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

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Abstract

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

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

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

1. 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 (Potts, 1994). Desiccation leads to water exit from cells, induces structural modifications, and causes osmotic and oxidative stresses (Dupont et al., 2014; França et al., 2007; Potts, 1994). In addition, the rehydration phase could lead to membrane alterations (Mille et al., 2003). 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 (Billi and Potts, 2002; Esbelin et al., 2018; Pointing and Belnap, 2012; Potts, 1994). In many organisms, desiccation survival is correlated with the accumulation of protective molecules, in particular trehalose (Crowe et al., 1998). However, desiccation tolerance involves other mechanisms that are mostly unknown except for extreme desiccation tolerant bacteria (anhydrobiotes) such as such as cyanobacteria (Potts, 1999), yeast (Dupont et al., 2014), resurrection plants (Crowe, 2014) and microscopic animals (Wełnicz et al., 2011).

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 (Chen and Jiang, 2017; Deng et al., 2012; Finn et al., 2013; Gruzdev et al., 2012; Li et al., 2012; Maserati et al., 2017). 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 (Cytryn et al., 2007; Higo et al., 2007; LeBlanc et al., 2008) 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 mutagenesis approaches were developed to identify genes for resistance to desiccation in *Listeria monocytogenes* (Hingston et al., 2015) and *Salmonella enterica* (Mandal and Kwon, 2017). For *L. monocytogenes* screening, genes were involved in energy production, membrane transport, amino acid metabolism, fatty acid metabolism and oxidative damage control. In *S. enterica*, in comparison to previous transcriptional studies, several genes were related to amino acid metabolism and more than 20% encoded hypothetical proteins. Recently, changes in proteomic expression were investigated for *S. enterica* during desiccation and rehydration (Maserati et al., 2018). The proteins with higher expression levels in dried samples were mainly ribosomal proteins whereas flagellar proteins, membrane proteins, and export systems as well as stress response proteins were identified in rehydrated samples.

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

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

Considering the importance of the *L.casei/paracasei* group for health and industrial applications, we compiled a non-redundant, annotated transposon mutant library of *L. paracasei* (Licandro-Seraut et al., 2014; Scornec et al., 2014) based on the P_{junc}-TpaseIS*1223* system, a random mutagenesis tool specifically designed for the *Lactobacillus* genus (Licandro-Seraut et al., 2012). Recently, using this approach we determined five *Lactobacillus pentosus* mutants sensitive to olive brine due to multifactorial stress (Perpetuini et al., 2013). In addition, new genetic determinants were identified during the early stage of *L. paracasei* establishment in the gut (Licandro-Seraut et al., 2014) and during monofactorial perturbations of mild intensities (Palud et al., 2018). In the present work, the *L. paracasei* transposon mutant library was screened to identify genes involved in the adaptation of this LAB to desiccation and rehydration. The expression of corresponding genes was studied by RT-qPCR during the two-step process to draw a chronological transcriptional profile during hydric fluctuations.

2. Materials and Methods

2.1. Strains and growth conditions

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

were mixed (30 s, 700 rpm, Eppendorf MixMate) and 10 µL of each well was used to inoculate 190 µL of MRS in new 96-well plates. Individual mutants were grown in tubes for 48h (2 ml) and vortexed to inoculate at a dilution of 1/100 in new tubes. Plates and tubes were incubated for 24h at 37°C to obtain cells in stationary phase (concentration between $1.0x10^9$ and $2.0x10^9$ UFC/mL) because it was the most convenient to obtain the same growth phase for the 1287 mutants, and thus to compare viability after drying.

