

# Complete Genome Sequencing of *Lactobacillus plantarum* UNQLp 11 Isolated from a Patagonian Pinot Noir Wine

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***Lactobacillus plantarum* UNQLp 11 strain was isolated from a Patagonian Pinot noir wine at the oldest commercial winery (110 years old) in General Roca, North Patagonia, Argentina, and has demonstrated its ability to survive during winemaking processes and successfully carry out malolactic fermentation. This work aimed to obtain the whole assembled genome of the UNQLp 11 strain, analysing its architecture and the possible functions of the predicted genes from the oenological properties of this strain. The genome size is 3 534 932 bp, with a mean GC content of 44.2%, 3 412 CDS, 80 transposons and 148 tandem repeats. A comparison between the genome size and gene content of 14 *Lb. plantarum* strains from different origins was performed, and UNQLp 11 exhibited the largest size. The *in silico* genome-wide analysis allowed us to confirm the existence of genes encoding enzymes involved in the synthesis of several metabolites of oenological interest, in addition to bacteriocins and exopolysaccharides. Furthermore, it is possible to speculate on this strain's adaptation to different environments, as it is able to use diverse substrates for its growth. All these features suggest the potential of UNQLp 11 to be a good starter culture for malolactic fermentation.**

## INTRODUCTION

*Lactobacillus plantarum* (recently reclassified in its genus as *Lactiplantibacillus plantarum*, Zheng *et al.*, 2020) is a species belonging to the lactic acid bacteria (LAB) group, which is found in different ecological niches such as vegetables, meat, fish and dairy products, as well as in the gastrointestinal, vaginal and urogenital tracts (Siezen *et al.*, 2010). This species is a facultative heterofermentative LAB that can utilise a broad range of fermentable carbon sources, hence their application in the production of a variety of foods and beverages, as well as to obtain vitamins, bacteriocins, probiotics, antifungals and potential anticaries agents (Evanovich *et al.*, 2019). Genome sequencing and comparative genomics of different *Lb. plantarum* strains have revealed high genomic diversity, versatility and flexibility, which ensure its success in various niches and applications (Siezen & Van Hylckama Vlieg, 2011; Jiang & Yang, 2018; Evanovich *et al.*, 2019). This versatility and

metabolic capacities make this LAB species highly relevant for industrial applications, and many strains are marketed as starter cultures for fermented foods and beverages (Molin, 2001; Weinberg *et al.*, 2004; Luxanani *et al.*, 2009).

In general, LAB have reduced genomes, but *Lb. plantarum* presents a larger genome with numerous genes that were acquired by horizontal gene transfer (HGT), mainly via mobile elements (Hubert & Kammerer, 1994; Kleerebezem *et al.*, 2003). The habitat diversity of *Lb. plantarum* might be related to abundant gene functions, resulting in increased genome size (Bringel *et al.*, 2001; Kant *et al.*, 2011). Genomic analysis has facilitated a more comprehensive characterisation of the genetic characteristics of some bacterial strains (Lasek *et al.*, 2017). In the past decade, several LAB strains were subjected to genome sequencing to further assess their physiological functions and environmental adaptation mechanisms. To date, more

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than 50 complete genome sequences of *Lb. plantarum* strains are available in the NCBI GenBank database (Yao *et al.*, 2020). Most of these complete genomes correspond to strains with probiotic properties (Kleerebezem *et al.*, 2003; Zhang *et al.*, 2010; Siezen *et al.*, 2010; Liang *et al.*, 2019). In contrast, only a few genomes of oenological strains have been published (Lamontanara *et al.*, 2015; Zhao *et al.*, 2016) or are available from the NCBI GenBank database (CP017363.1).

*Oenococcus oeni* is the main LAB species responsible for the malolactic fermentation (MLF) of wine, which normally follows the alcoholic fermentation (AF) produced by yeasts (Davis *et al.*, 1985). Also, it is probably the LAB species best adapted to overcome the harsh environmental conditions of wine, and therefore represents the majority of commercial MLF starter cultures. Due to global warming, the harvesting of grapes of higher maturity has resulted in higher pH of the musts. Under these high-pH conditions, *Lb. plantarum* bacteria have shown especially interesting results for their capacity to induce MLF and for minimising the risk of acetic acid production (Krieger-Weber *et al.*, 2020). Some *Lb. plantarum* strains can also tolerate the high alcohol and SO<sub>2</sub> levels normally encountered in wine and are responsible for MLF in various wine regions and cellars (Guzzon *et al.*, 2009; González-Arenzana *et al.*, 2012; Valdés la Hens *et al.*, 2015). Wine pH has been increasing gradually over the past several years, and red wines with a pH of over 3.5 to 3.6 are occurring more frequently (Martínez de Toda & Balda, 2014). At these pH levels, it is possible to observe a very fast growth in various indigenous microorganisms, some of which are spoilage bacteria that can cause a loss of wine quality (Krieger-Weber *et al.*, 2020). Among these species, *Lb. plantarum* strains have shown the most interesting results for their capacity to induce MLF under conditions of high pH, their facultative heterofermentative properties that avoid acetic acid production from hexose sugars, and their more complex enzymatic profile and different metabolism compared to *O. oeni*, which could play an important role in the modification of wine aromas (Du Toit *et al.*, 2011; Bravo-Ferrada *et al.*, 2013; Berbegal *et al.*, 2016; Iorizzo *et al.*, 2016; Krieger-Weber *et al.*, 2020).

Valdés la Hens *et al.* (2015) showed that Merlot and Pinot noir wines from the oldest cellar (more than 100 years old) in the North Patagonian region of Argentina, at different stages of spontaneous MLF, contained both LAB species, namely *O. oeni* and *Lb. plantarum*. In particular, the UNQLp 11 strain of *Lb. plantarum* was isolated from a Patagonian Pinot noir wine (vintage 2012), and has demonstrated its ability to survive AF and successfully carry out MLF (Bravo-Ferrada *et al.*, 2013, 2014; Brizuela *et al.*, 2017, 2018a, 2018b, 2018c, 2019), suggesting its potential to be employed as a starter culture. In addition, this strain can be grown in a low-cost medium and then used as a starter culture without any previous acclimation treatment (Cerqueira *et al.*, 2019).

