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# Analysis of the Genome Architecture of *Lacticaseibacillus paracasei* UNQLpc 10, a Strain with Oenological Potential as a Malolactic Starter

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**Abstract:** The *Lacticaseibacillus paracasei* UNQLpc 10 strain was isolated from a Malbec wine produced in North Patagonia, Argentina, and identified by 16S rRNA gene sequencing. The aim of this work was to obtain the fully assembled genome of the UNQLpc 10 strain, analyze its structure, and evaluate the possible functions of the predicted genes with regard to its oenological potential as a malolactic starter. UNQLpc10 is the first whole assembled genome of an oenological strain of *Lcb. paracasei* reported in databases. This information is of great interest inexpanding the knowledge of diversity of oenological lactic acid bacteria and in searching for new candidate species/strains to design starter cultures. The in silico genome-wide analysis of UNQLpc 10 confirms the existence of genes encoding enzymes involved in the synthesis of several metabolites of oenological interest, and proteins related to stress responses. Furthermore, when UNQLpc 10 was incubated in synthetic wine, it exhibited a very good survival and L-malic acid consumption ability.

**Keywords:** *Lacticaseibacillus paracasei*; wine; whole-genome sequencing analysis; cell survival and malic acid consumption



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## 1. Introduction

Malolactic fermentation (MLF) is the process responsible for the conversion of L-malic acid to L-lactic acid and $CO_2$, which reduces total acidity, improves biological stability and modifies the aroma profile of wine [1]. MLF takes place spontaneously during or after alcoholic fermentation (AF) and is carried out by one or more species of indigenous lactic acid bacteria (LAB) present in grapes and cellars [2–5]. The use of starter cultures of selected native LAB from each oenological region takes advantage of the natural adaptation of strains to the characteristics of wine, and maintains the regional *terroir* [1,6].

The LAB *Lacticaseibacillus paracasei* (formerly named *Lactobacillus paracasei*) [7] is a Gram-positive and facultative heterofermentative lactobacillus (non-spore-forming, rod-shaped, anaerobic bacterium). This bacterium is a member of the normal human and animal gut microbiota and is extensively used in the food industry as a starter culture of dairy products or as a probiotic. Strains of this species have also been isolated from a variety of fermented artisanal products such as fermented milk, cheese, sourdough bread, and fermented vegetables [8].

Although *Lactiplantibacillus plantarum* and *Oenococcus oeni* are the predominant species in MLF, the presence of *Lcb. paracasei* strains in wines from different geographical regions has been described by several authors [9–11]. Valdés La Hens et al. [9], for example, evaluated the LAB diversity in a Patagonian red Merlot wine in which MLF was spontaneous, and identified the species *Lcb. paracasei*.In addition, du Plessis et al. [10] reported the

presence of *Lcb. paracasei* in South African brandy-base wines, and Kačániová et al. [11] identified *Lcb. paracasei* strains, among other LAB, in grape, must, and wine samples collected from vineyards in Slovakia. More recently, López-Seijas et al. reported *Lcb. paracasei* as one of the predominant strains during MLF in Alvariño wines from Spain [12].

Since 2008, our research group has been studying native LAB from Patagonian red wines, looking for suitable strains to develop malolactic starter cultures, well adapted to the local winemaking practices and able to improve the fermentation process and enhance the quality and safety of wine. The key selection criteria that should be considered when designing effective malolactic starters are stress resistance, technological performance, and safety [13].

The formulation of native malolactic starter cultures requires the characterization and propagation of strains proven to be suitable for this purpose, applying different selection criteria at each stage and considering the possible positive innovative impacts of these microbial resources on the quality and sensory and functional properties of the wine.

In previous studies, we isolated several strains from Patagonian wines produced at a commercial winery located in General Roca, Río Negro, Argentine Patagonia [9,14–17]. Patagonia is the southernmost wine-producing region of Argentina and one of the most southern regions in the world. This region has optimal agroecological conditions for high-quality viticulture and a long winemaking tradition.

In previous work, the UNQLpc 10 strain was isolated from a Patagonian Malbec wine, vintage 2016, in which the AF was conducted by a native F8 strain of *Saccharomyces cerevisiae* and a blend culture of selected Patagonian native strains of *Lpb. plantarum* and *O. oeni* was inoculated to guide MLF [18]. Interestingly, UNQLpc10 (from native microbiota) was one of the predominant strains during MLF together with the *Lpb. plantarum* and *O. oeni* strains, added as malolactic starters. UNQLpc10 was then identified as *Lcb. paracasei*.

To ensure accurate taxonomic assignment of LAB strains while also providing genetic data concerning possible metabolic activities, complete genome sequence can be obtained. In the case of starter cultures, this technique can provide information regarding positive traits beneficial for their technological application as well as negative ones that would disqualify them from application in food production. In recent years, numerous complete genomes have become more accessible, and several works have used this technique for the analysis of oenological LAB strains. For winemaking purposes, it is relevant to search genes that are involved in wine fermentation and flavor, such as those coding for resistance to stress conditions, synthesis, or production of odorant compounds, and production of biogenic amines (which are undesirable because they affect wine quality and acceptability) [19–22].

