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Oxidation and Reduction of D-Xylose by Cell-Free Extract of *Hansenula polymorpha*

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Abstract: The mechanism of oxidation and reduction of D-xylose by cell-free extract of *Hansenula polymorpha* was studied in medium contain xylose as carbon source. Xylose dehydrogenase which NADP was detected dependent in the cell free extract. Both the oxidation and reduction were coupled in this enzyme and proved by HPLC as xylose; xylitol and xylonic acid. Xylose reductase, NADPH dependent in cell free extract that reduce xylose to xylitol. Both D-xylose and L-arabinose can be used as substrate Xylitol dehydrogenase was also found in the cell free extract which is NAD dependent and xylulose produced in the reaction was identified and it was found to oxidize a variety of polyol sugars.

Key words: *Hansenula polymorpha*, Xylose dehydrogenase- cell free extract- Xylose reductase- Xylitol dehydrogenase- NADP-NADPH-NAD-NADH-Xylose.

INTRODUCTION

Lignocellulosic material containing cellulose, hemicellulose, and lignin is an abundant renewable organic resource that can be used for the production of energy and biochemicals (Weber *et al.*, 2010). The conversion of both the cellulose and hemicellulose fractions for producing biochemicals is being intensively studied. Between 23% to 40% of the lignocellulosic biomass consists of hemicelluloses, the main component being xylose in most hardwoods and annual plants (Lee *et al.*, 1979). Although abundant, D-xylose is not considered to be fermentable (to ethanol) by yeasts (Barnett, 1976). Numerous studies have been carried out on various aspects of D-xylose bioconversion (Winkelhausen and Kuzmanova, 1998; Townsend and Howarth, 2010). In some yeasts and fungi, conversion of D-xylose to D-xylulose more often occurs by two enzymatic steps. First, D-xylose is reduced by a NADPH/NADH-linked xylose reductase (XR) to xylitol, whereupon the latter is oxidized to xylulose by an NAD-linked xylitol dehydrogenase (XDH) (Bruinenberg and Van Dijken, 1983). D-xylulose is subsequently phosphorylated to D-xylulose-5-phosphate by D-xylulokinase before entering the pentose phosphate, Embden-Meyerhof, and phosphoketolase pathways (Skoog and Hahn-Hagerdal, 1988). Some yeasts as in *Candida shehatae* and *Pichia stipitis* (Dupreez *et al.*, 1986); and *Pachysolen tannophilus* (Morimoto *et al.*, 1986) an unusual pathway for xylose oxidation to xylonic acid by xylose dehydrogenase enzyme found in cell free extract of *Pichia quercuum* described by (Suzuki and Onishi, 1975). Recently, recombinant *Saccharomyces cerevisiae* expressing a fungal pentose gen able to ferment all sugars present, including the pentose sugars L-arabinose and D-xylose (Bettiga *et al.*, 2009) to produce ethanol.

The aim of this work is to study the mechanism of formation of xylitol, xylonic and xylulose from corn cops (as a source of xylose) by cell free extract of *H. polymorpha*.

MATERIALS AND METHODS

Hansenula polymorpha was obtained from Microbial and Natural Products Chemistry Lab National Research Centre, Cairo, Egypt.

Maintenance Medium:

The organism was maintained on YPD medium containing 10g of yeast extract, 20g of peptone, 50g of D-glucose and 10g of agar per liter of distilled water.

Preparation of Inocula:

A loopful of *H. polymorpha* cells from a YPD medium agar slant was transferred to 20ml of medium containing 0.67% (w/v) yeast nitrogen base (YNB; Difco) without amino acids and 2% (w/v) xylose. The culture was incubated at 30°C in a loosely capped 125ml Erlenmeyer flask which was agitated at 200 rpm in a New Brunswick shaker for 48h (Lee *et al*., 1986).

Growth Medium:

Inoculums 2ml of *H. polymorpha* culture for each was transferred to 50ml of medium containing the following gradient (g/l): $(NH_4)_2SO_4$, 0.1; yeast extract, 4.0; NaCl, 2.5; xylose, 10.0; KH_2PO_4, 1.5 and K_2HPO_4, 1.0 and pH was adjusted to 6.0. The culture was incubated at 30°C in a loosely capped 125ml Erlenmeyer flask

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