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

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## Table of Contents

	Page
Abstract	i
Acknowledgements	ii
Table of contents	iii
List of Tables	vi
List of Figures	vi
Chapter 1	1
Introduction to the Thesis	1
Chapter 2	3
Literature Review	3
2.1 Arginine catabolism in wine lactic acid bacteria	3
2.2 Carbon source effects on enzyme induction	5
2.3 Other products of arginine catabolism	7
2.4 Arginine catabolism in non wine lactic acid bacteria	7
2.4.1 Occurrence of the arginine deiminase pathway enzymes	7
2.4.2 Regulation of the arginine deiminase pathway enzymes	7
2.4.3 Genetics of the arginine deiminase pathway system	8
2.5 Fluoride inhibition of bacteria	8
Chapter 3	10
General Methods and Materials	10
3.1 Lactic acid bacterial strains	10
3.2 Medium preparation, culturing and harvesting of wine LAB	10
3.3 Preparation of non-growing cells	11
3.4 Obtaining starved cells	12
3.5 Analytical methods	12
3.5.1 Determination of citrulline concentration	12
3.5.2 Determination of arginine concentration	13
3.5.2.1 The Sakaguchi reaction method	13
3.5.2.2 Staron-Allard (S-A) Method	14
3.5.3 Analysis of ammonia and glucose	15
3.5.4 Measurements of cell mass	15
3.5.5 Measurement of pH	15
Chapter 4	16
The Effect of Glucose on the Initiation of the ADI Pathway Enzymes	16
4.1 Introduction	16

4.2	Materials and Methods	16
4.2.1	Lactic acid bacterial strains	16
4.2.2	Experimental procedure	16
4.2.3	Culturing procedures and sampling regime	17
4.2.4	Chemical analysis of samples	18
4.3	Results	18
4.3.1	Comparison of organisms	18
4.3.2	pH monitoring	18
4.3.3	Arginine analysis	19
4.3.3.1	Comparison of the Sakaguchi reaction method and Staron Allard method	19
4.3.3.2	Arginine data	19
4.3.3.3	Arginine recovery	22
4.3.4	Ammonia data	23
4.3.5	Citrulline Output	23
4.3.6	Starved and Non starved Cells	23
4.4	Discussion	23
Chapter 5	The Effects of Inhibiting Glycolysis on the Observed Rate of Arginine Degradation	25
5.1	Introduction	25
5.2	Materials and Methods	25
5.2.1	Lactic acid bacterial strains	25
5.2.2	Experimental procedure	25
5.2.3	Culturing procedures and sampling regime	26
5.2.3	Analysis of samples	26
5.3	Results	26
5.3.1	pH monitoring	26
5.3.2	Arginine data	26
5.3.3	Ammonia data	29
5.3.4	Citrulline Output	29
5.3.5	Fluoride effects on glucose catabolism	29
5.5	Discussion	29

Chapter 6	The Effects of Fructose Addition on the Observed Rate of Arginine Degradation in Type II Wine LAB	31
	6.1 Introduction	31
	6.2 Materials and Methods	31
	6.2.1 Lactic acid bacterial strains	31
	6.2.2 Experimental procedure	31
	6.2.3 Culturing procedures and sampling regime	32
	6.2.4 Analysis of samples	32
	6.3 Results	32
	6.3.1 pH monitoring	32
	6.3.2 Arginine data	32
	6.3.3 Ammonia data	32
	6.3.4 Citrulline Output	35
	6.4 Discussion	35
Chapter 7	Summary and Conclusions	36
	Literature Cited	38

## List of Tables

Table	Page
2.1. Groupings of wine LAB by presence or absence of ADI pathway enzymes	6
4.1. Regression Analyses for Figure 4.2 Graphs	20
5.1. Regression Analyses for Figure 5.2 Graphs	27
6.1. Regression Analyses for Figure 6.2 Graphs	33

## List of Figures

Figure	Page
2.1 The arginine deiminase pathway	4
4.1. pH Overview of Cell Activity Before and After Addition of Glucose	20
4.2 Rates of Arginine Degradation and Ammonia Production Over Time Before and After Glucose Addition	21
5.1. pH Overview of Cell Activity Before and After Addition of Fluoride	27
5.2 Rates of Arginine Degradation and Ammonia Production Over Time Before and After Fluoride Addition	28
6.1. pH Overview of Cell Activity Before and After Addition of Fructose	33
6.2 Rates of Arginine Degradation and Ammonia Production Over Time Before and After Fructose Addition	34

## 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.