The aim of this work was to obtain the whole assembled genome of the UNQLp 11 *Lb. plantarum* strain so as to analyse its architecture and the possible functions of the predicted genes. The genome sequence of this native Patagonian strain will provide us with useful information

about the metabolic capabilities required to successfully carry out the MLF process and its ability to adapt to the stressful wine environment. Furthermore, a comparison with genomes of other *Lb. plantarum* strains, obtained from different sources, will reveal if UNQLp 11 has undergone specific genetic adaptations that allowed it to be a member of the native bacterial microbiome associated with wine grapes of the Patagonian Pinot noir variety, and to survive during the winemaking process, which would exhibit remarkable performance. The control, improvement and innovation of the MLF process demands detailed knowledge of the possible responses of the bacterium during fermentation and processing conditions.

## MATERIALS AND METHODS

### Strain information

The UNQLp 11 *Lb. plantarum* strain was obtained from a Patagonian Pinot noir wine (vintage 2012), in which AF and MLF were spontaneous. This wine was produced in a commercial cellar located in General Roca, Río Negro Province, Argentina. UNQLp 11 was chosen for its significant technological and oenological characteristics (Brizuela *et al.*, 2017, 2018a, 2018b, 2019).

### Bacterial growth and DNA extraction

The UNQLp 11 strain was grown in MRS broth at 28°C for two days. To obtain DNA, 1 mg/ml of lysozyme with 1% sodium dodecyl sulphate was used. Proteins were removed with 0.1 g/ml of proteinase K, followed by phenol-chloroform-isoamyl alcohol (25:24:1) extraction. Sixteen µg of high-quality genomic DNA was used for library preparation and sequencing (Iglesias *et al.*, 2019).

### 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 1 268 593 327 reads (383,24-fold coverage and a polymerase read N<sub>50</sub> size of 21 044 bp), with an average length of 14 480 bp and an estimated accuracy of 85.5%, were used as input for de novo assembly with the Canu package v1.8 (Koren *et al.*, 2017). The Canu output consisted of a single circular contig without gaps. Prediction of the coding sequences was done with Gene Marks-2. Replication and terminus origins were identified using GC-skew analysis and ORF orientation shift.

For genome annotation, the NCBI Prokaryotic Genome Annotation Pipeline was used (Chaudhari *et al.*, 2016). Protein function prediction and KEGG analysis were done by Blast2GO 5.1.1 (Götz *et al.*, 2008). The presence/absence of exclusive genes in specific *Lb. plantarum* strains was evaluated by BPGA (Bacterial Pan Genome Analysis Pipeline) (Chaudhari *et al.*, 2016). BPGA determines the core (conserved), accessory (dispensable) and unique (strain-specific) gene pool of a species. COG analysis was done by WebMGA (BMC Genomics 2011, 12:444) (Altermann *et al.*, 2005).

## RESULTS AND DISCUSSION

**General genome features of UNQLp 11 *Lb. plantarum* strain**

The complete genome of *Lb. plantarum* UNQLp 11 strain (Gene Bank Accession Number CP031140) contains a single, circular chromosome of 3 534 932 bp with an average GC content of 44.2%, and no plasmid structures were found. Its main features are shown in Table 1. *In silico* analysis revealed the presence of 3 412 ORFs, 83% of which showed similarities to classified genes from other organisms, and 17% remained unknown. The UNQLp 11 genome also contains 80 transposons, 148 tandem repeats, five ribosomal RNA operons (rRNA), one clustered regularly interspaced short palindromic repeat (CRISPR) locus, and four ncRNAs. CRISPR loci represent a family of DNA repeats typically composed of short and highly conserved sequences (~30 bp), interspaced by variable sequences, often found adjacent to *cas* genes (CRISPR associated), which are involved in defence systems against the invasion of foreign genetic material, in particular phages (Barrangou *et al.*, 2007). They were also observed in 40% of the sequenced bacterial genomes, and their presence may increase the genome stability, and therefore the environmental adaptation, of bacteria (Sorek *et al.*, 2008).

Fig. 1 shows the chromosome atlas of the UNQLp 11 strain. Replication and terminus origins were identified and it was observed that they were located in almost symmetric chromosome positions. Genes encoded in the UNQLp 11 genome are predominantly transcribed in the replication direction, a feature observed in many Gram-positive genomes with low GC content (Kleerebezem *et al.*, 2003).

Fig. 2 shows a comparison between genome size and gene content in 14 *Lb. plantarum* strains from different origins (plasmid genes present in other strains were not evaluated). UNQLp 11 exhibited the largest size and contains 342 genes more than WCFS1 (control), 405 more than XJ25, 341 more than Lp90, 740 more than Zhang-LL, and 282 more than TMW 1.277 (strains of oenological origin). Higher gene count is not necessarily indicative of transcription followed by a translation into functional proteins.

The UNQLp 11 genome contains 79.3% of protein-encoding genes of the COG class that are involved in the major metabolic pathways and could be assigned to 25 functional categories. Annotation of the UNQLp 11 genome sequence revealed the presence of genes mainly corresponded to the categories transcription, replication, recombination and repair, cell wall/membrane/envelope biogenesis, carbohydrate transport and metabolism. The remaining genes were catalogued as unknown functional proteins. Fig. 3 shows the relative abundance of each COG category of the oenological strains and the control, namely UNQLp 11, Zhang-LL, Lp90, XJ25 and WCFS1.

Furthermore, comparative genomics (gene content) analysis of some *Lb. plantarum* strains isolated from different fermented foods is shown as a cladogram (Fig. 4). It is evident that the information contained in the genomes of the strains of the *Lb. plantarum* species of oenological origin cannot determine a relationship between them. It is currently accepted that properties of potential oenological significance are strain-dependent, and the genomic bases

have not yet been elucidated (Spano *et al.*, 2006; Capozzi *et al.*, 2012; Bravo-Ferrada *et al.*, 2013). Further studies on the gene regulation mechanisms of *Lb. plantarum* species are required.

In order to evaluate the similarity between the genomes of *Lb. plantarum* strains isolated from fermented beverages, we compared the protein coding genes of UNQLp 11 with WCFS1 (reference strain from human saliva), XJ25 (from red wine), Lp 90 (from red wine), Zhang-LL (from rice wine) and TMW1277 (from palm wine). This analysis revealed that the compared genomes share only 2 115 core genes (data not shown).

