown in tubes for 48h (2 ml) and vortexed to inoculate at a
348 dilution of 1/100 in new tubes. Plates and tubes were incubated for 24h at 37°C to obtain cells
349 in stationary phase (concentration between $1.0x10^9$ and $2.0x10^9$ UFC/mL). Cells were
350 rehydrated with bromocresol purple (BCP) medium which contains 5 g/L of tryptone, pepsic
351 peptone, yeast extract and sodium acetate, 2 g/L of ammonium citrate and dipotassium
352 phosphate, 1g/L of glucose, 1 ml of tween 80, 0.20 g/L of magnesium sulfate, 0.17 g/L of
353 bromocresol purple and 0.05 g/L of manganese sulfate.

354 **Drying conditions**. The drying chambers were hermetic plastic boxes (20 cm × 13 cm × 6
355 cm) containing 100 mL saturated potassium acetate (Sigma–Aldrich) solution to obtain 25%
356 RH at 25°C. Samples were placed on a rack in the drying chamber to keep them above the salt
357 solution and the atmosphere was maintained using a ventilator (Sunon, Radiospare, France).
358 Temperature and RH were controlled using an EASY Log USB tool (Lascar Electronics).
359 Drying was performed in a U bottom 96-well microplate (Evergreen, untreated) for mutant
360 library screening and on sterile polypropylene (PP) coupon of 15 mm × 10 mm × 2 mm
361 (Scientix, Fougères, France) for individual treatments. Drying solutions with or without
362 protector were used and as a function of the experiments.

363 **Screening of the mutant library for viability after desiccation.** Stationary growth phase
364 cultures were mixed (1 min, 1000 rpm) and 50 µL of cells were spotted in a U bottom 96-well
365 microplate and centrifuged (5 min, 4000g, 25°C). Pellets were suspended with 50 µL of 50

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366 g/L lactose (1 min, 1700 rpm) and incubated 15 min at 20°C. Then, only 10 µL of suspension
367 were placed in each well and the plates were placed in the ventilated chamber. After 24 h,
368 each well was rehydrated rapidly with 110 µL of (BCP) medium at 37°C. This dedicated
369 culture medium with pH indicator turns to yellow when bacteria metabolize sugars after
370 rehydration. Absorbance at 420 nm (A_{420nm}) was measured in a plate reader for 7h at 37°C
371 (Paradigm, Beckman Coulter). Mutants were considered as potentially sensitive when
372 absorbance at 7 h was lower than 1.75, which is the mean value of the WT minus twice the
373 standard derivation, for the two biological replicates. Mutants were selected as potentially
374 resistant when absorbance at 7h ($A_{7h)}$ was higher than 2.07, which is the mean value of the
375 WT plus twice the standard derivation, for two biological replicates. Mutant phenotypes were
376 confirmed, determining viability on plate counts (3 biological replicates).

377 **Determination of the viability of selected mutants after progressive and rapid**
378 **rehydration.** *L. paracasei* cultures in stationary phase (1 mL) were centrifuged (5 min, 4000
379 g, 25°C) and pellets were suspended by vortexing with one volume of drying solution
380 composed of lactose 50 g/L. After incubation for 15 min, 10 µL of cell suspensions were
381 placed onto a sterile polypropylene (PP) coupon of 15 mm × 10 mm × 2 mm (Scientix,
382 Fougères, France). Three coupons were prepared for each mutant. The inoculated coupons
383 were placed in the ventilated chamber in plastic petri dishes. For rapid rehydration, 110 µL of
384 BCP medium was deposited on the dried cells and the latter were resuspended by 15
385 successive cycles using a micropipette. For progressive rehydration, dried cells were
386 introduced into a hermetic chamber at 99% RH for 2 h at 25°C. Then, 110 µL of BCP
387 medium was deposited on the wet bacteria cells that were recovered by 15 successive cycles
388 using a micropipette. Colony enumeration by plate counts was averaged and the viability
389 percentage was obtained with the ratio of colony enumeration (in CFU/coupon) before
390 desiccation to that after desiccation. Mutants exhibiting a viability percentage significantly

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391 lower (or higher) than the WT were determined as sensitive (or resistant) (Student test, $p < 0.05$).
392

393 **Bioinformatics.** The putative operon organization of corresponding genes was established
394 using the Biocyc website. Then, genes were aligned using BLAST
395 (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against all bacteria to determine their specificity.

