CHARACTERISATION OF WINE MALOLACTIC BACTERIA AND ACETIC ACID FROM FRUCTOSE METABOLISM

Department of Microbiology and Genetics
Massey University

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Robert John van Duivenboden
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ABSTRACT

Twenty-four strains of wine Lactic Acid Bacteria from the genera *Leuconostoc, Lactobacillus* and *Pediococcus* were characterised with respect to their growth responses to ethanol, temperature, pH, ability to degrade wine organic acids and utilisation of carbon sources. A novel single broth culture (HFA) was developed for the determination of heterofermentation, mannitol formation and ammonia production. Some strains of *Leuconostoc oenos* were found to produce ammonia from arginine. The implications of this are discussed. The production of mannitol from fructose by heterofermentative strains indicated potential acetic acid (volatile acid) spoilage risk for wines.

To investigate this risk, semi-synthetic media were devised to simulate "stuck" yeast alcoholic fermentation and the spoilage potential was evaluated under conditions of pH, substrate availability and ethanol concentration. Acetic acid production was analysed in the media by HPLC and found to occur at high levels from growth in the presence of fructose, but not glucose. The production was not affected by low pH or ethanol concentrations, or their combined effect. This indicated that acetic acid spoilage could occur under wine conditions. Other mechanisms of acetic acid production relative to this experiment are discussed. Erythritol and glycerol were detected in fermentation media but not quantified by HPLC. Their presence supported evidence of the activity of a novel glucose fermentation pathway in *Lc. oenos*.

FIGURE AND TABLES

1.0 INTRODUCTION

Grapes grown in cool climate regions around the world yield musts with high acid content. Alcoholic fermentations by yeasts such as *Saccharomyces cerevisiae* do little to remove acidity and consequently the wines produced from these musts are tart and unbalanced. Unless this acidity problem is addressed, most consumers will find these wines unpalatable and therefore they would be considered commercially unacceptable.

Certain strains of lactic acid bacteria are capable of degrading one of the predominant acids found in grapes, malic acid. This degradation, commonly known as malolactic fermentation (MLF), involves the biological conversion of L-malic acid to L-lactic acid and carbon dioxide. The formation of the weaker acid, lactic acid, and the release of carbon dioxide result in a more palatable wine of lower acidity and higher pH. The growth of the bacteria also modifies the composition of the wine by producing minor products from metabolism such as diacetyl which are recognised factors in determining flavour complexity.

In the past, winemakers have depended on MLF occurring spontaneously by the natural flora present in the musts and winery equipment. Although usually successful, such practices often incurred lengthy delays of many months before MLF was completed. Today, it is more common in the modern wineries around the world to add malolactic bacteria as starter cultures, as is practised in the dairy industry with other lactic acid bacteria. Such starters are introduced as actively growing bulk cultures prepared from lyophilised or frozen cultures. This process offers more control over MLF as to its initiation, length of time to completion and selection of the strain of bacteria. This also allows for control over the subtle complexity factors produced by growth of the selected bacterial strain.

Major endproducts from metabolism of grape sugars (glucose and fructose) such as lactic acid and carbon dioxide are expected from heterofermentative lactic acid bacteria, while lactic acid alone is produced by homofermentative strains. The amounts and nature of minor metabolic byproducts during growth of malolactic bacteria are difficult to quantify and will vary with the composition of the wine and the strain of starter culture used. For example, some heterofermentative strains may utilise fructose present in the must to gain additional energy for growth, but produce acetic acid in the process (Pilone *et al.*, 1991; Tracey and van Rooyen, 1988).

Acetic acid formation can be deleterious to wines if concentrations become too great. The formation of acetic acid by heterofermenters may occur when fructose is used as a hydrogen acceptor during grape sugar catabolism. The reduction of some fructose to mannitol by mannitol dehydrogenase (Martinez *et al.*, 1963) may be used to reoxidise reduced coenzymes formed in the heterolactic fermentation of these sugars. As shown in Figure 1.1, this allows acetyl phosphate formed from the phosphoketolase reaction to be hydrolysed, instead of having to be reduced to ethanol for this coenzyme reoxidation (Keenan, 1968). Hydrolysis of acetyl phosphate by acetate kinase, then, may result in extra beneficial energy (ATP) formation for the bacteria. In order for this to occur, fructose must be present in excess of requirements for fermentation. This would happen in situations of early inoculations of starter bacteria in musts where the grape sugars are in abundance, or during "stuck" or "sluggish" fermentations where yeast alcoholic fermentation is incomplete or very slow.