2.2. Drying and rehydration conditions

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

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

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

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

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

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

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

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

2.7.Bioinformatics

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

2.8.RT-qPCR

The transcriptional analysis was performed using mid-exponential growth phase cells (OD_{600} between 0.5 and 0.6) which are the most metabolically active and which generally present the strongest and the fastest gene regulation. Cells were centrifuged and concentrated to an OD_{600} of 20. Cell pellets were suspended with phosphate buffer (10 mM pH 6.5) supplemented with 50 g/L lactose except for control. The suspension was incubated for 15 min at 25°C and 1 mL was deposited on a 0.22 µm polyvinylidene membrane in a glass Petri dish to prevent cell adhesion**.** Cells were dried in ventilated chambers and RNA extractions were performed at two different stages of desiccation: partial desiccation D1 (76% of water evaporated after 1 h) and pronounced desiccation D2 (96% of water evaporated after 2 h) which corresponded to completely dried cells (Figure 2). As rehydration kinetics is decisive for bacterial survival, rapid and progressive rehydration were applied for RNA extractions after 2 h. Rehydrated cells were subsequently detached from the membrane using a cell lifter. Total RNA isolation, cDNA synthesis and qPCR were performed as previously described (Licandro-Seraut et al., 2008) using TRI Reagent (Sigma Aldrich), DNase I (Roche), iScript™ Reverse Transcription Supermix (Bio-Rad) and SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad). Primers were designed by using Primer3Plus (Untergasser et al., 2007) (Table 1). Quantitative PCR were performed using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) in triplicate, in a 20 µL-reaction mixture. Cq (threshold value) calculation was determined by a regression model of the CFX Manager™ Software. The relative transcript levels of genes were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). In order to select appropriate reference genes, 10 potential housekeeping genes (*fusA, ileS, lepA, leuS, mutL, pcrA, pyrG, recA, recG* and *rpoB*) (Landete et al., 2010) were tested with all the experimental conditions and analyzed using the CFX Manager™ Software. The genes *fusA*, *lepA* and *rpoB* were selected as the references because they displayed the lowest M values (0.22) and

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

3. Results

3.1. Selection of a protective drying solution for the screening

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

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

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

for a putative ribonucleotide diphosphate reductase (LSEI_1468) and for a hypothetical protein (LSEI_ 1316). Two mutants disrupted for a putative PTS (LSEI_0178) and for a putative alpha/beta fold family hydrolase (LSEI_0756) were categorized as ++. Nine genes out of the 16 belonged to putative operons (Table 4). Seven genes were specific to *L. paracasei* or related species, four to *Lactobacillus* genus and five were well conserved among Gram (+) bacteria. Two genes among the three encoding hypothetical proteins presented a transmembrane domain (LSEI_0040 and LSEI_1045). All hypothetical protein genes were specific to the *L. paracasei* group.

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

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

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

To assess if the identified genes were involved during dehydration whatever the rehydration 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 ± 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

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

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

4. Discussion

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

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

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

4.1. Metabolism and transport

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

Next, we identified a membrane associated protein (LSEI_1009) predicted to bind polyamines before their transport into the cell by the ABC transporters encoded by other genes of the 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

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

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

4.4. DNA related enzymes

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

Author Contributions

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

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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	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
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	ATCGTTGGCTTTGTCGTCTT
1316-F	CGCCGCATGAAGTATGTTTA	1316-R	GAGGGCTCAACACGTTTAACA
1468-F	CAATGGTTCGTGCTTTGACTT	1468-R	TGCTGTGAGCCTGGTGATTA
1659-F	TGGATGAAGGCAGTCACATC	1659-R	CCCATACCGATACCGACAAA
1754-F	CCGCCAATCCACGAATACTA	1754-R	GCACTTGCTTGAGTTGTTCAG
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	(+)	*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_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_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_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	(-)	*L.casei/paracasei/rhamnosus*

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 $2^{-\Delta\Delta Ct}$ 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