The aim of this work was to obtain the fully assembled genome of the UNQLpc 10 strain, analyze its structure, and evaluate the possible functions of the predicted genes with regard to its oenological properties and its ability to conduct the MLF. A core genome phylogeny was also used to determine the evolutionary distance between UNQLpc 10 and other 12 *Lcb. paracasei* strains from different fermented foods and beverages. In addition, cell survival and malic acid consumption (MAC) of the UNQLpc 10 strain were evaluated in conditions similar to those of the cellar.

## 2. Materials and Methods

### *2.1. Strain Information*

The *Lcb. paracasei* UNQLpc 10 strain was recovered from implantation control assays in a Patagonian Malbec wine vintage 2016, in which the AF was conducted by a native F8 strain of *Saccharomyces cerevisiae* and the MLF was guided by a blend culture of selected Patagonian native strains of *Lpb. plantarum* and *O. oeni* [18]. UNQLpc10 was identified by sequence analysis of the 16S rRNA gene, following Delfederico et al. [23].

### *2.2. Bacterial Growth and DNA Extraction*

UNQLpc 10 was grown in MRS broth (Biokar Diagnostics, Beauvais, France), at 28 °C, pH 6.5, in aerobic conditions, for two days. To obtain DNA, 1 mg/mL of lysozyme with 1% sodium dodecyl sulfate was used. Proteins were removed with 0.1 g/mL of proteinase K, followed by phenol-chloroform-isoamyl alcohol (25:24:1) extraction [22]. Then, 16 µg of high-quality genomic DNA was used for library preparation and sequencing.

### *2.3. Genome Sequencing, Assembly, and Bioinformatics Analysis*

A whole-genome shotgun library was constructed using a 20-kb SMRTbell version 1.0 template prep kit, followed by single-molecule real-time (SMRT) sequencing, conducted on an RS II (Pacific Biosciences) sequencer (Macrogen). A total of 967,156,791 base reads (31,688-fold coverage and a polymerase read N50 size of 20,860 bp), with an average length of 14,257 bp and an estimated accuracy of 85.6%, was used as input for the *de novo* assembly with the Canu package [24]. The Canu output consisted of a single circular contig without gaps. Coding sequences were predicted with Gene MarkS-2. Replication and terminus origins were identified using GC-skew analysis and ORF orientation shift. Genome annotation was done using the NCBI Prokaryotic Genome Annotation Pipeline. Protein function was predicted using Blast2GO 5.1.1 [25]. For exclusive presence/absence of genes in specific strains, a Bacterial Pan Genome Analysis Pipeline (BPGA) was used [25]. BPGA determines the core (conserved), accessory (dispensable), and unique (strain-specific) gene pool of a species. Finally, the database of Clusters of Orthologous Groups of proteins (COGs) was analyzed using WebMGA (BMC Genomics 2011, 12:444) [26].

### *2.4. Cell Survival and L-Malic Acid Consumption in Synthetic Wine*

#### 2.4.1. Cell Culture and Acclimation

The UNQLpc 10 strain was grown in MRS broth (Biokar Diagnostics, Beauvais, France) [27]. Bacterial cells at early stationary phase (approximately $10^9$ CFU/mL) were collected by centrifugation (10 min, at 5000× *g*) and suspended in the same volume of an acclimation medium (50 g/L MRS, 40 g/L D(-) fructose, 20 g/L D(-) glucose, 4 g/L L-malate, 1 g/L Tween 80, and 0.1 mg/L pyridoxine, adjusted to pH 4.6) supplemented with 6% *v*/*v* ethanol [28]. Cultures were incubated at 21 °C for 48 h and cells were harvested by centrifugation and then inoculated in a synthetic wine.

#### 2.4.2. Vinification Assays at Laboratory Scale

Acclimated and non-acclimated cells were inoculated ($1 \times 10^8$ CFU/mL) in a synthetic wine (5 g/L tartaric acid, 2.0 g/L L-malic acid, 0.6 g/L acetic acid, 2 g/L glucose, 2 g/L fructose, and 10 or 14.0% *v*/*v* ethanol), and incubated at 21 °C for 15 days. For each ethanol concentration, the pH values were adjusted to 3.6, using concentrated HCl (6 M). Viable cells were determined by plating on MRS agar and then sampling on days 0, 2, 4, 7, and 15. The remaining L-malic acid was measured with an L-malic acid enzymatic kit (L-Malic Acid Enology enzymatic kit, BioSystems SA, Barcelona, Spain). The percentage of L-malic acid consumed (MAC) on day 15 was calculated by Equation (1):

$$MAC\% = (100 - ([MA_f]\ 100/[MA_0]) \quad (1)$$

where $[MA_0]$ is the initial concentration of malic acid in the wine used and $[MA_f]$ is the final concentration of malic acid measured in the wine.

