MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF THE INDIGENOUS *Saccharomyces cerevisiae* GL15 STRAIN RESPONSIBLE OF TRADITIONAL PLUM JUICE FERMENTATIONS

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Introduction

A number of plum varieties (*Prunus salicina* Lindl.) are cultivated in the rural zone of Berisso (Argentina) and are used by local producers for elaborating a typical plum beverage. The biochemical characteristics of plum juice are quite singular as it is very acid (pH 2.9-3.2; 0.8-1.8% as malic acid) and it contains large amounts of polyphenols with high antioxidant capacity. Moreover, fermentation temperatures reach 35 ºC and high osmotic pressure occurs due to the addition of sugar to promote alcoholic fermentation, which is conducted in presence of sulphur dioxide as antioxidant and antibacterial agent.

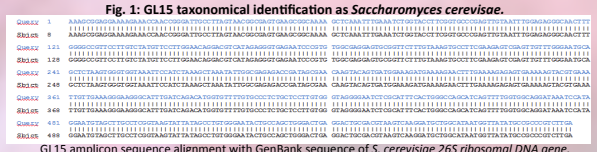
Aim of the study: Taxonomic identification and characterization of the indigenous yeast responsible of plum juice fermentations.

Materials and Methods

- Samples were taken from fermentation tanks of local familiar enterprises during three consecutive harvests.
- Yeasts were isolated on WL-agar plates (1) and reisolated until purity on GPY (glucose, peptone, yeast extract-agar) plates. Apparently one single yeast strain GL15 was present in all samples.
- Taxonomic identification by DNA-based methods: PCR (2) amplicon of the 26s ribosomal DNA of strain GL15 was sequenced in both strands and the consensus sequence obtained from the alignment was compared (BLAST search tool) with sequences of the GenBank database for species determination.
- A chemically defined synthetic broth (CDM) was designed for the optimal culture of strain GL15 to obtain biomass, and its sugar fermentation activity in liquid culture was studied.
- Nitrogen metabolism of GL15 in liquid culture with urea as the sole nitrogen source was analysed. Reverse phase h.p.l.c after derivatization with the reagent DEMM (diethyl ethoxy-methylene malonate)(3) was carried out. Assays were performed in triplicate.

Results

Fig. 1: GL15 taxonomical identification as *Saccharomyces cerevisae*.



GL15 amplicon sequence alignment with GenBank sequence of *S. cerevisiae 26S ribosomal DNA gene.*
Query cover = 100 %; E value=0

The CDM for optimal culture of strain GL15 with aeration contained: glucose, salts and urea, and required the following vitamins: biotin, thiamine, pyridoxine and calcium pantothenate. Under anaerobic conditions inositol was also required.

References

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(2) Kurtzman, C.P. and Robnett, C.J. 1998. *Ant. van Lee. 73*: 331-371.
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Results

Table 1: Strain GL15 fermentation activity

Nutrients	fermentation
glucose	+
sacarose	+
galactose	+
maltose	+
trehalose	-
lactose	-
rafinose	-

Fig. 1: GL15 growth in the optimized culture broth

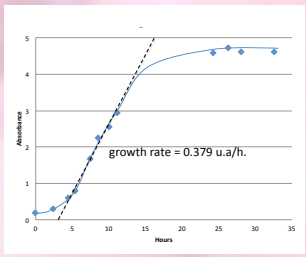
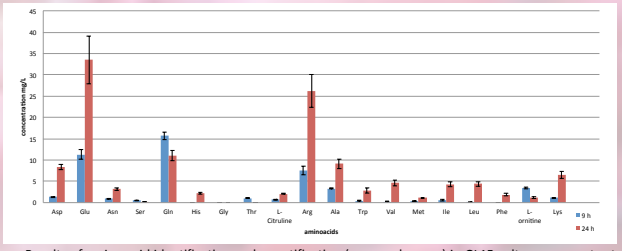
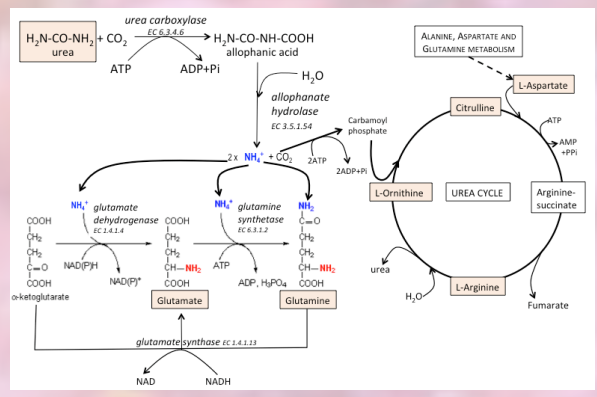


Fig. 3: Results of h.p.l.c. analysis of GL15 nitrogen metabolism



Results of amino acid identification and quantification (mean values +σ) in GL15 culture supernats at middle and stationary growth phase of GL15 in culture with urea as the unique N source



Conclusions

- The indigenous yeast strain GL15 was identified as *Saccharomyces cerevisiae*.
- GL15 leads plum juice fermentation and it can grow under extreme conditions of high acidity and osmotic pressure.
- GL15 was able to grow in a minimal culture broth containing glucose, salts and urea as the unique nitrogen source. In addition biotin, thiamine, pyridoxine and calcium pantothenate were required for growing under aeration conditions, and inositol was also required under anaerobiosis.
- The most abundant aminoacids at both middle and stationary growth phase of strain GL15 were: Glu, Gln and Arg, whose interconversion pathways constitute the yeast central nitrogen metabolism.
- Citrulline and ornithine, non-proteinogenic aminoacids that occur in the urea cycle, were generated together with proteinogenic aminoacids by strain GL15.
- No biogenic amines (histamine, agmatine, putrescine, spermidine) were detected in the culture broth after GL15 growth on urea as the sole nitrogen source, which shows GL15 fermentates suitable for consumption.