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Genomic perspective of Lachancea thermotolerans in wine bioacidification

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Abstract. We have sequenced two commercial strains of Lachancea thermotolerans (Lt) from the company Lallemand: Laktia™ y Blizz™. We intend to know information about the genes that control the acidification by Lt during grape fermentation to make wine. The production of lactic acid from sugars by Lt is regulated by several genes of LDH that are variable between strains. To perform whole genome sequencing (WGS) of the strains, they were grown in Yeast Extract Peptone Dextrose for 24h at 28ºC. DNA was extracted using the Yeast DNA extraction kit (ThermoFisher Scientific). Sequencing was done with Illumina and a read size of 150 Laktia™ and Blizz™ a genome draf of 10.45M and 10.44M bp respectively were obtained. A total of 5124 genes were detact in Blizz™ and 5120 genes in Laktia™. As previously observed, both strains carry three copies of the LDH gene encoding the enzyme L-lactate dehydrogenase, which cleaves pyruvate to lactate, two in tandem and the other located in other part of the genome. The genomic data obtained in this study will be key to correlate the phenotypic characteristics of these strains with wine fermentation. Also, we are currently studying the genes involved in the production of glycerol, and fermentative aromatic compounds, especially benzenoids and thiols. The regulation of the acyl transferase enzymes in Lt influences the formation of fruity and floral esters with high impact in the sensory profile of the wine. Additionally, it has been observed positive effects of Lt fermentations in the release of aromatic thiols, we have explored the presence and regulation of β-lyase enzymes in Lt to favor the revelation of 4MMP and 3MH from CYS-4MMP and CYS-3MH.

1. Introduction

Wine bioacidification is a feasible and sustainable biotechnology to decrease wine pH, and to control microbial spoilage, physicochemical estability and sensory profile. In the current global warming scenario grapes reach maturity with high pH, unbalanced composition and flat aroma [1]. The use of non-*Saccharomyces* yeasts is a powerful tool to modify the sensory profile of wines during fermentation. Our team together with others in Spain has been leaders in the isolation and selection of non-*Saccharomyces* yeasts in the last years [2].

Among the key non-*Saccharomyces* is specially significant the specie *Lachancea thermotolerans* for its ability to metabolize sugars to lactic acid by mean of the lactate DH (LDH) enzyme reaching concentrations of several g/L (1-10) and easily decreasing the pH 0.5 units [3-5]. Lactic acid is formed from sugars so pH reduction is simultaneous to a slight decrease of alcoholic degree (0.2-0.5%vol) depending on the acidification level [3] (**Figure 1**). Additionally, other potential positive effects strain- dependant have been descrived as the production of fermentative esters, the low production of volatile acidity [3-5], the release of volatile tiols [3, 6] and the production of succinic acid [5].

The production of lactic acid is regulated by LDH which metabolize pyruvate to lactate, codified by the LDH gene. Most of the strains have 3 copies of this gene in the genoma 2 of them in tandem and the third in other part of the genoma.

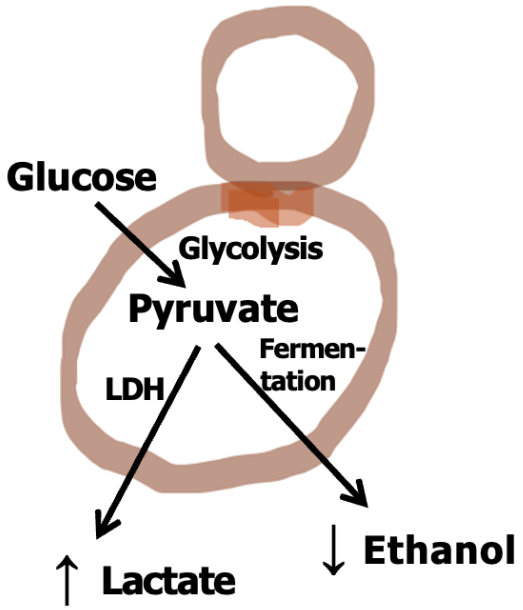

Figure 1. Effet of *L. thermotolerans* in acidification and alcohol reduction.

L. thermotolerans also shows a medium fermentative power (6-9% ABV) [3], and should be used in mixed or sequential fermentation with *Saccharomyces cerevisiae* to completely deplete the sugars and to produce dry wines. The other drawback of *L. thermotolerans* is the high sensitivity to sulfites being necessary levels lower than 20 mg/L for successful implantations. The shape of L. thermotolerans is ellipsoidal with multipolar budding (**Figure 2**).