The analysis of unique genes revealed that they belonged to the categories transcription, replication, recombination and repair, and transport and metabolism of carbohydrates. These results allowed us to assume that a greater gene number in these mentioned categories could increase the ability of UNQLp 11 to employ different sources of nutrients, and to better regulate the transcription processes. UNQLp 11 contains 325 unique genes (10% of its genome), and the smallest percentage of exclusively absent genes (0.1%). These data show that, among the compared genomes, UNQLp 11 contains the highest number of single proteins coding genes and the lowest number of exclusively absent genes (Fig. 5A). A COG analysis of the 10% of unique genes showed that 25% of them could not be classified in any of the COG categories (Fig. 5B). Furthermore, 13% of the 325 unique genes correspond to phage-related genes.

Among the strain-specific genes found in the UNQLp 11 genome are those coding for ATP-dependent Clp proteases, alpha-glucosidases, ABC transporters, PTS (sugar phosphotransferase system) sugar transporters, and USP (universal stress protein). ATP-dependent Clp proteases are

TABLE 1  
Genome characteristics of the UNQLp 11 *Lactobacillus plantarum* strain.

Genome size, bp	3 534 932
Overall G+C content, %	44.2
Number of genes	3 504
Number of coding genes	3 241
Number of CDS (total)	3 412
Number of coding CDS	3 241
Number of rrn operons	92
ncRNAs	4
CRISPR array	1
Pseudogenes (total)	171
Pseudogenes (ambiguous residues)	0 of 171
Pseudogenes (frameshifted)	101 of 171
Pseudogenes (incomplete)	51 of 171
Pseudogenes (internal stop)	38 of 171
Pseudogenes (multiple problems)	16 of 171

involved in several cellular processes, such as degradation of misfolded proteins, regulation of short-lived proteins and housekeeping removal of dysfunctional proteins (Porankiewicz *et al.*, 1999). They are also implicated in the control of cell growth and in the stress response in

low-GC Gram-positive bacteria (Fiocco *et al.*, 2010; Russo *et al.*, 2012). Alpha-glucosidases have been suggested to be involved in the degradation of yeast-derived macromolecules as a nutrient source for cell growth (Guilloux-Benatier *et al.*, 1993). The expression of the USP protein is associated

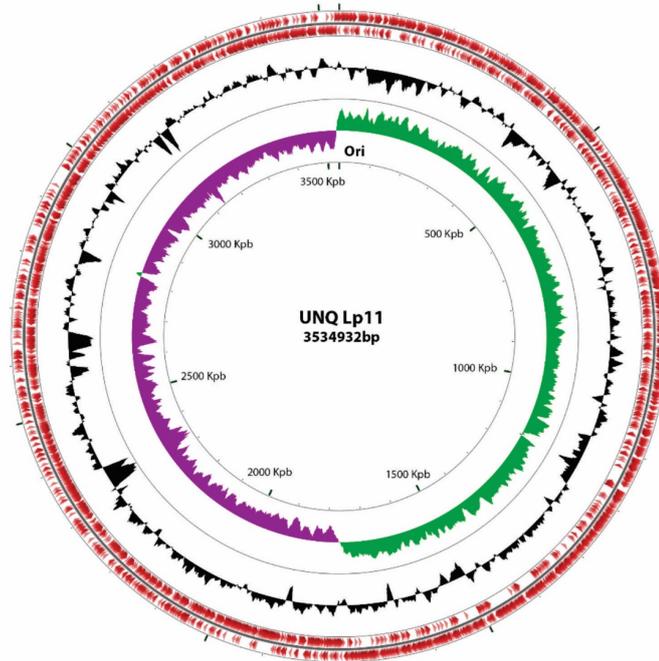


FIGURE 1

Chromosome atlas of *Lb. plantarum* UNQLp 11 strain generated using CGView v1.0 (Grant & Stothard, 2008). The GC content is illustrated in the black circle; positive and negative GC skew in the green and purple circles respectively; and CDS (CoDing Sequence) in the red circle. Genome positions in kbp are also shown in this atlas.

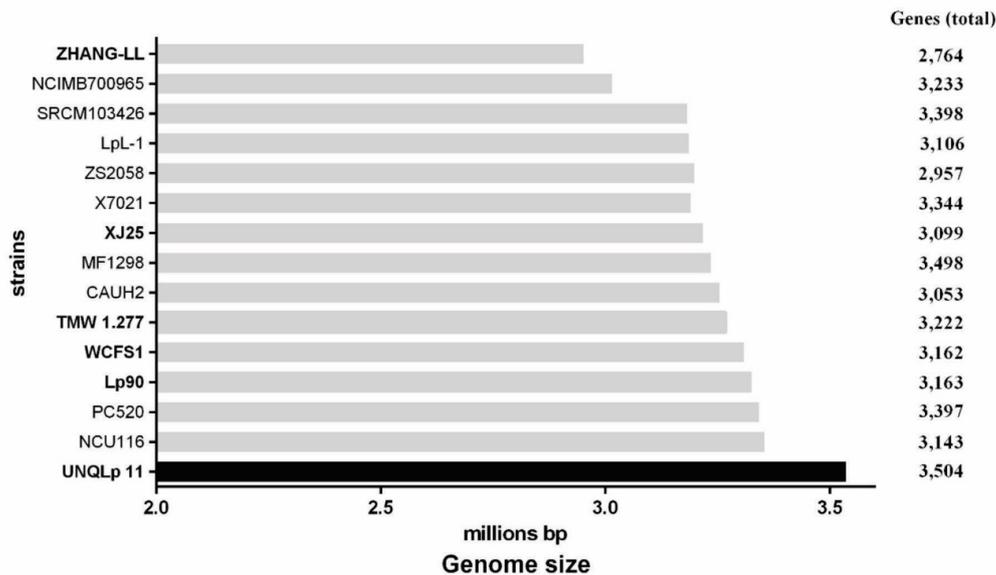


FIGURE 2

Comparison of genome size of 14 *Lb. plantarum* strains from different sources (sequences obtained from NCBI – GeneBank database). The source of each compared strain is: Zhang-LL (rice wine), NCIMB70096 (cheese), SRCM103426 (fermented food), LpL-1 (fermented fish), ZS2058 (sauerkraut), X7021 (tofu brine), XJ25 (red wine), MF1298 (fermented sausage), CAUH2 (vegetable pickles), TMW 1.277 (palm wine), WCFS1 (human saliva), Lp90 (red wine), PCS20 (pickles), NCU116 (pickles), and UNQLp 11 (Patagonian red wine).

with better stress responses by mechanisms still poorly understood (Tkaczuk *et al.*, 2013). The PTS and ABC transporters play a central role in the uptake of different sugars, both in *Lb. plantarum* and many other bacteria, and their presence is linked with a better capture of nutrients from the environment. Another strain-specific gene also found in UNQLp 11 was *mutS*, which is involved in the bacterium's repair systems (García-González *et al.*, 2012).