At the same time, an exponential one-phase equation model was used to fit the performed MAC kinetics during fermentation in synthetic wine. Equation (2) for this model was obtained by the GraphPad Prism® software(GraphPad SoftwareInc, San Diego, CA, USA):

$$MAC_t = (MAC_i - MAC_f)\ e^{-Kt} + MAC_f \quad (2)$$

where $MAC_t$ is the malic acid consumed at time = t in %, $MAC_f$ is the malic acid consumed of plateau equation and K is the kinetics rate constant.

### *2.5. Reproducibility of Results*

All determinations were the average of two independent replicate assays. Data are shown as mean values. The statistical analyses were carried out using GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA, 2007). Means were compared by one-way ANOVA, and if $p < 0.05$, the difference was considered statistically significant.

## 3. Results

### *3.1. General Features of the Genome*

Results showed that the complete genome of UNQLpc 10 (GenBank Accession code CP029686.1) consists of a single, circular chromosome (Figure 1), and no plasmid structures. Its main features obtained by in silico analysis are shown in Supplementary Materials Table S1. Results showed that both origins are located in almost symmetrical chromosome positions. The genes encoded by the UNQLpc 10 genome are predominantly transcribed in the direction of replication, which is a feature observed in many Gram-positive genomes with low G+C content [29].

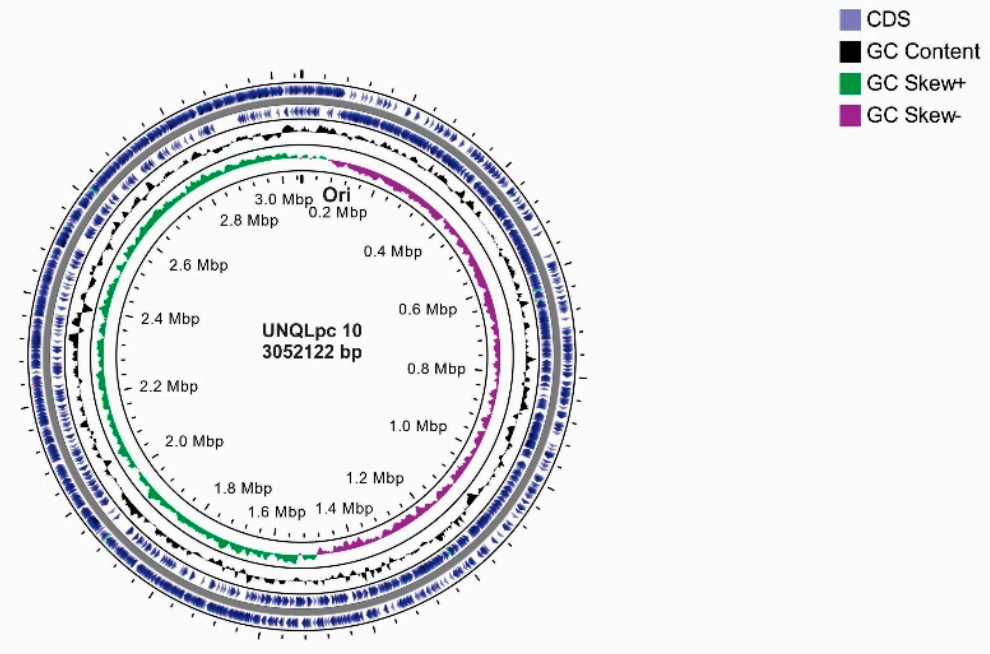


**Figure 1.** Chromosome atlas of the *Lacticaseibacillus paracasei* UNQLpc 10 strain generated using CGView v1.0 (Grant & Stothard, 2008). The GC content is illustrated in the black circle; the positive and negative GC skew is illustrated in the green and purple circles, respectively; and the CDS (Co Ding Sequence) is illustrated in the blue circle. Genome positions in kbp are also shown.

The results also showed that the UNQLpc 10 genome contains 91.0% of protein-encoding genes of the COG class involved in major metabolic pathways. These genes could be assigned to 25 functional categories. The remaining genes were cataloged as unknown functional proteins. COG data analysis showed that these genes belong mainly to the following categories: transcription, replication, recombination and repair, cell wall/membrane/envelope biogenesis, and amino acid transport and metabolism. The relative abundance of genes associated with general COG functional categories found in the UNQLpc 10 strain is shown in Figure 2.

As mentioned, to know the evolutionary distance between UNQLpc 10 and other 12 *Lcb. paracasei* strains from different fermented foods and beverages, we used a core genome phylogeny. Core genome phylogenies are widely used to build the evolutionary history of individual prokaryotic species [30].In the present study, the phylogeny produced from UNQLpc 10 and *Lcb. paracasei* strains isolated from different fermented foods showed that there is an evolutionary distance between a basal clade formed by ACUH35 (Koumis

isolation) and the other 12 strains of *Lcb. paracasei* (Figure 3). However, it is evident that the information contained in the genomes of the strains of the *Lcb. paracasei* species of fermented food origin cannot determine any other relationship between them.