1.

Figure 2. Atomic Force Microscopy of a budding cell of *L. thermotolerans*.

The aim of this research is to fully sequence the genoma of two strains of *L. thermotolerans* available at the market (Blizz™ and Laktia™, Lallemand, Canada), the first selected by our team (enotecUPM), to better understand the molecular mechanisms involved in lactic acid production from sugars and the enological performance of these non-*Saccharomyces* yeasts.

2. Materials and methods

2.1. Whole Genome Sequencing (WGS)

The whole genome sequencing (WGS) of the strains (details and applications in **Table 1**) was performed growing them in Yeast Extract Peptone Dextrose for 24h at 28ºC. DNA was extracted using the Yeast DNA extraction kit (ThermoFisher Scientific). WGS was done with Illumina NovaSeq X, 150bp paired end reads .

Table 1. *Lachancea thermotolerans* strains studied.

Strain (*L. Thermotolerans*)	Application
Laktia™ (Lallemand)	Wine bioacidification (launched 2019)
Blizz™ (Lallemand) Selected by our team	Wine bioacidification (launched 2024)

2.2. Bioinformatics

After quality control, *de novo assembly was performed with SPAdes*. BUSCO software was used for quantitative assessment of genome assembly and annotation completeness. Protein-coding genes prediction was performed combining different annotation tools and results combined using EVM v1.1.1 software. For genome functional annotation, predicted genes were compared to KOG, KEGG Swiss-Prot, TrEMBL, and Nr using BLAST v2.2.29 to obtain gene functional annotations. CAZy software was used to carbohydrate active enzymes genes.

3. Results and discussion

3.1. Genoma draft

Table 2 shows the global figures of the genoma for both strains of *L. thermotolerans* in comparation with *S. cerevisiae*. Similar genoma length has been found for both strains (≈10.4M bp) with 4 extra genes in Blizz™.

Table 2. Genoma of the *Lachancea thermotolerans* strains studied.

Strain (*L. Thermotolerans* / *Sc*)	Base pairs	GC%	Genes
Laktia™	10,454,053	47.25	5,120
Blizz™	10,445,369	47.27	5,124
S. cerevisiae S288C	12,100,000	38.50	6,021

3.2. Lactate DH (LDH) genes

The acidification in *L. thermotolerans* is performed by the LDH enzyme. Both strains have 3 copies of the LDH gene, 2 of them in tandem (**Figure 3**) and the third located in other part of the genoma. The acidification level is strain dependant ranging 1 to more than 16 g/L according to

literature [3], being the Blizz™ strain specially effective in pH control by the production of lactic acid under enological conditions. The study of the level of expression of these genes controlled by their promotors and regulatory mechanisms will report useful information to control the acidification process during fermentation. Also it has been located the lactate membrane transporter JEN1 involved in the extrusion of the lactate to the outside media.

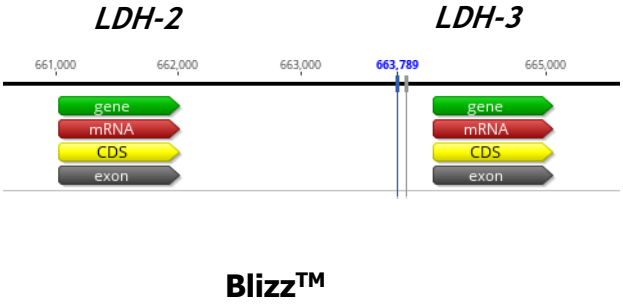

Figure 3. Location of LDH-2 and LDH-3 enzymes in the genome.

3.3. Influence on aroma profile and the revelation of volatile thiols

It has been previously published the effecto of *L. thermotolerans* in the release of volatile thiols [3], and we have also observed the effectivity of Blizz™ on this process [6]. The WGS has shown the presence of a β-lyase enzyme: Pectate lyase (E.C 4.2.2.2); which can be involved in the revelation of thiols, allowing the presence of higher contents of this molecules during the fermentation with Blizz™ of thiolic varieties such as Verdejo or Sauvignon blanc.

4. Conclusions

The Whole Genome Sequencing of selected strains of *L. thermotolerans* help to better understand their performance under enological conditions. We have located the main genes depending on the bioacidification process and also those involved in the release of volatile thiols. The detailed analysis of the genome will give us a lot of practical information to improve the performance of these strins during wine fermentation.

5. References

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