View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Massey Research Online

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Carbohydrate Effects on the Inducement of the Arginine Deiminase Pathway Enzymes in Wine Lactic Acid Bacteria

A Thesis Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Science in
Microbiology

at

Massey University
Palmerston North
New Zealand

Peter R. F. Church

1998

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction to the Thesis

Characterised by a fermentative sugar metabolism resulting in lactic acid as a major end product, the lactic acid bacteria (LAB) may be isolated from a broad range of sources. Dairy products, fermented vegetables, meats and baking products such as sourdough bread involve these organisms in a consistent and intentional manner in present times, no matter how accidental or fortuitous their initial involvement may have been. Alcoholic beverages such as beer, cider and, most pertinently here, wine are also affected by the presence of particular LAB. As conditions differ between nutrient environments so do the LAB found in wine differ to those isolated elsewhere - being both ethanol tolerant to the degree where growth is capable in 10% v/v ethanol and aciduric, able to maintain an active presence at acidic levels as great as pH 4 or less. This ability to remain viable during the primary yeast fermentation of juice into wine leads to these LAB being of no small practical interest in the wine industry. The process of malolactic fermentation (MLF) involves the wine LAB altering the raw materials present in the juice and wine further, increasing the intricacies of the winemaking and final product. Primarily encouraged due to its effect of reducing wine acidity, MLF also alters flavour and aroma in what is generally thought to be an advantageous manner when applied correctly. Another factor thought to be of significance is an increase in biological stability. Found, for example, among the lactobacilli, pediococci and leuconostocs, the wine LAB are classed as either homofermentative or heterofermentative. Homofermenters commonly produce two moles of lactic acid per mole of glucose fermented, while heterofermenters form one mole each of lactic acid and carbon dioxide and varied quantities of ethanol and acetic acid from one mole of glucose.

Natural or chance occurrences of wine LAB, whether as part of the microbiological community on the raw materials or from other sources - such as inoculation from

contaminated equipment - were the original manner in which these organisms were introduced into the vinification equation. With the predilection towards quality control, standardisation and safety in the present day, the use of pure microbial starter cultures to initiate MLF has become increasingly widespread. In order to optimise the manipulation of wine LAB in both the laboratory and industry a thorough insight into their physiology and metabolism is an obvious necessity. Certain areas of interest have undergone more intensive study than others, with, for example, the catabolism of carbohydrates in both wine (Davis *et al.*, 1986) and model wine systems (Liu *et al.*, 1995a) having had a considerable amount of research compared to less primary sources of energy such as nitrogen metabolism.

Utilisation of L-Arginine, a major amino acid found in grapes and wine, occurs in some wine LAB, these being most heterofermentative lactobacilli and leuconostocs (Liu, 1993). The arginine deiminase (ADI) pathway enzymes, namely arginine deiminase, ornithine transcarbamylase and carbamate kinase, are present and active to varying degrees in these heterofermenters, but not inducible in homofermenters. In the process of degradation arginine is converted into ornithine, carbon dioxide, ammonia and adenosine triphosphate (ATP), indicating that arginine is a potential source of energy for wine LAB. Frequently the high ethanol environment in which wine LAB are found tends to be low in sugars; thus an alternate source of ATP could be of particular use in maintaining the continued desired effect of the organisms in wine over time.

With the identification of the arginine deiminase pathway as that responsible for the catabolism of arginine in heterofermentative wine LAB the process of further investigation into factors affecting arginine metabolism begins. Endeavouring to answer in part or in whole questions raised in previous studies (Liu, 1993), should increase understanding of the process and the bacteria in which it is found. It has been noted that arginine utilisation is not initiated in wine LAB in the absence of a fermentable sugar (Liu *et al*, 1995b) and also that some sugars, such as glucose, are concurrently utilised with arginine, whereas others, such as fructose, are preferentially catabolised, causing arginine to be left until the sugar levels are low. This research project concentrates primarily on the effects of the carbohydrates glucose and fructose on the inducement of the ADI pathway enzymes in heterofermentative wine LAB.