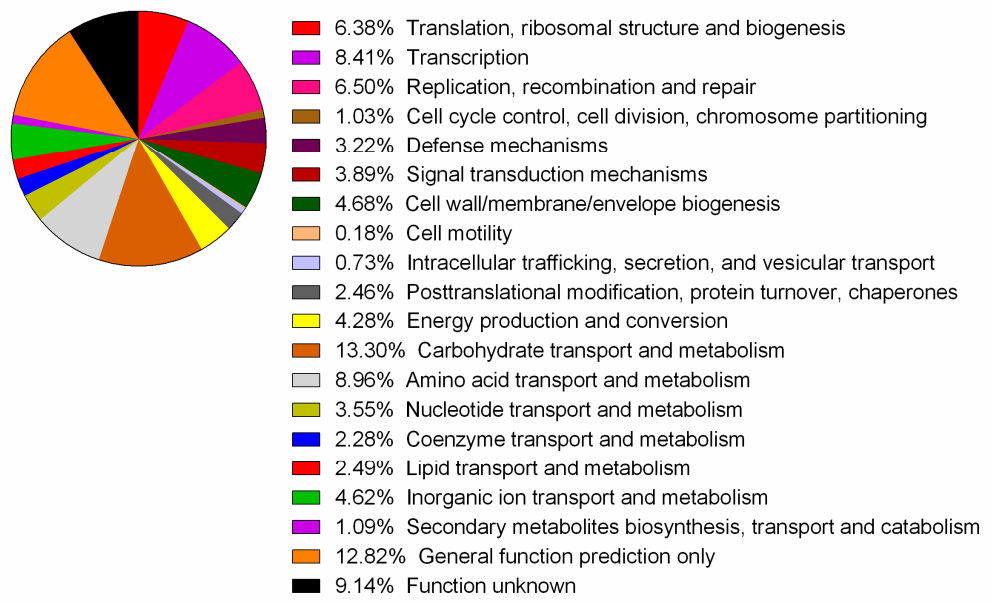


**Figure 2.** Relative abundance of genes associated with general clusters of orthologous groups(COG) functional categories in the UNQLpc10 strain.

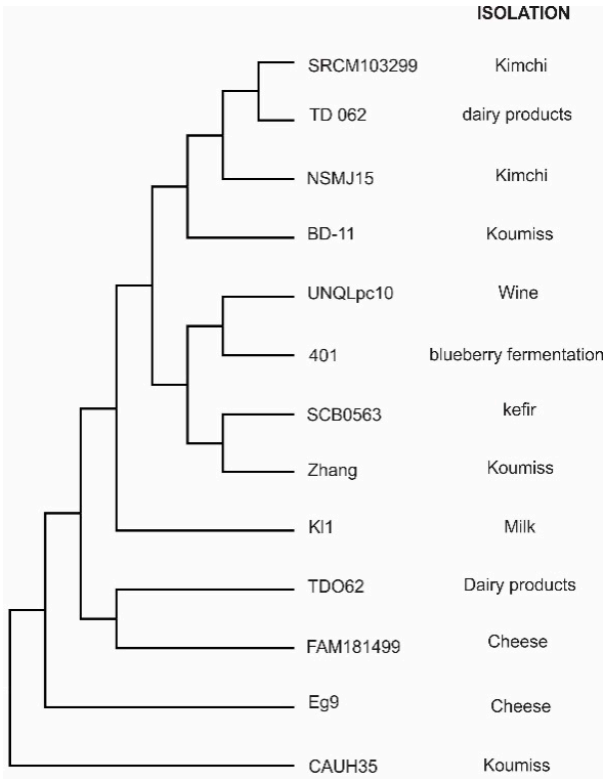


**Figure 3.** Core genome phylogenies, obtained by using the BGA software, from the gene content analysis of UNQLpc 10 (Patagonian red wine), and complete genomes of *Lcb. paracasei* strains isolated from different fermented foods (available from the NCBI GenBank database).

Finally, comparison of the protein-coding genes of UNQLpc 10 with those of the other 12 strains of *Lcb. paracasei* from different fermented foods, using the BPGA software,

showed that the genome of UNQLpc 10 contains 89 unique genes. A COG analysis of those unique genes is shown in Figure S1 of the Supplementary Materials.