Finally, we performed a comparative genomic analysis of four complete genomes, namely UNQLp11, Lp90, Zhang-LL and XJ25, and the reference strain WCFS1, which showed that genes found only in the oenological strains belong to the category of transport and metabolism of carbohydrates. An interesting finding, in the UNQLp 11 genome, was the identification of 27 genes belonging to the family of regulatory proteins of the xenobiotic-sensitive element (Xre), 17 more than in the reference strain, WCFS1 (data not shown). Xre is a critical regulator when the bacterium is in stressful environments, e.g. related to acid tolerance in *Lb. acidophilus* (Azcárate-Peril *et al.*, 2004) and to ethanol levels in *O. oeni* (Olguín *et al.*, 2015).

### Genes encoding proteins related to carbohydrate transport and metabolism

The *Lb. plantarum* species, due to its facultatively heterofermentative properties, is heterofermentative for pentoses and homofermentative for hexoses (Dick, 2006). Wine contains many monosaccharides and disaccharides, with glucose, fructose and arabinose being the main sugars metabolised by this LAB species (Hedberg *et al.*, 2008). Numerous genes related to carbohydrate transport and metabolism were found in the UNQLp 11 genome, including 67 phosphoenol pyruvate sugar-transferase systems

(PTS) and five sugar-ABC transporters. Among the PTS systems, six families of PTS permeases were identified, namely PTS glucose-glucoside (Glc), PTS fructose-mannitol (Fru), PTS lactose-N,N0-diacetylchitobiose-b-glucoside (Lac), PTS glucitol (Gut), PTS galactitol (Gat), and PTS mannose-fructose-sorbose (Man). As expected, the genome analysis of UNQLp 11 revealed the presence of genes corresponding to whole phosphoketolase and glycolysis pathways. This genome contains five transduction sites (locus tags = DVH03\_15355, DVH03\_16150, DVH03\_06425, DVH03\_07445, DVH03\_11680) for the L-lactate dehydrogenase protein. This observation supports the relevance of pyruvate-dissipating capacity in this strain.

During the catabolism of readily fermentable sugars such as glucose, the synthesis of enzymes involved in the catabolism of other sugars is repressed, a global regulatory phenomenon termed carbon catabolite repression (CCR) (Brückner & Titgemeyer, 2002). The dominant mechanism of global carbon control in low-GC Gram-positive bacteria is one involving HPr, the bifunctional HPr kinase/phosphatase (HPrK), and the catabolite control protein A, CcpA (Henkin *et al.*, 1991). CcpA is a member of the LacI-GalR family of bacterial regulator proteins and regulates the transcription of genes at a global level by binding to a *cis*-acting DNA sequence designated a catabolite responsive element (*cre*) (Hueck & Hillen, 1995; Miwa *et al.*, 2000). Catabolite control by CcpA involves both transcriptional activation and repression, and the CcpA regulon is commonly scattered throughout the entire bacterial genome, which has been confirmed by the comparative whole-genome transcriptome analyses in the Gram-positive species *Bacillus subtilis*, *Lb. lactis*, and *Lb. plantarum*, and their *ccpA* mutant derivatives (Titgemeyer & Hillen, 2002). Also identified

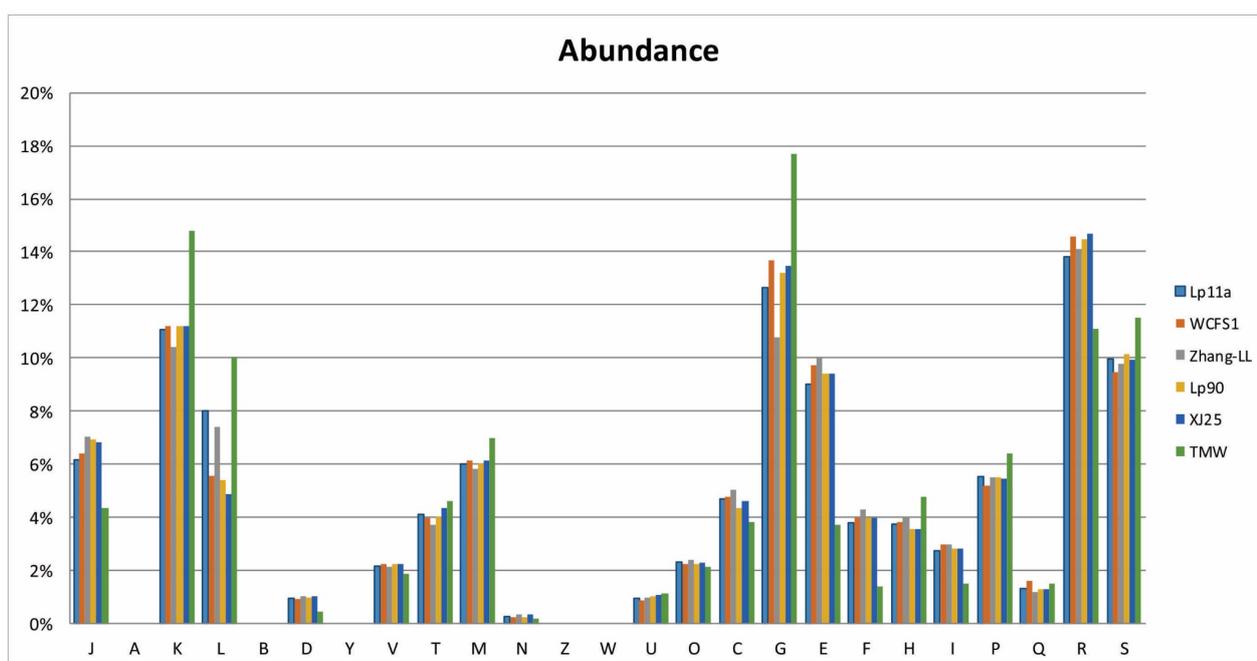


FIGURE 3

Relative abundance of genes associated with general COG functional categories in the UNQLp 11, ZhangLL, Lp90, XJ25 and WCFS1 *Lb. plantarum* strains.