396 **RT-qPCR.** Cells in mid-exponential growth phase (OD_{600} between 0.5 and 0.6) were
397 centrifuged and concentrated to an OD_{600} of 20. Cell pellets were suspended with phosphate
398 buffer (10 mM pH 6.5) supplemented with 50 g/L lactose except for control. The suspension
399 was incubated for 15 min at 25°C and 1 mL was deposited on a 0.22 µm polyvinylidene
400 membrane in a glass Petri dish to prevent cell adhesion**.** Cells were dried in ventilated
401 chambers for 2 h and then subjected to either rapid or progressive rehydration. Rehydrated
402 cells were subsequently detached from the membrane using a cell lifter. Total RNA isolation,
403 cDNA synthesis and qPCR were performed as previously described (60) using TRI Reagent
404 (Sigma Aldrich), DNase I (Roche), iScript™ Reverse Transcription Supermix (Bio-Rad) and
405 SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad). Primers were designed by
406 using Primer3Plus (61) (Table 6). Quantitative PCR were performed using a CFX96 Touch™
407 Real-Time PCR Detection System (Bio-Rad) in triplicate, in a 20 µL-reaction mixture. Cq
408 (threshold value) calculation was determined by a regression model of the CFX Manager™
409 Software. The relative transcript levels of genes were calculated using the $2^{-\Delta\Delta CT}$ method (62).
410 In order to select appropriate reference genes, 10 potential housekeeping genes (*fusA, ileS,*
411 *lepA, leuS, mutL, pcrA, pyrG, recA, recG* and *rpoB*) (63) were tested with all the experimental
412 conditions and analyzed using the CFX Manager™ Software. The genes *fusA*, *lepA* and *rpoB*
413 were selected as the references because they displayed the lowest M values (0.22) and

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414 coefficients of variation (0.09), meaning that they have the most stable expression in the
415 tested conditions.
416
417 **Conflict of Interest Statement**
418 The authors declare that the research was conducted in the absence of any commercial or
419 financial relationships that could be construed as a potential conflict of interest.
420
421 **Author Contributions**
422 Study conception and design: AP, HL, LB and JFC. Acquisition of data: AP. Analysis and
423 interpretation of data: AP, HL, LB and JFC. Drafting of manuscript: AP. Critical revision:
424 AP, HL, LB and JFC. All authors read and approved the final manuscript.
425
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433
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610 **Tables**

611 **Table 1. *L. paracasei* ATCC 334 viability after a pronounced desiccation period followed**
612 **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

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

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623 **Table 2. Table 2. *L. paracasei* mutants sensitive to a pronounced desiccation period**
624 **followed by rapid rehydration and their corresponding A_{420nm} at 7h and viability**
625 **percentage.**

Disrupted gene	Predictive function	A_{420nm} at 7 h 2 repetitions	Viability % 3 repetitions	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	23 ± 3	+
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_0733	Hypothetical protein	1.74 / 1.75	31 ± 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_0806	Hypothetical protein	1.46 / 1.59	29 ± 3	+
LSEI_0934	DNA-binding response regulator	1.48 / 1.59	28 ± 4	+
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 ± 6	+++
LSEI_1324	Membrane metal-binding protein	1.70 / 1.63	33 ± 3	+
LSEI_1468	Ribonucleotide diphosphate reductase	1.10 / 1.11	4 ± 1	+++
LSEI_1539	ppGpp	1.35 / 1.56	29 ± 5	+
LSEI_1659	Glucokinase	1.65 / 1.66	25 ± 8	+
LSEI_1709	DNA polymerase I	1.65 / 1.60	28 ± 6	+
LSEI_1754	SAICAR synthase	1.75 / 1.46	23 ± 4	+
LSEI_2505	Phosphate-starvation-inducible protein	1.71 / 1.75	32 ± 3	+
LSEI_2725	Sorbitol transcription regulator	1.74 / 1.70	27 ± 5	+
LSEI_A05	PTS cellobiose-specific	1.59 / 1.44	31 ± 2	+

626 Cells in stationary growth phase were air dried for 24 h at 25% RH and 25°C with 50 g/L
627 lactose solution in microplates or on PP coupons. Survival was determined by monitoring
628 A420nm of dried cells suspended in BCP medium. Mutants were classified into three
629 categories according to their viability: + (mean viability at least 1.2-fold less), ++ (mean
630 viability at least 2-fold less) and +++ (mean viability at least 3-fold less than WT.

