

**CHARACTERISATION OF CARBOHYDRATE
METABOLISM BY INDUSTRIAL STRAINS OF
SACCHAROMYCES CEREVISIAE YEAST**

by

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THESIS SUMMARY

In the brewing industry, the rapid and efficient utilisation of fermentable carbohydrates are considered important attributes of the culture yeast. However, fermentation capabilities vary widely between brewing strains of yeast, especially with regard to the utilisation of individual sugars. Accordingly, this study was performed to determine the physiological and genetic basis for the differences in fermentation performance by brewing strains of the yeast *Saccharomyces cerevisiae* when presented with a composite of carbohydrates typically present in wort. Additionally, our investigation involved the determination of whether a desirable attribute of one strain is transferable to other strains. A model fermentation system employing a defined medium has been used to study sugar transport and catabolising capabilities. An emphasis has been placed on characterising the kinetics of utilisation of the predominant wort carbohydrate maltose as influenced by the presence of fructose, glucose and sucrose.

The first phase of our study involved a survey of twenty eight strains of *Saccharomyces cerevisiae* consisting of brewing, baking, distilling, wine and laboratory strains, with regard to their fermentation kinetics and pattern of sugar utilisation. Stronger correlations were established between shorter duration of fermentation and biomass formation and rates of maltose or monosaccharide removal. Conversely, neither high rates of sucrose hydrolysis nor the early onset of maltose utilisation had a strong influence on overall rates of fermentation. The survey also revealed three patterns of maltose and sucrose utilisation: repressible, constitutive and non-utilisers. Furthermore,

sucrose utilisation by brewing strains was seen in two forms: cell-associated and non-cell-associated.

In order to explain the divergent maltose and sucrose utilisation patterns exhibited by the strains under investigation, the presence and distribution of genes of the *MAL* loci as well as the *SUC2* gene was determined. Sequences corresponding to the *MAL1* (*AGT1*), *MAL2*, *MAL3* and *MAL4* locus were detected in the strains studied. Multiple copies of the invertase gene were also detected in the industrial strains using *SUC2* sequence as probe. Strains that exhibit the ability to utilise maltose in the presence of repressing sugar glucose and/or fructose, displayed high to moderate and largely consistent expression of the *AGT1* gene compared to non-constitutive maltose utilisers. The pattern of expression is in agreement with the pattern of maltose removal from the medium by these industrial strains, thereby suggesting that the permease encoded by *AGT1* plays a more active role in maltose uptake compared to those encoded by other *MALx1* genes. The kinetics of *AGT1* expression was, however, seen to be independent of the timing and levels of expression of the transcriptional activators, *MAL33* and *MAL13*. The pattern of sucrose utilisation correlated poorly to the kinetics of *SUC2* expression, suggesting that other *SUC* gene(s) play a greater role in the utilisation of this sugar.

To examine the importance of consistent and strong expression of the *AGT1* gene with regards to non-repressible maltose utilisation, we expressed the gene from a constitutive strain (NCYC 1681) in a laboratory strain (209) displaying a repressible maltose phenotype. The recipient strain was found to constitutively express *AGT1* and exhibited a two-fold increase in the maximum rates of maltose transport compared to the control (vector only) strain. Moreover, the pattern of maltose depletion was similar to that of the donor strain, whereby maltose was removed simultaneously with glucose in the experimental system used. However, the transformant failed to mirror the complete utilisation of maltose seen for NCYC 1681, instead the maltose taken up was apparently released back into the medium following the depletion of glucose.

In assessing the basis for differences in the kinetics of *AGT1* expression between brewing strains displaying a constitutive (NCYC 1681) or non-constitutive (CBL 1B) maltose phenotype, the structural gene as well as its promoter region were cloned and sequenced. In addition, a comparison was also made between the *MAL31* permease gene and flanking sequences of both strains. All sequences were compared with those already reported for the laboratory strain S288c in the *Saccharomyces* genome database. Nucleotide sequence analysis of the *MAL31* gene and its upstream regulatory region in both brewing strains showed them to be closely matched to that of the corresponding region of S288c. However, we did find that with the exception of a few single base pair changes, only 318 bp of sequence immediately upstream of *AGT1* was identical between the brewing and laboratory strain. Further upstream, the sequence became different from that of the laboratory strain and there was no corresponding *MAL12* gene identified.

Instead an extra non-coding sequence, which runs for at least 1500 bp was detected. Additionally, we suspect that the strong levels of *AGT1* expression by the constitutive strain was likely to be due to the absence of nucleotide sequence from its promoter, which correspond to the recognition site of the repressor protein encoded by *MIG1*.

The project is now at the stage of being able to make use of these initial findings to construct strains that have the potential to show optimised fermentation attributes.

TABLE OF CONTENTS

Statement of authorship	ii
Thesis summary	iii
Acknowledgements	vii
Publications	ix
Abbreviations	x
Table of Figures	xix
Table of Tables	xxii
CHAPTER 1: Literature review	1
1.0 Introduction	2
1.1 Brewing process	3
1.2 Brewing yeasts	6
1.3 Patterns of wort carbohydrate utilisation	9
1.3.1 Sucrose, glucose and fructose utilisation	11
1.3.2 Maltose utilisation	11
1.3.3 Maltotriose and dextrin utilisation	13
1.4 Transport kinetics of wort carbohydrates	15
1.5 Enzymatic activity of brewing yeasts	17
1.6 Genes involved in wort carbohydrate utilisation	19
1.6.1 <i>SUC</i> and <i>HXT</i> genes	19
1.6.2 <i>MAL</i> genes	21
1.7 Structure of maltose permease, maltase and regulatory genes	25
1.8 Regulation of maltose utilisation	26
1.8.1 Transcriptional control	26
1.8.2 Post-transcriptional and post-translational control	29
1.9 Aims of this study	30