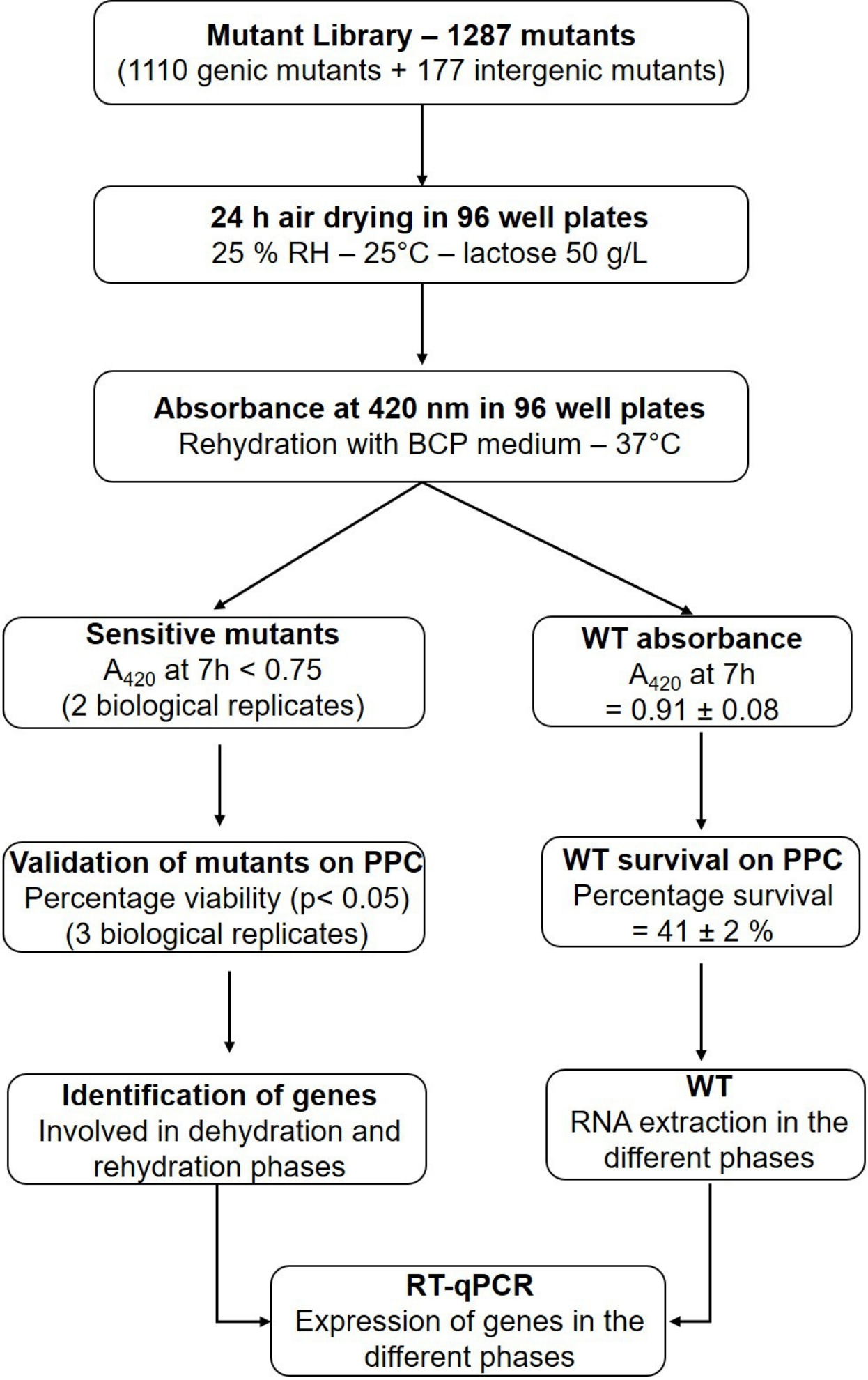
upregulation and in dark red to upregulations > 2.0; values in light blue correspond to downregulation and in dark blue to down regulation < -2.0.