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631 **Table 3. Operon organization and specificity of the 24 genes involved in adaptation to**

632 **pronounced desiccation followed by rapid rehydration.**

Gene	Predictive function	Putative operon	DNA strand	Specificity
LSEI_0040	Hypothetical protein	No	(+)	*L.casei/paracasei/rhamnosus*
LSEI_0167	Diadenosine tetraphosphatase	No	(-)	*L.casei/paracasei/rhamnosus*
LSEI_0178	PTS	0178-0180	(+)	*L.casei/paracasei/rhamnosus*
LSEI_0238	PST transporter	0238-0240	(+)	*L.casei/paracasei/rhamnosus*
LSEI_0363	Pyridoxine 5'-phosphate oxidase	No	(-)	*Lactobacillus*
LSEI_0397	NAD (FAD)- dehydrogenase	No	(-)	*Lactobacillus*
LSEI_0733	Hypothetical protein	0731-0733	(-)	Gram +
LSEI_0749	Cation transport ATPase	No	(-)	*Lactobacillus*
LSEI_0756	Alpha/beta fold family hydrolase	No	(+)	*Lactobacillus*
LSEI_0758	Aldo/keto reductase related enzyme	0757-0758	(-)	Gram +
LSEI_0806	Hypothetical protein	0805-0806	(+)	*L.casei/paracasei/rhamnosus*
LSEI_0934	DNA-binding response regulator	0933-0941	(+)	*Lactobacillus*
LSEI_1009	Spermidine/putrescine-binding protein	1005-1009	(+)	Gram +
LSEI_1045	Hypothetical protein	No	(-)	*L.casei/paracasei/rhamnosus*
LSEI_1316	Hypothetical protein	1314-1319	(+)	*L.casei/paracasei/rhamnosus*
LSEI_1324	Membrane metal-binding protein	1320-1326	(+)	*L.casei/paracasei/rhamnosus*
LSEI_1468	Ribonucleotide reductase	1467-1470	(+)	Gram +
LSEI_1539	ppGpp synthase	1537-1539	(-)	Gram +
LSEI_1659	Glucokinase	1658-1661	(-)	Gram +
LSEI_1709	DNA polymerase	1704-1709	(-)	Gram +
LSEI_1754	SAICAR synthase	1746-1756	(-)	Gram +
LSEI_2505	Phosphate-starvation-inducible protein	No	(+)	*L.casei/paracasei/rhamnosus*
LSEI_2725	Sorbitol transcription regulator	2720-2726	(-)	*L.casei/paracasei/rhamnosus*
LSEI_A05	PTS	A04-A06	(-)	*L.casei/paracasei/rhamnosus*

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636 **Table 4. *L. paracasei* mutants sensitive to pronounced desiccation followed by**
637 **progressive rehydration and their corresponding viability percentage among mutants**
638 **identified as sensitive to pronounced desiccation followed by rapid rehydration.**

Disrupted gene	Predictive function	Viability % 3 repetitions
WT		**99 ± 3**
LSEI_0040	Hypothetical protein	86 ± 7
LSEI_0167	Diadenosine tetraphosphatase	60 ± 0
LSEI_0178	PTS	82 ± 7
LSEI_0397	NAD (FAD)-dependent dehydrogenase	41 ± 6
LSEI_0749	Cation transport ATPase	57 ± 4
LSEI_1468	Ribonucleotide diphosphate reductase	14 ± 2
LSEI_1754	SAICAR synthase	87 ± 8

639 Cells in stationary growth phase were air dried for 24 h at 25% RH and 25°C with 50 g/L
640 lactose solution on PP coupons. Progressive rehydration was performed in a closed chamber
641 with RH adjusted at 99% for 2 h and the addition of water to obtain the mass before drying.
642 Viability percentages were determined by suspending dried cells with BCP medium.