1.1 Objectives

Because of the deleterious effects of high acetic acid content in wine, it would be useful for the winemaker to know which strains of malolactic bacteria are capable of mannitol formation and consequently are potential acetic acid producers. In addition, it would be helpful to understand under what conditions this might occur and to minimise

the risk by proper strain selection. This study, therefore, looks at the characteristics of the bacteria and investigates some of the endproducts and parameters under which they are formed. Most of the malolactic bacteria used in this investigation are commercial strains used world-wide and therefore the results will be of international interest.

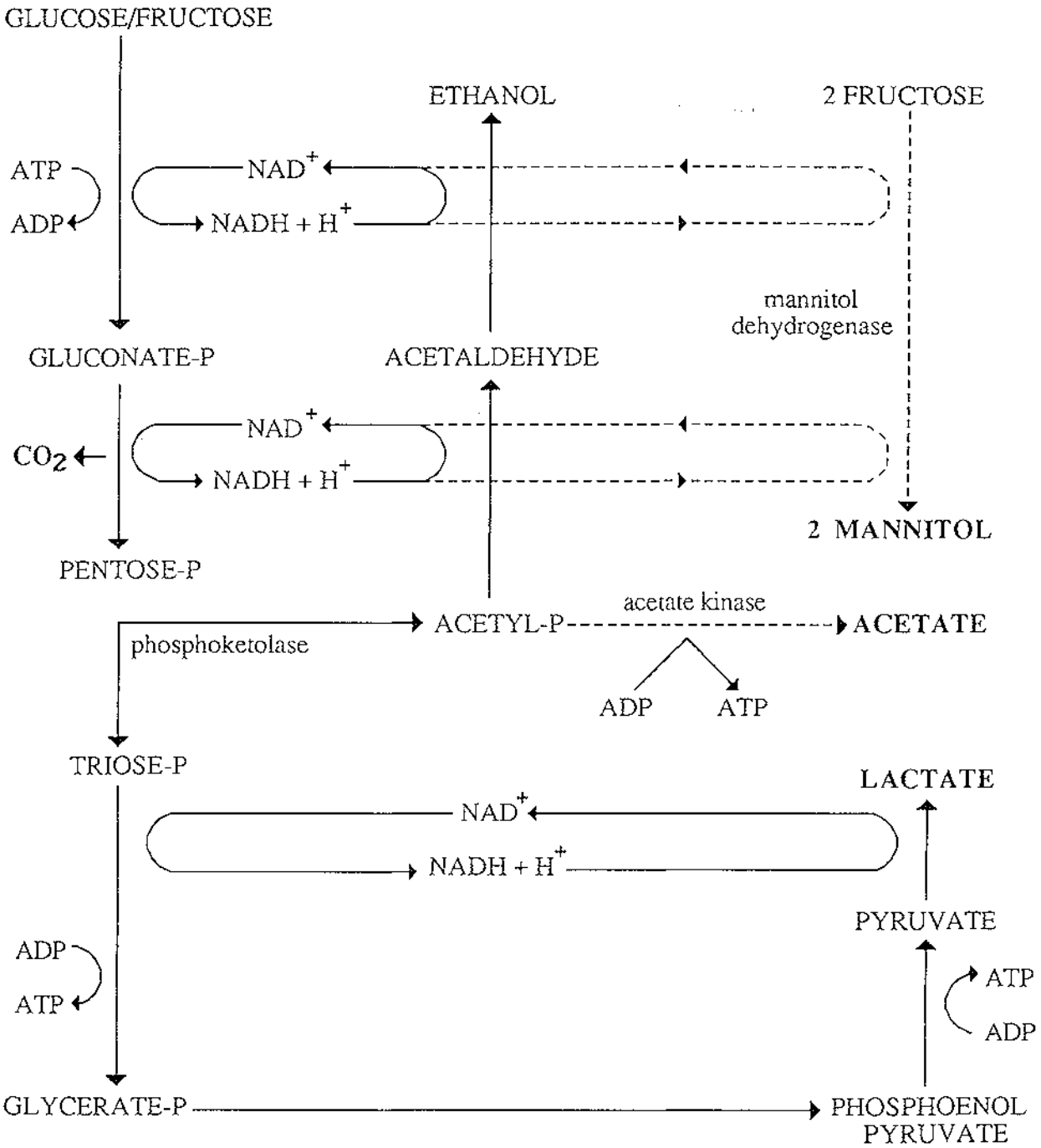

Fig. 1.1 Fructose reduction to mannitol with formation of acetic acid and ATP in heterofermentatative lactic acid bacteria. After Pilone, 1988.