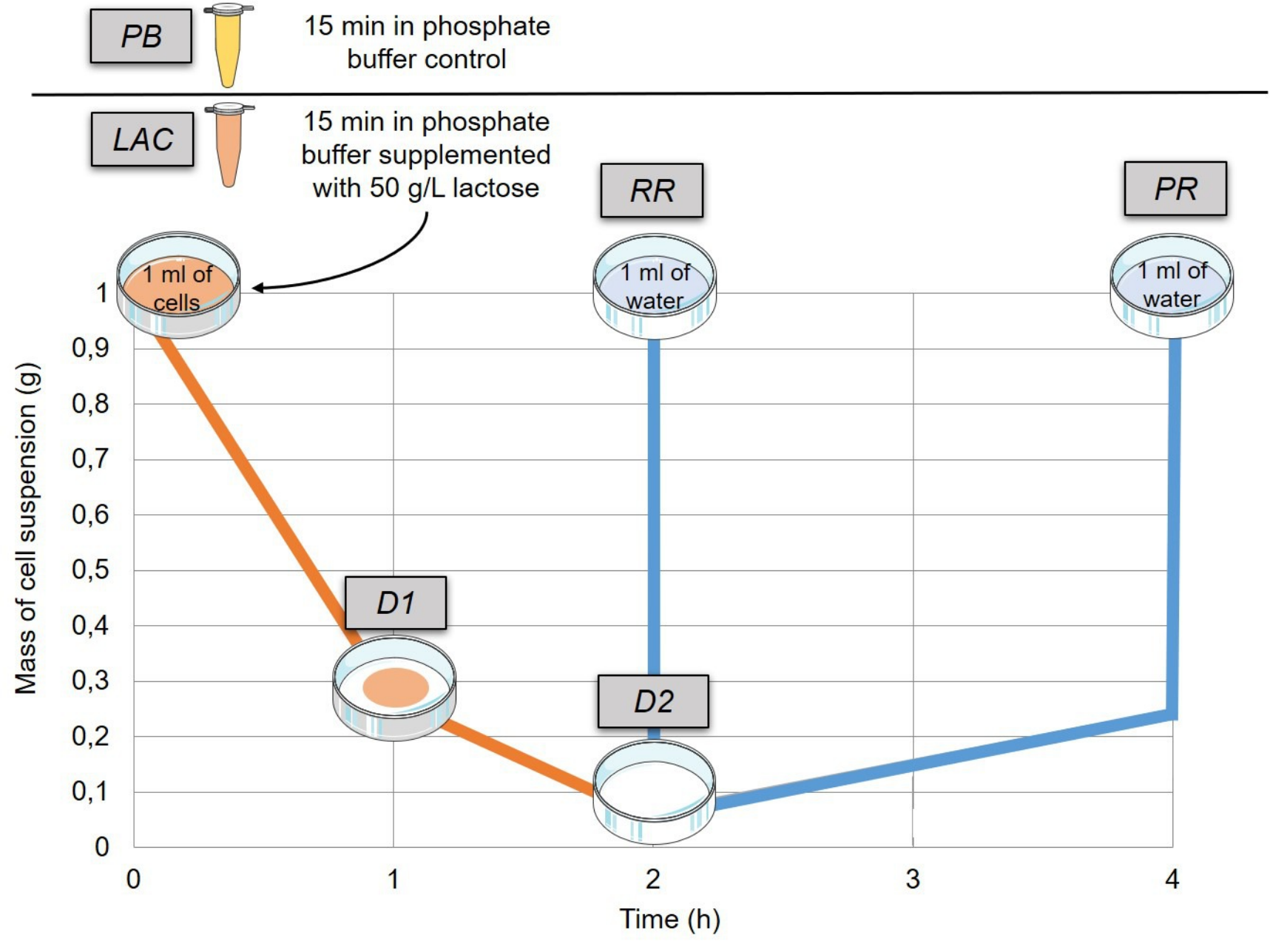
Figure legends

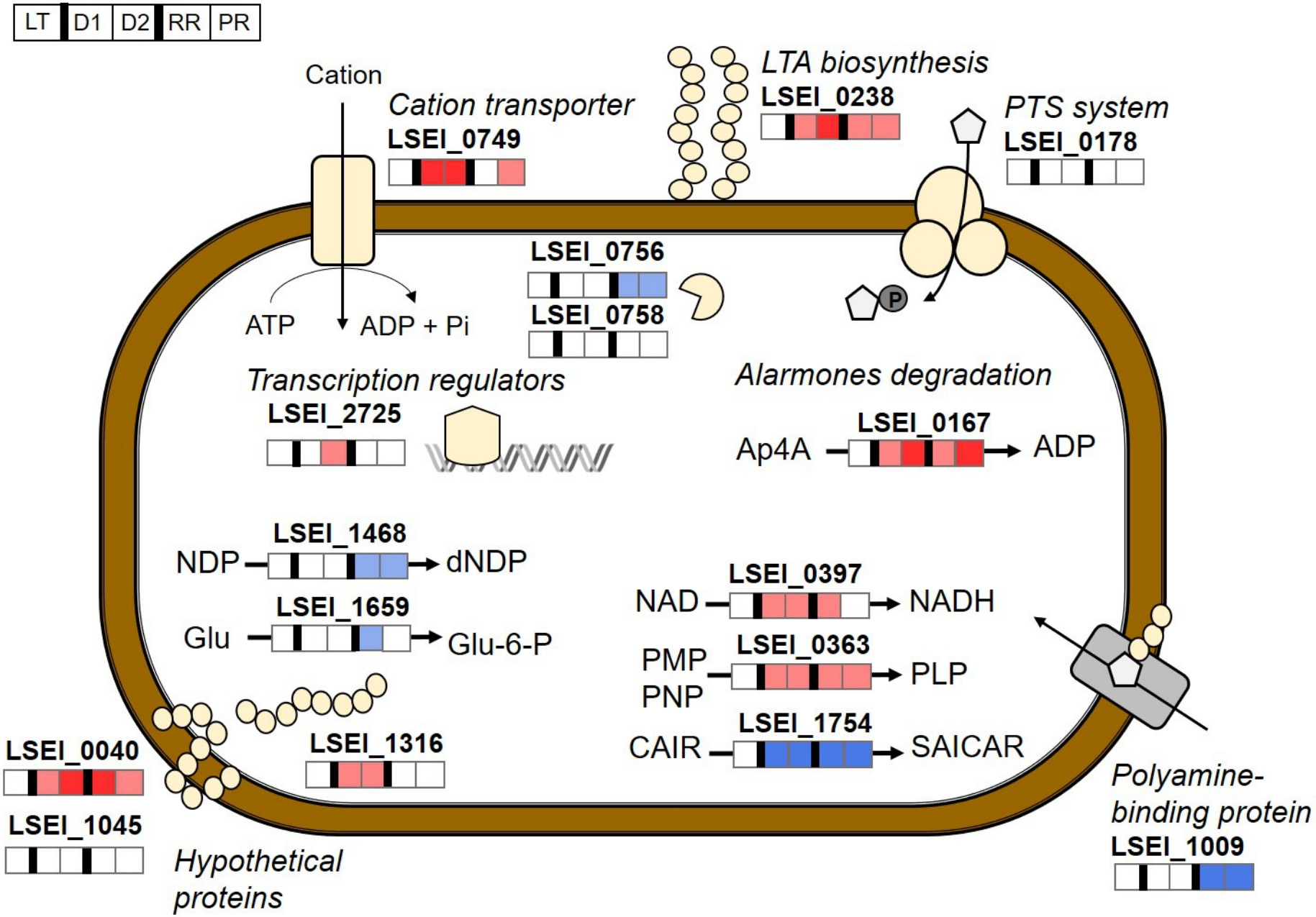
Figure 1. Organizational chart of the strategy developed in this work to identify genetic determinants of *L. paracasei* resistance to desiccation and rehydration. PPC, polypropylene coupons.

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


LT
D1
D2
RR
PR
Cation
Cation transporter
LSEI_0749
LTA biosynthesis
LSEI_0238
PTS system
LSEI_0178
LSEI_0756
LSEI_0758
ATP
ADP + Pi
P
Transcription regulators
LSEI_2725
Alarmones degradation
Ap4A
LSEI_0167
ADP
LSEI_1468
NDP
dNDP
LSEI_1659
Glu
Glu-6-P
LSEI_0397
NAD
NADH
PMP
PNP
LSEI_0363
PLP
LSEI_1754
CAIR
SAICAR
LSEI_1316
LSEI_0040
LSEI_1045
Hypothetical proteins
Polyamine-binding protein
LSEI_1009