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653 **Table 5. Relative transcript level of *L paracasei* genes after desiccation (1 h and 2 h) and**
654 **rehydration (rapid or progressive) for the 24 genes identified after library mutant**
655 **screening.**

Gene	Fonction	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_0733	Hypothetical protein	1.1 ± 0.1	-1.1 ± 0.2	1.3 ± 0.2	1.1 ± 0.1	1.2 ± 0.3
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_0806	Hypothetical protein	-1.1 ± 0.1	1.7 ± 0.5*	2.3 ± 0.3*	2.0 ± 0.2*	2.4 ± 0.4*
LSEI_0934	DNA-binding response regulator	-1.1 ± 0.1	1.2 ± 0.2	1.6 ± 0.2*	1.2 ± 0.1	1.4 ± 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_1324	Membrane metal-binding protein	-1.1 ± 0.1	-1.4 ± 0.2	-1.3 ± 0.1	-1.4 ± 0.2*	-1.3 ± 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_1539	ppGpp synthase	1.0 ± 0.1	-1.3 ± 0.2	-1.1 ± 0.1	-1.1 ± 0.1	-1.3 ± 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_1709	DNA polymerase	1.0 ± 0.2	1.0 ± 0.3	1.2 ± 0.2	1.2 ± 0.1	1.3 ± 0.3
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_2505	Phosphate-starvation-inducible protein	-1.1 ± 0.1	-1.1 ± 0.4	1.8 ± 0.2*	1.2 ± 0.2	1.1 ± 0.3
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
LSEI_A05	PTS	1.0 ± 0.1	3.5 ± 0.2*	4.1 ± 0.2*	2.3 ± 0.1*	2.1 ± 0.3*
Total of genes differentially expressed		**0**	**10**	**13**	**13**	**11**

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656 LAC (cells incubated 15 min with lactose), D1 (cells dehydrated for 1 h), D2 (cells
657 dehydrated for 2 h), RR (rapid rehydration with 1 mL of distillated water), PR (progressive
658 rehydration in a closed chamber with RH adjusted at 99% for 2 h). RTL were calculated using
659 $2^{-\Delta\Delta Ct}$ method. For the phosphate buffer control condition, a gene expression value of 1.0 was
660 attributed and RTL of genes in stress condition were calculated as a function of this value.
661 Positive values (> 1.0) represent upregulation and negative values (< 1.0) represent
662 downregulation. *, significant changes in gene expression ($p<0.05$) compared to the
663 phosphate buffer condition (4 biological replicates). Values in light red correspond to
664 upregulation and in dark red to upregulations > 2.0; values in light blue correspond to
665 downregulation and in dark blue to down regulation < -2.0.

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689 **Table 6. Primers designed for RT-qPCR.**

