INVESTIGATIONS ON THE HEXOSE-PHOSPHORYLATING ENZYMES IN THE PENTOSE-FERMENTING YEAST, *PACHYSOLEN TANNOPHILUS*

A thesis presented in partial fulfilment

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DAVID NEIL WEDLOCK

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ABSTRACT

Mutants of *Pachysolen tannophilus*, resistant to 2-deoxyglucose, the toxic analogue of D-glucose, have been isolated and characterised. Their growth characteristics on hexose and pentose sugars, resistance to 2-deoxyglucose and cellular hexose-phosphorylating activities were investigated. Loss of hexose-ATP-kinase activity was found to correlate with loss of ability to grow on hexose sugars and increased resistance to 2-deoxyglucose. The growth of these mutants on D-xylose was not affected.

A further series of fructose-negative and glucose-negative mutants were isolated by selecting for increased resistance to 2-deoxyglucose and by UV mutagenesis. Mutants, defective in each of the three hexose-phosphorylating enzymes found to be present in this yeast, were completely negative for growth on D-glucose, but could slowly convert this sugar to D-fructose. The conversion of D-glucose to D-fructose was hypothesised to be catalysed by the enzymes xylose reductase and xylitol dehydrogenase and experiments were conducted to investigate this possibility.

Cell-free extracts from the wild type strain and several of the glucose-negative mutants were chromatographed on DEAE-cellulose. The results of hexokinase assays and anion-exchange chromatography confirmed the existence of three hexose-phosphorylating enzymes in *P. tannophilus*. Two hexokinases which phosphorylated both D-glucose and D-fructose, exhibited F/G ratios of 1.3/1.0 and 3.0/1.0,

while a glucokinase specific for D-glucose was also present. These enzymes were referred to as hexokinase A and B and glucokinase.

Examination of the hexose-ATP-kinase profiles on DEAE-cellulose of the wild type extract from cells grown on D-glucose, D-xylose and glycerol indicated that the glucokinase and hexokinase B were constitutive, while hexokinase A was inducible.

Glucose repression of xylose reductase and xylitol dehydrogenase was found to require an active hexokinase A enzyme. This enzyme was purified from a glucokinase defective mutant by DEAE-cellulose chromatography, followed by affinity chromatography on Cibacron Blue F3G-A Sepharose (Blue Sepharose) and examined further. The Km values for D-glucose and D-fructose were 0.36 and 2.28 mM respectively. An estimated Vmax fructose/Vmax glucose was 1.5/1.0. When incubated with D-xylose in the presence of $MgCl_2$ and ATP, the enzyme was inactivated.

A strain of *Pachysolen tannophilus*, defective in all three hexose-phosphorylating enzymes, was transformed with a plasmid carrying the cloned PII hexokinase gene from *Saccharomyces cerevisiae*. The gene was expressed and the presence of the enzyme within the cells was demonstrated by DEAE-cellulose chromatography of a cell-free extract.

As part of the overall plan to attempt genetic improvement in *P. tannophilus*, two superior ethanol producing mutants were hybridised and the segregants made available for fermentation trials at the Forest Research Institute.

Hexose-negative mutants able to ferment D-xylose in the presence of D-glucose were selected for and subjected to fermentation trials. Several of these mutants produced promising concentrations and yields of ethanol from the fermentation of D-xylose, both as a sole carbon source and in a mixture of D-glucose and D-xylose.

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To my Mother

and in memory of my Father

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