INFLUENCE OF NON-*SACCHAROMYCES* YEAST GROWTH ON THE METABOLISM OF NITROGENOUS COMPOUNDS IN LACTIC ACID BACTERIA FROM WINE

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***Abstract*-- A comparative study of the influence of *Hanseniaspora uvarum* metabolism on the growth and physiology of two lactic acid bacteria involved in vinification: *Lactobacillus hilgardii* 5w and *Oenococcus oeni* X_2L was carried out. At different yeast growth times in grape juice medium, fermented broth was inoculated with *L. hilgardii* or *O. oeni* and incubated for 48 h at 30°C. When *O. oeni* grown in the culture supernatant after 0, 3 and 8 hours of yeast growth (M1, M2 and M3 respectively), the growth rate and final biomass were not modified. With *L. hilgardii* a decrease of both parameters was observed. After 12, 24 and 34 h of yeast growth (M4, M5 and M6 respectively), the results for both microorganisms were similar: the growth rate increased and the final cellular mass decreased. When *O. oeni* was inoculated in M1, M2 and M3 media, an initial decrease of proteins directly related to amino acids production was observed. With *L. hilgardii* the amino acid and proteins concentrations diminished. In M4, M5 and M6 media *O. oeni* showed that the protein concentrations remained constant and the amino acid concentrations diminished. The results obtained with *L. hilgardii* were different with a higher protein consumption rate without significative amino acids modification. The amino acids increment in the first hours of *O. oeni* growth could regulate the protease production achieving the equilibrium between amino acids production and utilization. The high proteolytic activity in *L. hilgardii* could be responsible of the amino acids increase despite its consumption.**

***Keywords*-- Lactic acid bacteria, Non-*Saccharomyces* yeast, Wine.**

I. INTRODUCTION

Alcoholic and malolactic fermentations are the main processes that take place in wine winemaking. Yeasts transform sugars to ethanol, but at the same time play an important role in organic acid production and volatile end products in alcoholic beverages. The malolactic fermentation remains an imperfectly controlled process, since many nutritional and physic-chemical factors affect the growth and metabolism of lactic acid bacteria. Some of them depend on the yeast strain used, providing different amounts of amino acids, peptides and vitamins available acting as growth factors for lactic acid development, or on the presence of metabolic products which act as inhibitors. Several studies reviewed the importance of nitrogen from source, to transport, metabolic fate and influence on the fermentation carried out by the yeast *Saccharomyces cerevisiae.* Unfortunately, there is very little information about the nitrogen metabolism of non-*Saccharomyces* species of wine yeast. Recent quantitative studies on winemaking ecology showed that non-*Saccharomyces* species (especially *Hanseniaspora uvarum* (*Kloeckera apiculata*) and *Candida stellata*) survive during alcoholic fermentation at significant levels for longer periods than previously thought and grew to maximum populations of 10^6-10^7 cfu ml^{-1} (Fleet *et al*., 1984; Heard and Fleet, 1985; Pardo *et al*., 1989). Such growth was considered to be quantitatively significant and likely to influence the chemical composition of the wine.

We carried out a comparative study of the influence of *Hanseniaspora uvarum* metabolism on the growth and nitrogen physiology of two lactic acid bacteria involved in vinification. *Lactobacillus hilgardii* 5w, a detrimental microorganism, with negative ecological effect like hydrogen peroxide (Rodriguez and Manca de Nadra. 1995a, b) and histamine production (Farias *et al*., 1995; Farias *et al*., 1996), and *Oenococcus oeni* X_2L, a beneficial microorganism with optimal technological properties.

II. MATERIALS AND METHODS

A. Microorganisms and Culture Conditions

Lactobacillus hilgardii 5w, *Oenococcus oeni* X_2L and *Hanseniaspora uvarum* ca12 (*Kloeckera apiculata*) were isolated from Argentinean wines. Yeast and lactic acid bacteria were grown in basal medium containing per liter: yeast extract, 10 g; glucose, 5 g; tween 80, 1 ml and grape juice, 57 ml. When indicated, 1% KNO_3