Primers	Sequence (5' → 3')	Primers	Sequence (5' → 3')
0040-F	CCACAAATGAGTCAAACCATCT	0040-R	GGTCGTTATCGCACCTAATGA
0167-F	ATGCCGACTGCTGTTGAGTT	0167-R	CGGACCCACATTCGATTAAA
0178-F	TAGGCAACATTCGCAGTCAA	0178-R	ATAATCGGCAACGGCTTTC
0238-F	CGGATCGCTTCCCTTATTCT	0238-R	CCAACATCTGTGAGCCAGAC
0363-F	GGTCCCAAGGGTTCACTACA	0363-R	GCCAAATGGTTGTTCGAGAG
0397-F	GGCACGACGCAGGATTAT	0397-R	CCACGCATCAAGAAGACATT
0733-F	AATTGCTGGCTGGCAGTATC	0733-R	GTTTGAAATCCCGGTCGTAA
0749-F	ATGACTGGTGACGGTGTGAA	0749-R	AACGAAATCCGCAATAGAGC
0756-F	CACTGACAGCCACGATCAAA	0756-R	CTTGCTGCCGTGAATAAACA
0758-F	TGATTCAGGCACAACGATTT	0758-R	GCGTCACGTCGGTTTCTTTA
0806-F	GCTAGTGCCTTAGCCACCAC	0806-R	CGATCTGCCAAGTCTTCAGA
0934-F	GCCTAGAACTCGGAGCAGAT	0934-R	GTTTGCGGCTGACTCTGACT
1009-F	CAGGTGAAGCCAGTGAGATG	1009-R	CCGTCTTAGGGATGACCAAGT
1045-F	CAATACACAGACCGCCAGTG	1045-R	ATCGTTGGCTTTGTCGTCTT
1316-F	CGCCGCATGAAGTATGTTTA	1316-R	GAGGGCTCAACACGTTTAACA
1324-F	CCACTTGGTGCTGGATTGT	1324-R	AATAAGTCAAGCCCGCTGAA
1468-F	CAATGGTTCGTGCTTTGACTT	1468-R	TGCTGTGAGCCTGGTGATTA
1539-F	ACAAACATCAGCAGCCACAA	1539-R	CATGCAACACTTCGCAAGAT
1659-F	TGGATGAAGGCAGTCACATC	1659-R	CCCATACCGATACCGACAAA
1709-F	CAAGTGCCAGAAGACGATAAG	1709-R	TCCAAATCACCAGTCAAATCAG
1754-F	CCGCCAATCCACGAATACTA	1754-R	GCACTTGCTTGAGTTGTTCAG
2505-F	TGCTGGTTCGCTACATTGAG	2505-R	ACGAGCAGTTGGCGTAAGAT
2725-F	CAGATCGACCATTGACACACA	2725-R	GCCGATGCCCTTACCTTAGT
A05-F	TTCTGGTTCTGTGGTGTCCA	A05-R	ATGTTGCCCTGCCTGATA
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

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692 **Figure legends**

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

696 **Figure 2. Evolution of the cell suspension mass during desiccation (orange curve) and**
697 **rehydration (blue curve) on a polyvinylidene membrane**. Sampling times for RNA
698 extractions are represented by the grey boxes: LAC (1 mL of cells incubated 15 min with
699 lactose before drying), D1 (cells dried 1 h, partial desiccation), D2 (cells dried 2 h,
700 pronounced desiccation), RR (rapid rehydration with 1 mL of distillated water), PR
701 (progressive rehydration in a closed chamber with RH adjusted at 99% for 2 h and addition of
702 water to obtain the mass before drying).

703 **Figure 3. Schematic representation of genes differentially expressed in *L. paracasei***
704 **during desiccation and rehydration**. Gene expressions are represented by color boxes in
705 light red for upregulation, dark red for upregulations > 2.0, light blue for downregulation,
706 dark blue for downregulation < -2.0 and in white for constitutive regulation. For each gene,
707 boxes from left to right correspond to: lactose incubation (LT), desiccation for 1h (D1),
708 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
$= 0.91 \pm 0.08$

Resistant mutants
A_{420} at 7h > 1.07
(2 biological replicates)