CHAPTER 2: A survey of industrial strains of <i>Saccharomyces cerevisiae</i> reveals numerous altered patterns of maltose and sucrose utilisation	32
2.0 Introduction	33
2.1 Materials and Methods	36
2.1.1 Yeast strains and maintenance	36
2.1.2 Media	36
2.1.3 Fermentations	39
2.1.4 Analysis	39
2.1.5 Determination of rate of fermentation	40
2.1.6 Measurement of extracellular invertase activity	40
2.2 Results	42
2.2.1 Growth characteristics	42
2.2.2 Sugar utilisation patterns	44
2.2.2.1 Sucrose	44
2.2.2.2 Glucose and fructose	46
2.2.2.3 Maltose	47
2.2.2.4 Maltotriose	48
2.2.3 Fermentation performance	48
2.2.4 Constitutivity and solubility of invertase	51
2.2.5 Constitutive maltose utilisation	57
2.3 Discussion	60

CHAPTER 3: Expression patterns of genes and enzymes involved in sugar catabolism in industrial <i>Saccharomyces cerevisiae</i> strains displaying novel fermentation characteristics	65
3.0 Introduction	66
3.1 Materials and Methods	68
3.1.1 Yeast strains	68
3.1.2 Media	68
3.1.3 Fermentations	68
3.1.4 Residual sugar analysis	69
3.1.5 RNA extraction and Northern (slot) blot analysis	70
3.1.6 CHEF electrophoresis	71
3.1.7 Primers used for karyotyping and Northern (slot) blot analysis	74
3.1.8 Determination of maltose uptake rates	74
3.1.9 Determination of maltase activity	75
3.2 Results	76
3.2.1 Growth characteristics	76
3.2.2 Sugar utilisation pattern	79
3.2.3 Maltose permease and maltase activities	82
3.2.4 Detection of genes involved in maltose and sucrose utilisation	85
3.2.5 Kinetics of <i>MAL31</i> and <i>AGT1</i> expression	92
3.2.6 Expression of <i>MAL32</i> and <i>MAL12</i>	95
3.2.7 Expression of <i>MAL33</i> and <i>MAL13</i> activator genes	95
3.2.8 Expression of <i>SUC2</i> , the invertase structural gene	96
3.3 Discussion	97

CHAPTER 4: The transformation of a laboratory strain of <i>Saccharomyces cerevisiae</i> for constitutive <i>AGTI</i> expression	104
4.0 Introduction	105
4.1 Materials and Methods	106
4.1.1 Organisms	106
4.1.2 Culture conditions	106
4.1.3 Preparation of plasmid DNA	108
4.1.4 Construction of plasmid pET13:1_ <i>AGTI</i>	108
4.1.5 Preparation of chemically competent cells	111
4.1.6 Transformation of competent <i>E. coli</i> cells	113
4.1.7 Extraction of plasmid DNA from <i>E. coli</i> cells	113
4.1.8 Colony hybridisation	114
4.1.9 Yeast transformation	115
4.1.10 Verification of positive yeast transformants	116
4.2 Results	117
4.2.1 Introduction of <i>AGTI</i> _{NCYC 1681} into a laboratory strain	117
4.2.2 Growth characteristics	117
4.2.3 Sugar utilisation patterns	119
4.2.4 Maltose permease activities	120
4.2.5 Kinetics of <i>AGTI</i> expression	120
4.3 Discussion	123

CHAPTER 5: Cloning and sequencing of the <i>AGT1</i> and <i>MAL31</i> genes from constitutive and non-constitutive maltose utilising strains	126
5.0 Introduction	127
5.1 Materials and Methods	128
5.1.1 Yeast genomic DNA extraction	128
5.1.1.1 Ten Minute Extraction protocol	128
5.1.1.2 Genomic-Tip procedure	129
5.1.2 Primer combinations used for cloning <i>AGT1</i> and <i>MAL31</i> coding and non-coding regions	130
5.1.3 Conditions for PCR amplification of genomic DNA sequences	130
5.1.4 Adaptor oligonucleotide ligation	134
5.2 Results	137
5.2.1 Cloning of <i>AGT1</i> promoter by standard PCR technique	137
5.2.2 Cloning of <i>AGT1</i> promoter by adaptor-ligation technique	150
5.2.3 DNA sequence of the <i>AGT1</i> promoter	152
5.2.3.1 Identification of putative regulatory elements in the <i>AGT1</i> promoter of the representative strains	158
5.2.3.2 Localisation of active elements regulating <i>AGT1</i> expression	162
5.2.4 DNA Sequence of <i>MAL31</i>	164
5.2.4.1 Identification of putative regulatory elements in the <i>MAL31</i> promoter of the representative strains	168
5.2.4.2 Localisation of active elements regulating <i>MAL31</i> expression	170
5.2.5 DNA sequence analysis of the <i>AGT1</i> gene	171
5.2.6 Amino acid sequence analysis of the <i>AGT1</i> gene	177
5.2.7 DNA sequence analysis of the <i>MAL31</i> gene	182
5.2.8 Amino acid sequence analysis of the <i>MAL31</i> gene	186
5.3 Discussion	190

CHAPTER 6: Conclusions and future directions	196
6.0 Conclusions	197
6.1 Physiological characteristics	197
6.2 Genetic and enzymatic characteristics	198
6.3 Future directions	201
REFERENCES	202