CITRIC ACID PRODUCTION FROM YEASTS:

COMPARISON OF A PARENT AND A MUTANT STRAIN OF *CANDIDA GUILLIERMONDII*, AND SUBSEQENT REVERSION OF THE MUTANT

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ABSTRACT

Citric acid production from yeasts has been studied widely owing to the short duration of fermentation, the broad choice of carbon source and the better yields obtained when compared to the currently used submerged or surface fermentation with *Aspergillus niger*.

In this work two strains of *Candida guilliermondii* were compared for their citric acid-producing capabilities, these being parent strain *Candida guilliermondii* NRRL Y-448, and mutant strain *Candida guilliermondii* IMK1. The mutant was previously selected for its ability to produce much higher concentrations of citric acid than the parent. These strains were grown under various nutrient limitations to determine if nutrient limitation had an effect on the amount of citric acid produced.

Several differences were observed between the non-citric acid-producing parent and the citric acid-producing mutant. The mutant generally consumed less glucose ($g.g^{-1}$), produced less biomass ($g.L^{-1}$) and produced much higher levels of citric acid – the best production (7.34 $g.g^{-1}$) seen from the culture grown under phosphorus-limited (0.15 mM) conditions. Upon assessment of enzyme activities it was found that the mutant also exhibited reduced activity of the enzyme NAD-ICDH (NAD-dependent isocitrate dehydrogenase), a recognised control point for the over-production of citric acid. NAD-ICDH is inhibited by increased concentrations of ATP - these are associated with the accumulation of citric acid in the cell in the stationary phase of growth. This reduction in NAD-ICDH activity correlated with a dramatic increase in the activity of NADP-ICDH (NADP-specific isocitrate dehydrogenase), the activity of which was thought to compensate for the loss of activity of NAD-ICDH. However, in a subsequent experiment, the mutant was found to have reverted - losing its ability to produce citric acid. This loss of productivity occurred before the levels of adenine

nucleotides in the cell could be assessed, meaning that the suggested inhibition of NAD-ICDH by elevated levels of ATP could not be confirmed.

Upon analysis of the revertant, it was found that glucose consumption (grams per gram of cells) had increased, as had the production of biomass ($g.L^{-1}$). Even though the revertant failed to consume as much glucose as the parent, in many instances it produced higher levels of biomass. Upon analysis of enzyme activity, it was found that the activity of NAD-ICDH had increased, so reducing the accumulation of citric and isocitric acids. The activity of NADP-ICDH had decreased somewhat, but activity of this enzyme remained at significant levels. It is proposed that the activity of NADP-ICDH in the revertant was responsible for the increased efficiency of biomass production.

In conclusion, it is suggested that overproduction of citric acid in *Candida guilliermondii* IMK1 was due to the consumption of lowered levels of glucose combined with the reduced activity of the enzyme NAD-ICDH, which it is speculated was due to elevated concentrations of ATP in the cell.

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When I started on this journey of discovery, who would have known where the next few years were to take me. However, this particular little journey has finally been completed. I would like to thank my Mum and Dad for all of their love and support, which helped to keep me going when all was not going as planned. I would also like to thank John Brooks - his support and encouragement was greatly appreciated. I can't forget the other technical staff in the Food Technology department, most of whom at some time have offered advice and encouragement. Lastly I want to acknowledge myself – I finally made it!

A cloud does not know why it moves in such a direction and at such a speed
It feels an impulsion……this is the place to go now
But the sky knows the reasons and patterns behind all clouds
And you will know too – when you lift yourself high enough to see beyond horizons

Richard Bach
Illusions

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CHAPTER 1

INTRODUCTION

Citric acid is currently produced industrially using the submerged or surface fermentation with *Aspergillus niger*. Citric acid has a wide range of uses, but the majority of citric acid produced is used in the food and pharmaceutical industries.

Recently attention has been focused on the use of yeasts for the production of citric acid. Yeasts have certain advantages over fungi, some of these being the shorter duration of fermentation, broad choice of carbon source and better yields.

Much work has been performed on the mechanism of accumulation of citric acid in yeasts. The organisms most commonly used are those of the genus *Candida, C. lipolytica* (Synonym: *Yarrowia lipolytica, Saccharomycopsis lipolytica*) being the most favoured. It has been found that the optimum production of citric acid can vary depending on the type of carbon source, the medium pH, aeration and also the presence or absence of trace elements.

It is generally accepted that for citric acid production to occur, the culture must be under conditions of nutrient limitation - usually nitrogen limitation. However, other nutrient limitations have been assessed successfully for the production of citric acid.

The object of this research was to compare two strains of *Candida guilliermondii* - the parent (NRRL Y-448) and a mutant strain (IMK1). The mutant was chosen for its increased production of citric acid. Comparisons were made between the parent and the mutant to attempt to identify the differences that were responsible

for the increased production of citric acid from the mutant. Comparisons were made of glucose consumption, rates of production of citric acid, levels of intermediates and enzymes of the tricarboxylic acid (TCA) cycle.

Unfortunately, the mutant proved to be unstable and citric acid producing activity was lost after a period of time. Attempts were made to revive this mutant and to isolate a new citric acid producing mutant, but this proved to be unsuccessful.