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

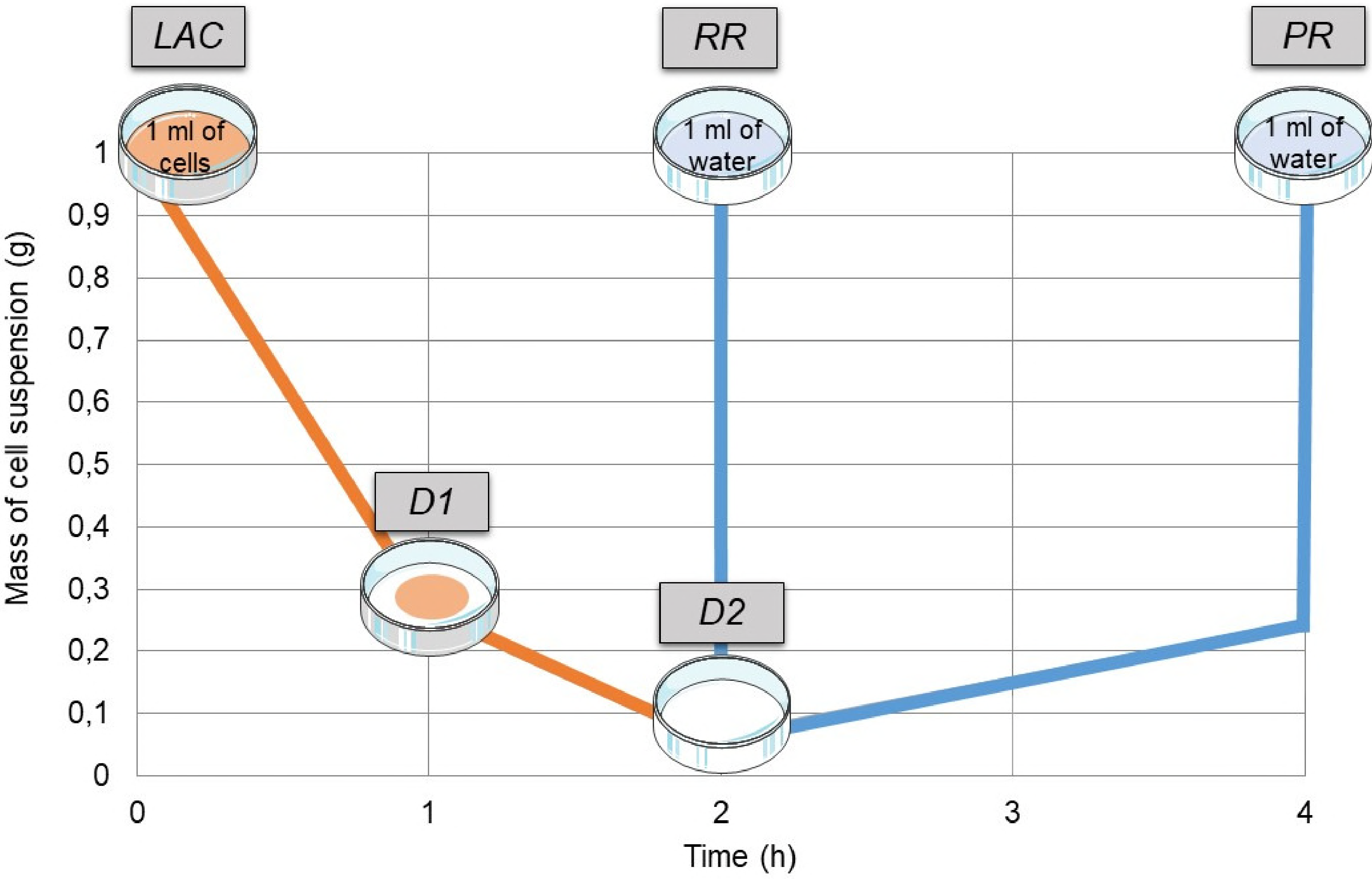
WT survival on PPC
Percentage survival
$= 41 \pm 2$ %

