Arginine Metabolism in Malolactic Wine Lactic Acid Bacteria and Its Oenological Implications

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ABSTRACT

L-Arginine is a major amino acid found in grapes and wine which is degraded by some wine lactic acid bacteria (LAB). The mechanism of this degradation and its oenological implications were examined in this research.

It was found that wine LAB able to degrade arginine do so by means of the arginine deiminase pathway, demonstrated by measuring the enzyme activities in cell-free extracts: arginine deiminase, ornithine trans-carbamylase and carbamate kinase. These enzymes were present in most heterofermentative lactobacilli and leuconostocs, but were absent in homofermentative lactobacilli and pediococci.

The presence of arginine increased the activities of arginine deiminase pathway enzymes in heterofermenters, but failed to induce these enzymes in homofermenters even under conditions of low glucose concentration (1 g/L). Glucose did not repress arginine utilisation but fructose appeared to do so, as fructose and arginine were metabolised sequentially, with arginine being metabolised mainly after utilisation of the fructose.

Detailed studies on *Leuconostoc oenos* OENO, *Lactobacillus buchneri* CUC-3 and *Lactobacillus brevis* 250 showed that arginine was converted stoichiometrically to ammonia and ornithine as the major end-products and that arginine catabolism could supply energy (ATP) to support growth. It was also demonstrated that citrulline was excreted during arginine catabolism by both the lactobacilli and the leuconostoc. Some of the excreted citrulline was reassimilated and catabolised after arginine depletion by the lactobacilli, but not by the leuconostoc.

The implication of citrulline excretion for the wine industry was explored by studying the formation of the carcinogen ethyl carbamate (urethane) in a synthetic wine and a white wine, since citrulline is a known precursor of ethyl carbamate. During growth of *Lc. oenos* OENO and *Lb. buchneri* CUC-3 in the synthetic wine and wine, significant amounts of ethyl carbamate were

found in the two wine types upon heat treatment of samples. The formation of ethyl carbamate correlated well with arginine degradation and citrulline excretion. Citrulline excretion during arginine degradation is of concern to the winemaker, since the reaction of citrulline and ethanol to form ethyl carbamate has been shown by other workers to occur even at normal wine storage temperatures. Winemakers, therefore, should avoid using arginine-degrading LAB starter cultures for inducing malolactic fermentation (MLF). In addition, spontaneous MLF in wine by undefined LAB strains should be discouraged, as this may lead to formation of ethyl carbamate precursors.

Ammonia detection with Nessler's reagent provides a simple, rapid test to assess arginine degradation by wine LAB in a complex medium, but is useful only for strains showing strong ammonia formation. The more sensitive enzymatic determination of ammonia is required for strains showing weak ammonia formation.

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