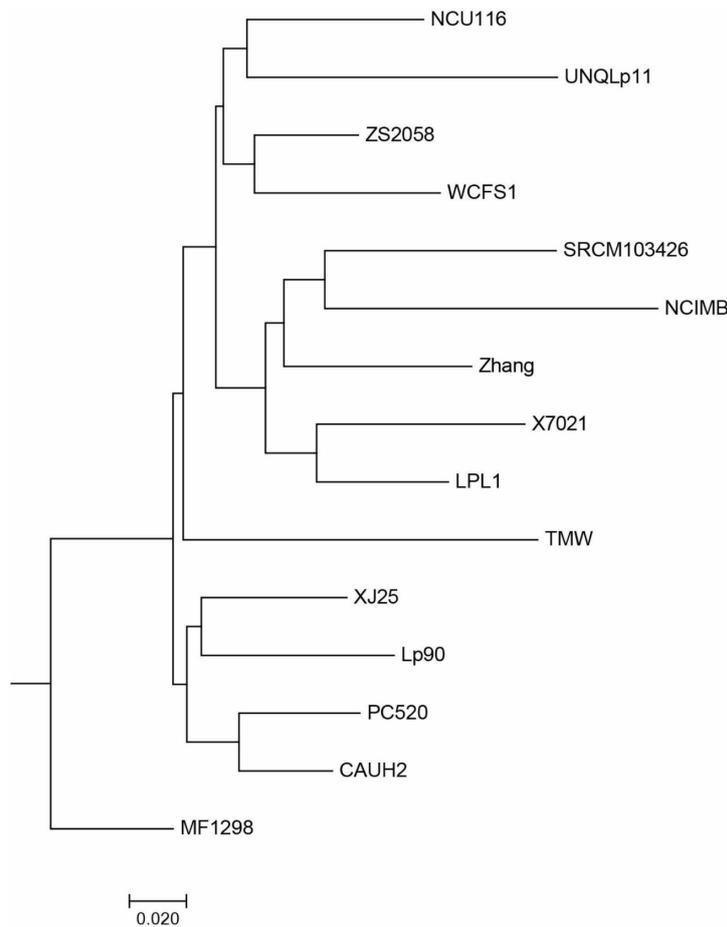


FIGURE 4

Cladogram, obtained by using BGA software, from the gene content analysis of UNQLp 11 (Patagonian red wine), WCFS1 (human saliva), XJ25 (red wine), Lp90 (red wine), Zhang-LL (rice wine), and TMW1277 (palm wine) *Lb. plantarum* strains.

were coding genes for the regulatory proteins HPr, CcpA, and HPrK/P (locus tags = DVH03\_11560, DVH03\_14365, DVH03\_06855).

#### Genes encoding proteins related to amino acid biosynthesis

LAB generally inhabit protein-rich environments (including milk) and are equipped with protein-degradation machinery to create a selective advantage for growth under these conditions (Kleerebezem *et al.*, 2003). *Lb. plantarum* has uptake systems (Opp and Dtp) for peptides and, once internalised, these peptides are degraded by a variety of peptidases, which have been studied extensively in *lactococci* and *lactobacilli* (Ferain *et al.*, 1996). Despite this elaborate protein-degradation machinery, the UNQLp 11 genome contains the genes for the whole biosynthetic pathways of 13 amino acids, namely alanine, aspartate, glutamine, lysine, arginine, cysteine, isoleucine, leucine, methionine, serine, threonine, valine and glycine. For the remaining seven amino acids (asparagine, glutamate, histidine, phenylalanine, tryptophan, tyrosine and proline), the biosynthetic pathways were incomplete, according to the KEGG analysis of maps (data not shown).

#### Genes encoding proteins related to flavour-development enzymes

*Lb. plantarum* has a diverse array of enzymes that could have positive effects on aromatic and sensorial wine properties (Du Toit *et al.*, 2011). Besides the malolactic enzyme itself (locus tags = DVH03\_12300, DVH03\_12380 in the UNQLp 11 genome), other enzymes involved in flavour development include glycosidases, esterases, phenolic acid decarboxylases and citrate lyases (Matthews *et al.*, 2004; Grimaldi *et al.*, 2005). Some (polygalacturonase, pectin methyl esterase) are involved in colour improvement in red wines, or reduce problems associated with wine filtration, such as tannase.

An *in silico* analysis was performed to look for genes encoding aminopeptidases, glutamate dehydrogenase and phosphotransacylase, among other enzymes (Table 2). Genes that code for alcohol dehydrogenase (AlcDH) and aldehyde dehydrogenase (AldDH) were identified. These enzymes catalyse the conversion of aldehydes to alcohols (AlcDH) or to carboxylic acids (AldDH), which are involved in the fruit flavour development in red wines (Styger *et al.*, 2011). Likewise, the gene coding PAD (phenolic acid decarboxylase) was also found in the UNQLp 11 genome (Table 2, EC: 4.1.1.102). This enzyme participates in the metabolism of phenolic acids from must and wine (Cavin *et al.*, 1993; Swiegers *et al.*, 2005; Mtshali *et al.*, 2010).