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

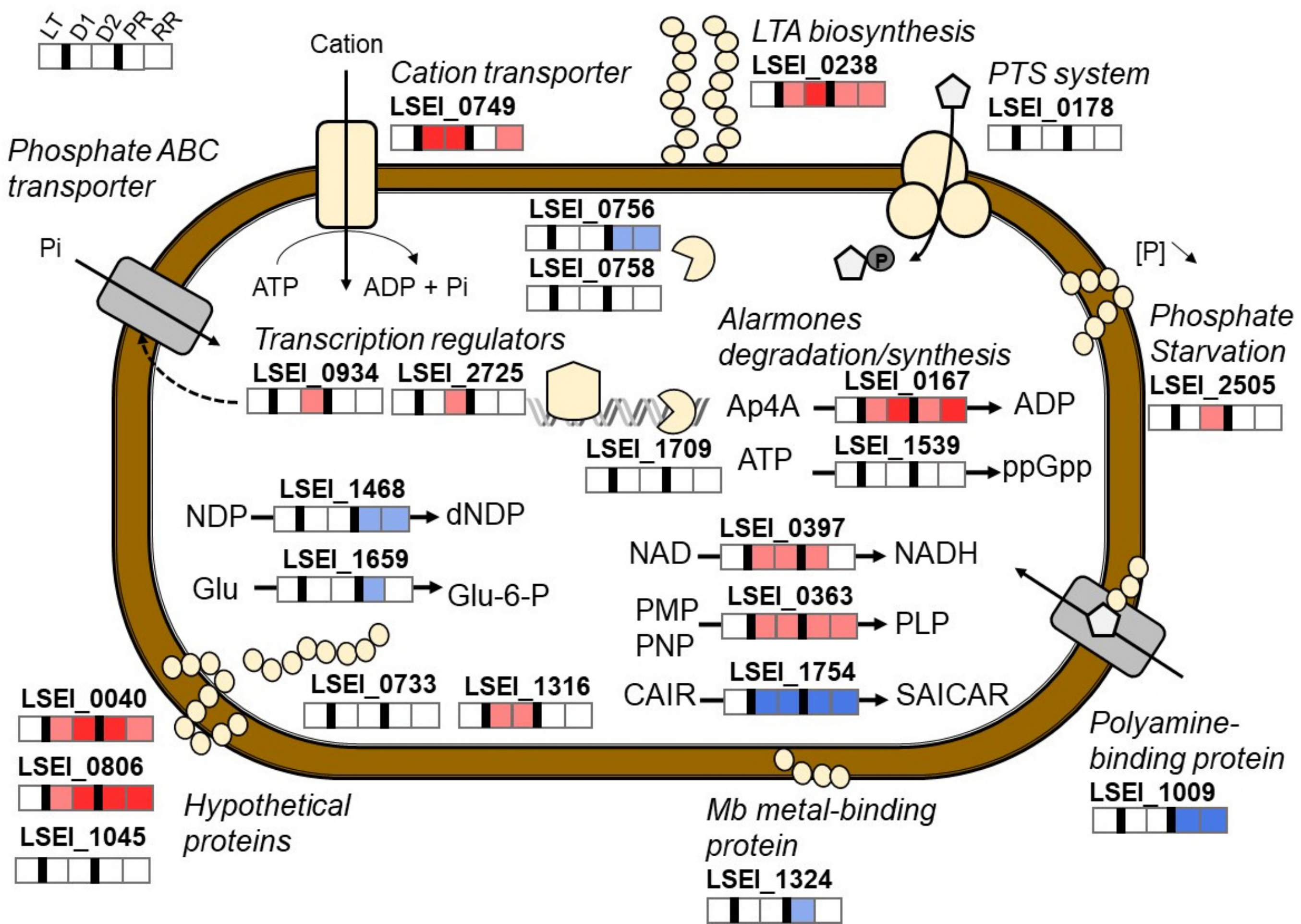
Identification of genes
Involved in dehydration and rehydration phases

WT
RNA extraction in the different phases

RT-qPCR
Expression of genes in the different phases



LAC
RR
PR
1 ml of cells
1 ml of water
1 ml of water
D1
D2
Mass of cell suspension (g)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
1
2
3
4
Time (h)



LT
D1
D2
PR
RR
Cation
Cation transporter
LSEI_0749
LTA biosynthesis
LSEI_0238
PTS system
LSEI_0178
Phosphate ABC transporter
Pi
ATP
ADP + Pi
LSEI_0756
LSEI_0758
P
[P]
Phosphate Starvation
LSEI_2505
Alarmones degradation/synthesis
Transcription regulators
LSEI_0934
LSEI_2725
Ap4A
LSEI_0167
ADP
LSEI_1709
ATP
LSEI_1539
ppGpp
NDP
LSEI_1468
dNDP
Glu
LSEI_1659
Glu-6-P
NAD
LSEI_0397
NADH
PMP
PNP
LSEI_0363
PLP
CAIR
LSEI_1754
SAICAR
LSEI_0733
LSEI_1316
LSEI_0040
LSEI_0806
LSEI_1045
Hypothetical proteins
Mb metal-binding protein
LSEI_1324
Polyamine-binding protein
LSEI_1009