### *3.2. Genes Encoding Enzymes Related to the Winemaking Process*

Table 1 shows the results of the in silico analysis of the UNQLpc 10 genome looking for genes coding enzymes related to the winemaking process. This analysis showed the presence of numerous genes related to carbohydrate transport and metabolism, including 28 phosphoenol–pyruvate sugar transferase systems (PTS) and four sugar-ABC transporters. Among the PTS systems, six families of PTS permeases were identified, namely PTS glucose–glucoside (Glc), PTS fructosemannitol (Fru), PTS lactose/cellobiose (Cel-Lac), PTS glucitol (Gut), PTS galactitol (Gat), and PTS mannose–fructose–sorbose (Man). Concerning the metabolic pathways related to carbohydrate metabolism, genes corresponding to whole phosphoketolase (PK) and glycolysis pathways were found. Furthermore, the UNQLpc 10 genome showed the presence of three transduction sites (locus tags = DMC16_04445, DMC16_09520, and DMC16_09845) for the L-lactate dehydrogenase protein, supporting the relevance of the pyruvate-dissipating ability in this strain.

**Table 1.** In silico analysis of the UNQLpc 10 genome looking for genes coding for enzymes of technological interest.

| Name | Function | Genes in UNQLpc10 |
|---|---|---|
| **Sugar Metabolism** | | |
| Phosphoenolpyruvate | Carbohydrate transport and metabolism | 28 |
| Sugar-ABC transporters | Mainly mediate the transport of nutrients and other molecules into cells or the pumping of toxins and lipids across membranes [31] | 4 |
| L-lactate dehydrogenase | Participates in anaerobic energy metabolism, reducing pyruvate (from glycolysis) to regenerate $NAD^+$ | 3 |
| **Related Enzymes of Flavor Development** | | |
| Branched-chain aminotransferase | Conversion of valine, leucine, and isoleucine into keto acid components. The keto acids are then further converted into aldehydes, alcohols, and esters, which are important aroma compounds [32] | 1 |
| Aromatic aminotransferase | Conversion of tyrosine, tryptophan, and phenylalanine into Keto acid components [32] | 1 |
| Aspartate transaminase | Conversion of aspartate into keto acid components. | 1 |
| Glutamate dehydrogenase | Catalyzes the deamination of glutamate to oxoglutaric acid related to amino acid (branched-chain amino acids, aromatic amino acids, and methionine) degradation Pathway [32] | 1 |
| D-hydroxyacid dehydrogenase | Catalyzes the reduction of two keto branched-chain acids to hydro acids of interest in flavor formation [33] | 1 |
| Esterase | Catalyze the biosynthesis of esters derived from short-chain fatty acids [32] | 1 |
| Homoserine dehydrogenase | Has homoserine trans-acetylase activity and involved in the Biosynthesis of methionine [32] | 1 |
| Homoserine kinase | Involved in the onset of methionine biosynthesis [32] | 1 |
| 6-phospho-beta-glucosidase | Hydrolytic activity in glycosylated compounds, acts on the glucosidic bonds β (1–4) [34] | 3 |

**Table 1.** *Cont.*

| Name | Function | Genes in UNQLpc10 |
|---|---|---|
| Alpha-glucosidase | Hydrolyticactivityonterminal, non-reducing(1→4)-linked alpha-D-glucose residues with release of D-glucose [34] | 5 |
| Malolactic enzyme | Involved in the malolactic fermentation of wine, which results in a natural decrease in acidity and favorable changes in wine flavors | 2 |
| **Other Enzymes of Oenological Interest** | | |
| Membrane intrinsic proteins | Regulate a large set of developmental and physiological processes and stress responses within cells. | 5 |
| Heat-shock genes | Environmental stress response | 8 |
| Ethyl stress response | Environmental stress response | 2 |

Two clusters of genes involved in L-malate metabolism were also identified. These clusters consist of three genes encoding a malolactic enzyme (mleS) (locus tags = AWR90332.1 and AWR91068.1) and an L-malate transporter (mleT) (locus tags = AWR90331.1 and AWR91067.1), and, oriented in the opposite direction, a LysR-type transcriptional regulator (mleR) (locus tag = AWR90333.1) or hypothetical protein (locus tag = AWR91069.1)(Figure 4). The last cluster is similar to those found in several *O. oeni* strains (Figure S2 Supplementary Material).

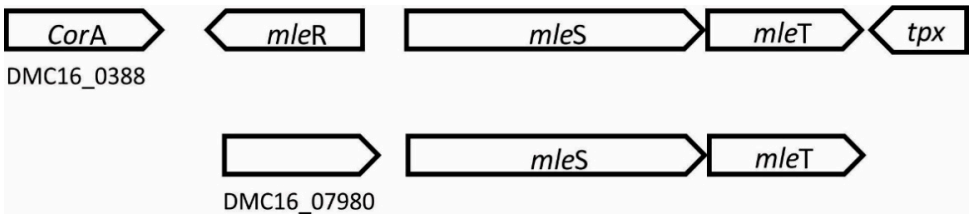


**Figure 4.** Schematic representation of the *mle* gene clusters in *Lcb. paracasei* UNQLpc 10.

Results also showed the presence of several genes encoding enzymes involved in the development of fruit flavor in red wines, including aminopeptidases, glutamate dehydrogenase, and phosphotransacylase [35] (Table 1), and three genes encoding β-glucosidase enzymes (locus tags = AWR90012.1, AWR91732.1, and AWR92112.1), and a typical citrate lyase gene cluster (citC, citD, citF, citX, citG) (locus tags = DMC16_13395, DMC16_13400, DMC16_13410, DMC16_13415, and DMC16_13435).