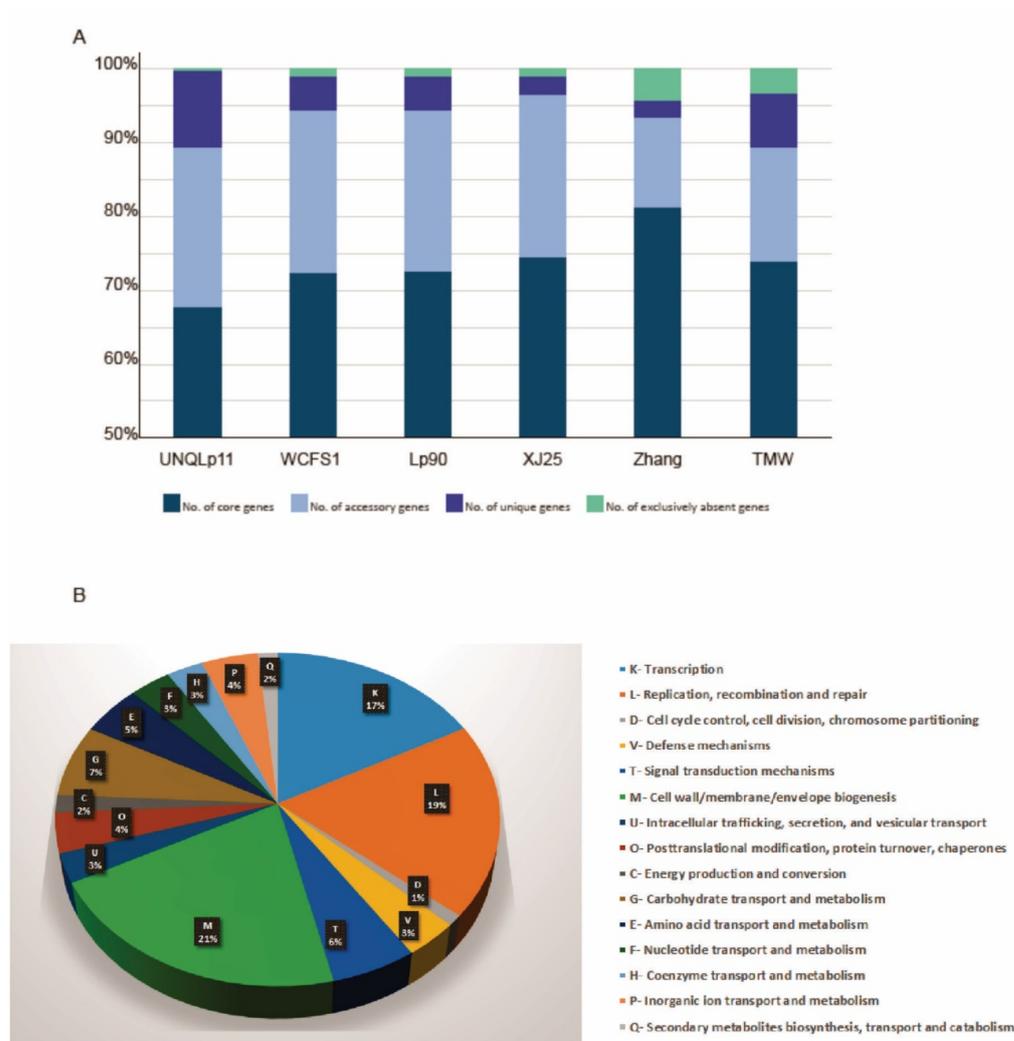


FIGURE 5

A: Ratio comparison of content in core genes (colour), accessory genes (colour), unique genes (colour) and exclusively absent genes (colour) among the *Lb. plantarum* strains UNQLp 11 (red wine), WCFS1 (human saliva), XJ25 (red wine), Lp90 (red wine), Zhang-LL (rice wine) and TMW1277 (palm wine).

B: COGs (clusters of orthologous groups) analysis performed on the 10% of unique genes found in the UNQLp 11 genome.

The beta-glucosidase enzyme catalyses the release of different aroma compounds by glycosidic bond cleavage, transforming terpenes, alcohols, fatty acids, etc. from bound to free forms (Grimaldi *et al.*, 2000; Spano *et al.*, 2005) (locus tags = DVH03\_01055, DVH03\_04430, DVH03\_04435, DVH03\_15725).

Citric acid is present in the wines in concentrations between 0.1 and 1 g/L. The lactic bacteria of the wine cleave it into a molecule of oxalacetate and another of acetate by way of the citrate lyase enzyme. Oxaloacetate is decarboxylated to pyruvic acid, and diacetyl, which is important from an organoleptic point of view because it gives buttery notes to wine, is formed as an intermediate metabolite in the reductive decarboxylation of pyruvic acid to 2,3- butanediol (Bartowsky & Henschke, 2004; Ribéreau-Gayon *et al.*, 2006).

A typical citrate lyase gene cluster (*citC*, *citE*, *citF*, *citX*) (locus tags = DVH03\_12375, DVH03\_12365, DVH03\_12360, DVH03\_12315) was observed in the

UNQLp 11 genome.

The gene coding for tannin acyl hydrolase (tannase) (EC: 3.1.1.20), which catalyses ester bond hydrolysis in hydrolysable tannins, such as tannic acid, releasing glucose and gallic acid (Lekha & Lonsane, 1997), was also found in the UNQLp 11 genome. Microorganisms with tannase activity are currently used in the food industry to remove the tannins responsible for undesirable effects in food processing, such as turbidity in wine or fruit juices (Vaquero *et al.*, 2004).

#### Genes encoding proteins related to stress responses

UNQLp 11 genome analysis allowed the identification of several genes involved in tolerance mechanisms to survive harsh conditions, as previously described in various works (Jobin *et al.*, 1999a; 1999b; Beltramo *et al.*, 2004; Spano & Massa, 2006; Van Bokhorst-Van de Veen *et al.*, 2011). The genes found include *clpX* (locus tag = DVH03\_07590), *clpLP* (locus tag = DVH03\_14965), and *trxA* (locus tag =

TABLE 2  
*In silico* analysis of the UNQLp 11 genome looking for genes coding for enzymes involved in flavor-forming pathways. A: amino acid degradation; \* involved in methionine and cysteine metabolism; G: glucosidases; O: others.

