INTRODUCTION

The first step in xylose metabolism by yeasts and mycelial fungi is reduction of xylose to xylitol, a reaction catalyzed by [Nicotin amid adenine dinucleatidephospho hydrogenate] NADPH-linked D-xylose reductase. This step is followed by the oxidation of xylitol to xylulose, which is catalyzed by a [Nicotin amid adenine dinucleatide] NAD-linked xylitol dehydrogenase.

The utilization of xylose, a major constituent of hemicellulose which comprises up to 30-40 % of the renewable biomass in nature, has been studied intensively for the production of valuable products such as SCP (**Feliu, *et al.*, 1990**), ethanol (**Delgenes, et al., 1990**), xylitol (**Furlan, et al., 1991**), and hydrogen (**Heyndrickx, et al., 1991**). Most studies have focused on ethanol production by natural and mutated xylose-fermenting yeasts (**Du Preez and Prior, 1985**), as well as by genetically improved *Saccharomyces* strains (**Sarthy et al., 1987**) which obtain xylose-utilizing capability.

Most xylose-assimilating yeasts convert xylose to xylulose via NADPH- and / or NADH-dependent xylose reductase and NAD-dependent xylitol dehydrogenase, whereas a single enzymatic conversion of xylose by xylose isomerase is performed in bacteria. In the case of *Saccharomyces cerevisiae* fermentation of xylose cannot proceed because of insufficient enzyme levels (**Batt, et al., 1986**) and an imbalance in the NAD / NADH redox system which is involved in xylose metabolism (**Bruinenberg, et al., 1984**). In view of the redox balance in xylose metabolism, xylose isomerase genes from several

bacteria have been cloned and transformed into *Saccharomyces cerevisiae*. The enzymes produced in *Saccharomyces cerevisiae* were, however, found to be inactive (**Amore et al., 1989**). The cloning and expression of the xylose reductase (**Takuma, et al, 1991**) and xylitol dehydrogenase genes (**Kötter, et al., 1990**) isolated from *Pichia stipitis*, possessing the dual cofactor (NADPH / NADH) specific xylose reductase have been investigated.

The yeast *Saccharomyces cerevisiae* is one of the simplest eukaryotes with a genome size only about three times that of *Escherichia coli* **(Clson, 1981)**. The nuclear genome consists of 16 chromosomes that are catalogued according to size as determined by chromosome separation by pulsed-field gel electrophoresis (PFGE), (**Schwartz and Cantor, 1984**).

Tetrad analysis may be defined as the genetic analysis of all the products of single meiotic event in organisms with (ordinarily) cytologically observable chromosomes (eukaryotes) and a standard meiosis. Tetrad analysis is possible in those organisms in which the four products (or derivatives) of a single nucleus which undergone meiosis are grouped together. Tetrad analysis is now routinely employed to assign newly found mutant genes to linkage groups (chromosomes) and then to map each gene in relation both to the chromosomal centromere and other genes on the same chromosome. This is accomplished by analyzing the segregation patterns of different heterozygous markers among the four haploid spores. Initially, such mapping was much more time-consuming than mapping *E. coli*. Genetic analysis has revealed

that the minimum number of chromosomes in haploid *Saccharomyces cerevisiae* strain is 17 (**Mortimer and Schild, 1980**).

Through the use of such "tricks" the number of mapped genes in *Saccharomyces cerevisiae* is now 568, roughly half the number of mapped *E. coli* genes, and is increasing rapidly. However, since yeast contains some four times more DNA than *E. coli*, the mapping of yeast genome still remain in relative infancy (**Mortimer and Schild, 1985** and **Watson et al., 1987**).

Passoth et al., (1992) determined the electrophoretic banding patterns of the chromosomes of *Pichia stipitis* and *Candida shehatae*. Both species exhibited six chromosomal bands, except for one strain of *Candida shehatae, which* had only five bands. Strains of the two species showed considerable chromosomal length polymorphisms.

Transverse agarose field electrophoretic (TAFE)-karyotyping of *Pichia stipitis* CSIR Y633 revealed a total of four chromosomes ranging from 0.93 to 1.86 Mb (**Selebane et al., 1993**).

An initial breakthrough was discovered that *Saccharomyces cerevisiae, Schizosaccharomyces pombe,* and some other yeasts could ferment the keto isomer of xylose and xylulose (**Wang et al., 1980**). This was significant because the commercial enzyme, xylose isomerase (= glucose isomerase), will produce xylulose from xylose. This approach served as the basis for fermenting xylulose with *Saccharomyces cerevisiae* (**Chiang et al., 1981 and Suihko and Poutanen, 1984**) *Schizosaccharomyces pombe* (**Ueng et al., 1981 and Lastick et al.,**

1989), *Candida tropicalis* (**Jeffries, 1981**), and other yeasts (**Gong et al., 1981,c**).

Researchers in four laboratories discovered the direct conversion of xylose to ethanol almost simultaneously. Two groups observed ethanol production directly from xylose following screens of yeasts for anaerobic xylose metabolism (**Schneider, et al., 1981 and Slininger et al., 1982**). One observation came from mutation and selection studies on a yeast strain known to assimilate xylose (**Gong et al.,1981**) and one came from chance observations of ethanol production from xylose / xylulose mixtures under aerobic conditions (**Jeffries, 1981**).

Sugars obtained by hydrolysis of lignocellulosic material represent an interesting substrate for ethanol production in terms of availability and cost. Depending on the hydrolysis process, two-stages or single-stage, glucose and xylose derived from lignocellulose can be converted into ethanol by separate fermentation or a co-culture process using in both cases a glucose-fermenting micro-organism and a xylose-fermenting yeast (**Du Preez et al., 1986**).

The aim of this work is an attempt to transfer genes of *Pichia stipitis* which is responsible for production of ethanol alcohol from xylose sugar to done *Saccharomyces cerevisiae* mutants strains in order to obtain a transformable strain of *Saccharomyces cerevisiae* having the ability to produce ethanol from xylose.