### *3.3. Genes Encoding Proteins Related to Stress Responses*

Bacteria protect themselves from changes in environmental osmolarity by using stretch-activated (or mechano-sensitive) channels that regulate a large set of developmental and physiological processes and stress responses within cells [22,36].The UNQLpc 10 strain showed the presence of genes encoding two channel protein families (the voltage-gated ion channel (locus tag = DMC16_03540) and the large conductance mechano-sensitive channel (locus tag = DMC16_07815)), as well as some aquaporins (locus tags = DMC16_03555, DMC16_04280, and DMC16_04830) of the membrane intrinsic protein(MIP) family, which may function primarily to protect the bacteria against osmotic stress [37]. Other genes involved in stress-tolerance mechanisms, including clpX (locus tag = DMC16_00815) and trxA (locus tag = DMC16_03605), were also identified. In addition, class I heat-shock genes (groES, groEL, and dnaK/dnaJ operons) (locus tags = DMC16_11375, DMC16_11380, DMC16_15210, and DMC16_15220), trxA and trxB homologs (involved in the disulphide-reducing pathway) (locus tags = DMC16_03605 and DMC16_02820), and HrcA and CtsR

(genes codifying proteins involved in the ethyl stress response) (locus tags = DMC16_15200 and DMC16_09980) were also identified.

### *3.4. Genes Encoding Proteins That Deteriorate Wine*

Our analysis also verified that the UNQLpc 10 genome does not contain genes encoding the enzymes histidine decarboxylase, putrescine carbamoyl transferase, or tyrosine decarboxylase, which are responsible for the synthesis of the main biogenic amines in wines (histamine, tyrosine, and putrescine).

### *3.5. Cell Viability and L-Malic Acid Consumption*

After the analysis and characterization of the genome structure of UNQLpc 10, it was interesting to confirm that this strain is able to survive and consume L-malic acid under hostile winemaking conditions (low pH and high content of ethanol). The performance of the UNQLpc 10 strain, with or without acclimation, was analyzed in synthetic wine. The number of viable cells and the MAC kinetics are shown in Figure 5.