Enzyme (EC)	Name	Pathway	Function	Genes in UNQLp11
2.6.1.42	branched-chain aminotransferase	A	Conversion of valine, leucine and isoleucine into ketoacid components. The ketoacids are then further converted into aldehydes, alcohols and esters, which are important aroma compounds (Liu et al., 2008)	1
2.6.1.57	aromatic aminotransferase	A	Conversion of tyrosine, tryptophan and phenylalanine into ketoacid components (Liu et al., 2008)	2
2.6.1.1	aspartate transaminase	A	Conversion of aspartate into ketoacid components	2
1.4.1.2	glutamate dehydrogenase	A	Catalyses the deamination of glutamate to oxoglutaric acid related of the generic amino acid- (branched-chain amino acids, aromatic amino acids and methionine) degradation pathway (Liu et al., 2008)	1
1.1.1.1	alcohol dehydrogenase	A	Catalyses the conversion of aldehydes to alcohols (Liu et al., 2008)	5
1.2.1.10	acetaldehyde dehydrogenase	A	Catalyses the conversion of aldehydes to carboxylic acids (Liu et al., 2008)	2
2.3.1.19	phosphotransbutyrylase	A	Involved in the first step for the conversion of acid keto to esters (Liu et al. 2008).	1
1.1.1.-	D-hydroxyacid dehydrogenase	A	Catalyses the reduction of two keto branched chain acids to hydro acids of interest in flavour formation (Chambellon et al., 2009)	2
3.1.1.1	esterase A	A	Catalyses the biosynthesis of esters derived from short-chain fatty acids (Liu et al., 2008)	1
2.3.1.30	serine acetyltransferase	A*	Is involved in the synthesis of cysteine from L-serine (Liu et al., 2008)	1
4.2.1.22	cystathionine beta-synthase	A*	Lyases that have an O-acetylserine-thiol-lyase (cysteine synthase) activity (Liu et al., 2008)	1
2.5.1.48	cystathionine gamma-synthase	A*	Catalyses an $\alpha,\gamma$ -elimination reaction, converting cystathionine to homocysteine or cysteine (Liu et al., 2008)	1
2.1.1.10	homocysteine S-methyltransferase	A*	Catalyses the methylation of homocysteine in the final stage of the biosynthesis of methionine (Liu et al. 2008)	1
2.1.1.14	homocysteine methylase	A*	Involved in the final stage of homocysteine methylation for methionine synthesis (Liu et al., 2008)	1
1.1.1.3	homoserine dehydrogenase	A*	Has homoserine trans-acetylase activity that is involved in the biosynthesis of methionine (Liu et al., 2008)	2
2.7.1.39	homoserine kinase	A*	Involved in the onset of methionine biosynthesis (Liu et al., 2008)	1
2.5.1.49	O-acetylhomoserine sulphydrolase	A*	Has homoserine trans-acetylase activity involved in the biosynthesis of methionine (Liu et al., 2008)	1
3.2.1.86	6-phospho-beta-glucosidase	G	Hydrolytic activity in glycosylated compounds, acts on the glucosidic bonds $\beta$ (1-4) (Grimaldi et al., 2005)	4
3.2.1.40	alpha-L-rhamnosidase	G	Hydrolytic activity on terminal non-reducing alpha-L-rhamnose residues in alpha-L-rhamnosides (Grimaldi et al., 2005)	2
3.2.1.20	alpha-glucosidase	G	Hydrolytic activity on terminal, non-reducing (1 $\rightarrow$ 4)-linked alpha-D-glucose residues with release of D-glucose (Grimaldi et al., 2005)	7
4.1.1.102	PAD	O	Catalyses the decarboxylation of phenylacrylic acids present in plant cell walls (Mtshali et al., 2010)	1
3.1.1.20	tannase	O	Catalyses the decarboxylation of phenacrylic acids present in plant cell walls (Rodriguez et al., 2008)	1
4.1.1.101	malolactic enzyme	O	Involved in the malolactic fermentation of wine, which results in a natural decrease in acidity and favourable changes in wine flavours	2

TABLE 2 (CONTINUED)

Enzyme (EC)	Name	Pathway	Function	Genes in UNQ Lp11
2.6.1.42	branched-chain aminotransferase	A	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 (Liu et al 2008).	1
2.6.1.57	aromatic aminotransferase	A	Conversion of tyrosine, tryptophan, and phenylalanine into keto acid components (Liu et al 2008).	2
2.6.1.1	aspartate transaminase	A	Conversion of aspartate into keto acid components.	2
1.4.1.2	glutamate dehydrogenase	A	Catalyzes the deamination of glutamate to oxoglutaric acid related of generic amino acid (branched-chain amino acids, aromatic amino acids, and methionine) degradation pathway (Liu et al 2008).	1
1.1.1.1	alcohol dehydrogenase	A	Catalyze the conversion of aldehydes to alcohols (Liu et al 2008).	5
1.2.1.10	acetaldehyde dehydrogenase	A	catalyze the conversion of aldehydes to carboxylic acids (Liu et al 2008).	2
2.3.1.19	phosphotransbutyrylase	A	It is involved in the first step for the conversion of acid keto to esters (Liu et al 2008).	1
1.1.1.-	D-hydroxyacid dehydrogenase	A	Catalyzes the reduction of two keto branched chain acids to hydro acids of interest in flavor formation (Chambellon et al 2009)	2
3.1.1.1	esterase A	A	Catalyze the biosynthesis of esters derived from short-chain fatty acids (Liu et al 2008).	1
2.3.1.30	serine acetyltransferase	A*	It is involved in the synthesis of cysteine from L-serine (Liu et al 2008).	1
4.2.1.22	cystathionine beta-synthase	A*	Lyase that have an O-acetylserine-thiol-lyase (cysteine synthase) activity (Liu et al 2008).	1
2.5.1.48	cystathionine gamma-synthase	A*	catalyze an $\alpha,\gamma$ -elimination reaction, converting cystathionine to homocysteine or cysteine (Liu et al 2008).	1
2.1.1.10	homocysteine S-methyltransferase	A*	It catalyzes the methylation of homocysteine in the final stage of the biosynthesis of methionine (Liu et al 2008).	1
2.1.1.14	homocysteine methylase	A*	It is involved in the final stage of homocysteine methylation for methionine synthesis (Liu et al 2008).	1
1.1.1.3	homoserine dehydrogenase	A*	It has homoserine trans-acetylase activity involved in the biosynthesis of methionine (Liu et al 2008).	2
2.7.1.39	homoserine kinase	A*	Involved in the onset of methionine biosynthesis (Liu et al 2008).	1
2.5.1.49	O-acetylhomoserine sulphydrolase	A*	It has homoserine trans-acetylase activity involved in the biosynthesis of methionine (Liu et al 2008).	1
3.2.1.86	6-phospho-beta-glucosidase	G	Hydrolytic activity in glycosylated compounds, act on the glucosidic bonds $\beta$ (1-4) (Grimaldi et al 2005).	4
3.2.1.40	alpha-L-rhamnosidase	G	Hydrolytic activity on terminal non-reducing alpha-L-rhamnose residues in alpha-L-rhamnosides (Grimaldi et al 2005).	2
3.2.1.20	alpha-glucosidase	G	Hydrolytic activity on terminal, non-reducing (1->4)-linked alpha-D-glucose residues with release of D-glucose (Grimaldi et al 2005).	7
4.1.1.102	PAD	O	Catalyzes the decarboxylation of phenacrylic acids present in plant cell walls ( Mtshali et al 2010)	1
3.1.1.20	tannase	O	Catalyses the decarboxylation of phenacrylic acids present in plant cell walls (Rodriguez et al 2008)	1
4.1.1.101	malolactic enzyme	O	It is involved in the malolactic fermentation of wine, which results in a natural decrease in acidity and favorable changes in wine flavors.	2

DVH03\_14965). In addition, class I heat shock genes (*groES*, *groEL* and *dnaK/dnaJ* operons) (locus tags = DVH03\_14475, DVH03\_14470, DVH03\_08265, DVH03\_08260), involved in the disulphide-reducing pathway (*trxA* and *trxB* homologs) (locus tags = DVH03\_06790, DVH03\_14250), as well as genes codifying proteins implicated in the ethyl stress response (HrcA and CtsR) (locus tags = DVH03\_08250, DVH03\_12730), were also identified.