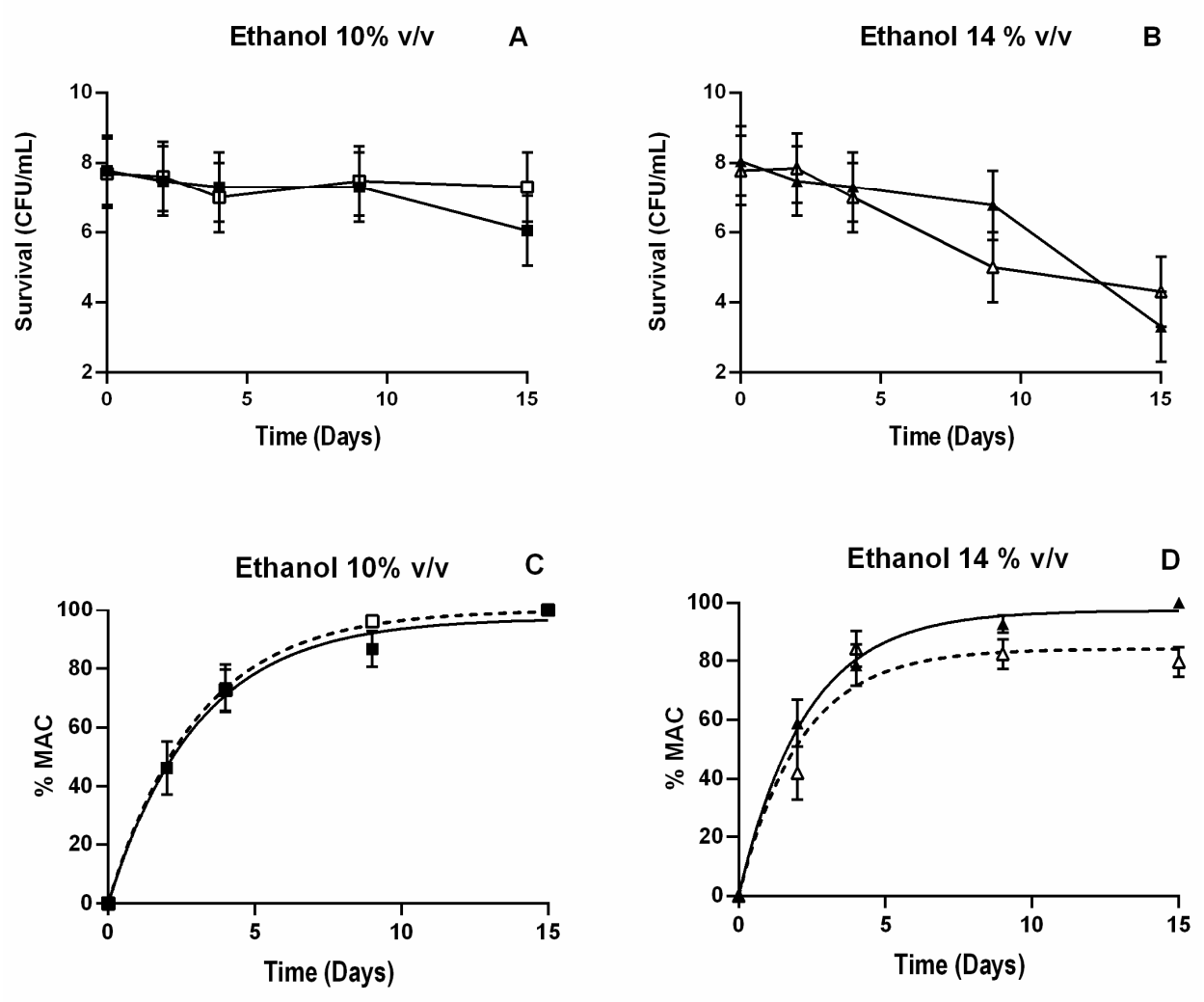


**Figure 5.** Survival of the *Lcb. paracasei* UNQLpc 10 strain (**A**,**B**) (filled symbols: previously acclimated culture, empty symbols: non-acclimated culture) and percentage of L-malic acid consumption (% MAC) during malolactic fermentation (MLF) (**C**,**D**) in synthetic wine with 10 or 14% $v/v$ ethanol (filled symbols: previously acclimated culture, empty symbols: non-acclimated culture). Data are shown as mean ± SD of two independent measurements.

When UNQLpc 10 was incubated in synthetic wine with 10% $v/v$ ethanol, the culture was able to maintain the number of viable cells at higher than 95% during 15 days of

fermentation with an MAC, and no significant differences were found in the performance of the previously acclimated or non-acclimated strains.

In contrast, when UNQLpc 10 was incubated in synthetic wine with 14% *v*/*v* ethanol, cells were not able to survive during fermentation, with a decrease of ~3–4 log CFU/mL. However, cells were able to consume malic acid in high percentage, being significantly higher when the culture was previously acclimated (97.12%) than when it was not previously acclimated (84.10%) (Table 2).

**Table 2.** Parameters obtained from Figure 5 by application of Equation (2).

| Wine | Ethanol 10% | | Ethanol 14% | |
|---|---|---|---|---|
| **Acclimation** | **No** | **Yes** | **No** | **Yes** |
| $\%MAC_f$ | 97.18 ± 3.58 | 100 ± 1.00 | 84.10 ± 7.11 * | 97.12 ± 2.16 |
| K | 0.328 ± 0.037 | 0.330 ± 0.013 | 0.475 ± 0.150 | 0.440 ± 0.035 |
| $R^2$ | 0.99 | 0.99 | 0.94 | 0.99 |

* Significantly lower than acclimated samples ($p < 0.05$). $\%MAC_f$:maximum malic acid consumption obtained by the plateau of Equation (2). K: constant of first order exponential decay. $R^2$: coefficient of determination.

## 4. Discussion

*Lactobacillus paracasei* belongs to the *L. casei* group and is found in diverse niches [38] including food (raw or fermented), plants [39], birds [40], and the gut of mammals [41], including human gut of healthy adults and of breast-fed infants [42,43]. The advance in molecular techniques and their application in winemaking has allowed for the identification of this species as part of the wine microbiota [2].

Previous studies in our laboratory have shown that LAB isolates of *Lpb. plantarum* and *O. oeni* are able to carry out MLF in synthetic and Patagonian wines. In this study, we analyzed a LAB of the species *Lcb. paracasei*, not typically used as malolactic starter in winemaking.

In addition, we analyzed its genome architecture. The description of the genes present in it allowed us to know their possible functions in the strain, characterize, in depth, the technological and oenological potential of the strain, and provide information on possible interactions in the fermentation ecosystem.

The COG database, which has been devised as a way to allow phylogenetic classification of proteins from complete microbial genomes [44], was used as a tool to classify proteins from the complete UNQLpc 10 genome by COG functional categories. Two functional categories of uncharacterized proteins, R (genes with only a generic functional prediction, typically of the biochemical activity) and S (uncharacterized genes) (22.0% of the genes), are particularly useful, as they reflect the current level of understanding of protein function at the proteome level [45].

Comparative genomics of different *Lcb. paracasei* strains (using complete sequence genomes of strains from fermented foods) revealed high genomic diversity and no relationship between them could be established. It is currently accepted that properties of potential technological significance are strain-dependent, and the genomic bases have not yet been elucidated [22,46,47].