Bacteria protect themselves from changes in environmental osmolarity by using stretch-activated (or mechano-sensitive) channels that respond to changes in membrane tension when cells expand. In the UNQLp 11 genome, genes encoding two-channel protein families (Voltage-gated ion channel (VIC) (locus tag = DVH03\_06830) and large conductance mechano-sensitive channel (MscL) (locus tag = DVH03\_11240) were found, as well as some aquaporins (locus tag = DVH03\_01360) of the MIP (membrane intrinsic proteins) family, which may function primarily to protect the bacteria against osmotic stress (Biggin & Sansom, 2003). In the work of Bienert *et al.* (2013), it is observed that the number of genes that encode MIP in *Lb. plantarum* is the largest of those reported in other BAL species, and is suggested to have potential flexibility for different substrates.

#### Genes encoding proteins related to exopolysaccharide production

Exopolysaccharides (EPSs) are high-molecular-weight carbohydrate polymers secreted extracellularly by many microorganisms (Zhou *et al.*, 2019). EPS are thought to protect bacterial cells against extreme environmental conditions, such as biotic and/or abiotic stresses, including temperature, light intensity, pH and osmotic stress (Donot *et al.*, 2012). The UNQLp 11 genome contains genes that are associated with surface polysaccharide production (locus tags = DVH03\_04750, DVH03\_09260, DVH03\_15400). It should be noted that EPSs have been reported to be responsible for the high viscosity of musts. Besides, after alcoholic fermentation, EPSs form aggregates that could block the filter system, delay spontaneous clarification by sedimentation and worsen wine filterability (Dimopoulou *et al.*, 2017). The amount and type of EPS depends on fermentation conditions, *Lactobacillus* strain and the growth stage of the microorganism (De Vuyst & Degeest, 1999; Zannini *et al.*, 2016). EPSs can be degraded by other organisms in a complex microbial consortium of wine (Salazar *et al.*, 2009). For these reasons, EPS production of UNQLp 11 in wine requires more studies.

#### Genes encoding proteins related to bacteriocins

Bacteriocins are ribosomally synthesised antimicrobial peptides or proteins, are ubiquitous in the microbial world and are produced by both Gram-positive and Gram-negative bacteria, have cationic properties and kill target cells by destabilising the integrity of the inner membrane envelope, causing disruption of the membrane potential and/or leakage of cellular solutes that eventually leads to cell death (Diep *et al.*, 2009). Different *Lb. plantarum* strains (NC8, WCFS1, J23 and J51) have been found to harbour mosaic *pln* loci in their genomes (Diep *et al.*, 2009). In the

UNQLp 11 genome, we have identified a region containing some bacteriocin putative genes (*plnJ*, *plnF* and *plnE*, locus tags = DVH03\_15860, DVH03\_15815, DVH03\_15810 respectively) implicated in the synthesis of the EF and JK plantaricins, and plantamicin A. The *pln* locus is widespread among *Lb. plantarum* strains of oenological origin and shows genetic diversity and plasticity (Sáenz *et al.*, 2009). The production of these bacteriocins could generate a competitive advantage over other microorganisms, thus favouring successful implantation in the wine environment.

#### Genes encoding proteins related to undesirable metabolites

Arginine is the major amino acid present in wine, with grape juice concentrations ranging from a few hundred mg l<sup>-1</sup> to approximately 2.5 g l<sup>-1</sup> (Liu *et al.*, 1995). One of the major concerns about arginine metabolism by wine LAB is the synthesis of ethyl carbamate (EC) precursors (Arena & De Nadra, 2005). Ethyl carbamate, also referred to urethane, is a known animal carcinogen found in fermented foods and beverages, including wine (Araque *et al.*, 2011). The *in silico* analysis showed that the *Lb. plantarum* UNQLp 11 genome does not contain enzymes coding genes involved in EC synthesis.

Also, the synthesis of biogenic amines (BA) by LAB during winemaking processes, should be avoided, because these compounds affect wine quality and acceptability (Lonvaud-Funel, 1999; Moreno-Arribas *et al.*, 2003), and they can also induce a variety of diseases when they are consumed (and absorbed) in high concentrations. Although the worldwide regulation is not uniform, wines containing high amounts of histamine are rejected in certain markets due to recommendations for or suggested limits of this compound (Smit *et al.*, 2008). In our analysis, it was possible to verify that the UNQLp 11 genome does not contain genes encoding the enzymes histidine decarboxylase (EC: 4.1.1.17), putrescine carbamoyltransferase (EC: 2.1.3.6), and tyrosine decarboxylase (EC: 4.1.1.25), which are responsible for the synthesis of the main BA found in wines (histamine, tyrosine, putrescine).

#### CONCLUSIONS

The analysis of the complete genome of the UNQLp 11 *Lb. plantarum* oenological strain revealed the presence of genes involved in the transport and catabolism of different sugars, the biosynthesis of 13 amino acids, the synthesis of wine aroma compounds, and the production of exopolysaccharides and bacteriocins. When unique genes were analysed, it was observed that they belonged to the categories transcription, replication, recombination and repair, and carbohydrate transport and metabolism. These findings allow us to speculate on their adaptation to different environments, as they are able to use diverse substrates for their growth.

The genome comparison of UNQLp 11 with fourteen other *Lb. plantarum* strains from fermented foods and beverages did not allow us to observe any genotypic relationship among the strains isolated from wine, revealing that the compared genomes shared only 2 115 core genes.

Although gene presence does not guarantee its

expression during winemaking, the genome sequencing and analysis of the native UNQLp 11 strain allowed us to associate phenotypes expressed by this strain under different winemaking conditions (Bravo-Ferrada *et al.*, 2013, 2014; Brizuela *et al.*, 2017, 2018a, 2018b, 2018c) and will improve our understanding of its performance in future winemaking assays on pilot scale.

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