Likewise, in this work, relevant genes involved in vinification were searched for. Wine contains many monosaccharides and disaccharides, with glucose, fructose, and arabinose being the main sugars metabolized by this LAB species [48]. Numerous genes related to carbohydrate transport and metabolism were found in the UNQLpc 10 genome, which is of relevance to the persistence of the bacterium in the harsh wine environment.

Our results showed the presence of genes of relevance to the cellular stress response. These included clusters related to the MIP family associated with osmotic stress, as well as genes coding for proteins involved in the ethanol stress response, and the clpX and trxA genes, which play a major role in the degradation of misfolded proteins accumulated following exposure to stress conditions. These genes are important since they are related to the survival of LAB in a hostile environment such as wine.

Additionally, we found that the UNQLpc 10 genome contains genes coding for enzymes that could have positive effects on aromatic and sensorial characteristics of the vinification process. The metabolism of L-malic acid by LAB has led to considerable interest [2], since the degradation of L-malate leads to a reduction in the acidity of wine and provides microbiological stability by preventing the secondary growth of LAB after bottling. Most LAB decarboxylate L-malate to L-lactate by a $NAD^+$- and $Mn^{2+}$-dependent malolactic enzyme [49]. This is the first work that describes two gene clusters involved in the metabolism of L-malate in a *Lcb. paracasei* strain. This type of cluster is closely related to the species *Lcb. rhamnosus* [49]. A comparison with *O. oeni* strains showed that one of the clusters of UNQLpc 10 is similar to the clusters found in *O. oeni* (Figure S2). The additional cluster for L-malate metabolism in UNQLpc 10 could be an advantage under harsh wine conditions, and, as we can see during fermentation in synthetic wine (Figure 5), a higher MAC value was observed regardless of survival or acclimation.

Our analysis also showed other genes of relevance in vinification, such as genes encoding β-glucosidase, an enzyme that catalyzes the release of different aroma compounds by glycosidic bond cleavage, transforming terpenes, alcohols, fatty acids, etc. from bound to free forms [50]. In addition, we found several clusters related to the enzyme citrate lyase, which cleaves this bond into oxalacetate and acetate. Oxaloacetate is then decarboxylated to pyruvic acid and diacetyl, the latter of which is formed as an intermediate metabolite in the reductive decarboxylation of pyruvic acid to 2,3-butanediol, and is important from an organoleptic point of view because it gives buttery notes to wine [51]. Several of these genes have also been reported in the complete genome analysis of other oenological strains [20–22].

No genes synthesizing biogenic amines were found in the complete genome of UNQLpc 10. This is an important result because the synthesis of biogenic amines by LAB during winemaking processes should be avoided, because these compounds affect wine quality and acceptability [2,52].

The performance of the *Lcb. paracasei* UNQLpc 10 strain was technologically analyzed in synthetic wine, which was inoculated with cultures previously acclimated or non-acclimated. The effect of the pre-acclimation treatment on ethanol tolerance of MLF starter cultures is widely reported [53–55]. In addition, in previous works, we reported the positive effect of acclimation of Patagonian *Lpb. plantarum* and *O. oeni* strains exposed to wine conditions [14,15]. In these works, we showed that the pre-exposure of cells to sub-lethal stress conditions has a positive effect on cell survival in wine, improving culture performance. Similarly, Costantini et al. [56] reported that ethanol acclimation leads to a significantly increased expression of genes related to MLF and stress response, showing that the acclimation with ethanol induces surface modifications such as exopolysaccharide-layer production and structural changes such as membrane-composition modification. However, our results showed that acclimation did not change the survival of *Lcb. paracasei* cells in synthetic wine fermentations, but was relevant to improving MAC under high ethanol concentration (Figure 5 and Table 2). This shows that UNQLpc 10 could be more tolerant to acid and ethanol stress than the *Lpb. plantarum* and *O. oeni* strains previously reported by our group [15,28]. However, more studies are necessary to corroborate its activities in vinification at pilot and industrial scale.

## 5. Conclusions

This is the first work describing the characteristics of the complete genome of a strain of *Lcb. paracasei* of oenological origin. The analysis of the complete genome revealed the presence of genes responsible for malic acid consumption and for the adaptation of the bacterium to different stress environments related to the wine-fermentation process. Genome analysis also revealed the absence of genes coding for biogenic amines (compounds that affect wine quality and acceptability) and the presence of genes responsible for the synthesis of aromas in wine. Vinification at laboratory-scale showed that UNQLpc 10 is able to survive and consume L-malic acid under high ethanol content, making it an

excellent candidate as a malolactic starter culture. Finally, UNQLpc 10 has genes that are of oenological interest and more investigation must be carried out to evaluate the potential of the strain in winemaking.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8120726/s1, Figure S1: Analysis of the COG database performed on the 89 unique genes found in the Lcb. paracasei UNQLpc 10 genome; Figure S2: Schematic representation of the mle gene clusters in Lcb. paracasei UNQLpc 10 and O. oeni strains from different origin; Table S1: Characteristics of the *Lpc.* UNQLpc10 genome.

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