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Investigation on *Candida Parapsilosis strain BKR1* for the Production of Xylose reductase enzyme andits Kinetic Parameter Appraisal

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Abstract: Xylitol is a valuable product with rising demands in market; it becomes significant to produce xylitol with economic justification due to its more insulin independent metabolism and anticariogenic properties. In the presentstudy, growth of *Candida parapsilosis strain BKR1 (NCBI accession no.:KC462059)* andits xylitol production capability were assessed. The enzyme xylose reductase (XR) was partially purified and estimated for its kinetic parameters were estimated. Under Optimized conditions, the specific activity of xylose reductase was found to be 0.464 U/mg of protein.The Km and Vm were estimated as 62.016 mg/l and 0.560 U/ml.
Keywords: xylose reductase (XR), xylitol, *Candida parapsilosis strain BKR1*

Introduction

The demand for Xylitol may be much in the country today due to lack of awareness and availability. The techno- economic feasibility for extracting Xylitol from bagasse (and using the residues for paper making or boiler fuel) may lead to a cheaper cost of the product compared to one obtained from birch wood or hard wood chips as practiced in the European countries.

Candida yeasts are promising strains in fermenting about 40% D-xylose in 24-48 h to yield xylitol.[1]According to Gong et al., out of ten yeast strains compared for xylose conversion, C. tropicalis HPX2 showed highest xylitol yield of 0.8 g/g of xylose.[2]The xylitol fermentation process can be influenced by the abiotic factors, initial substrate concentration and inoculum age, this affecting the metabolic activity and the viability of the cells.[3]India with over 400 sugar mills produces around 70 million tons of bagasse every year. The TIFAC (GoI) reportson 'System Approach to Bagasse Utilization'in detail the national and international trends and scenarios on various means of bagasse utilization.

Present investigation were undertaken to address the problem of xylitol, a natural sweetener, production through cost effective biotechnological means. It starts from the identification of novel yeast isolates to mass production of the natural sweetener for the benefit of biopharmaceutical and healthcare sectors. This first reported yeast strain is extracted for the xylose reductase and studied for kinetic parameters and specific activities.

Materials & Methods

Microbial Growth and Fermentation

The microorganism used in this study was isolated from sugarcane extracts processed in the Sathyamangalam, Erode District, Tamil Nadu, India. Among the 24 isolates, *Candida parapsilosis strain BKR1* (*KC462059*) was one of the prominent xylitol producing yeast which was used throughout this report. It was maintained on agar slant containing 3 g of malt extract, 3 g of yeast extract, 5 g of peptone, 10 g of xylose and 15 g of agar per litre at 37 °C for 48 hours. The modified minimal (MM) medium used contains: 5g K_2HPO_4, 1g KH_2PO_4, 0.5g $MgSO_4.7H_2O$, 0.1g $CaCl_2.2H_2O$, 1g Yeast Extract, 20g Xylose per litre.[4]

Studies on Enzyme purification and kinetic parameter estimation were carried out with MM medium grown yeasts described elsewhere. The fermentation was carried out at 30°C and pH 4.0 in MM Medium with xylose as sole carbon source and an agitation rate of 100 rpm.

Preparation of Crude Enzyme Extract

Cells were harvested by centrifugation at 6000 rpm and washed in phosphate buffer (50 mM, pH 7.2) and the cell pellets were stored in refrigerator (4°C). For enzyme assays, cell extracts were thawed and disrupted by sonicator (SONICS, USA) using ultrasound waves at 4°C for 20 min. Cell homogenates were centrifuged at 10000 rpm for 15 min (4°C) and the supernatant solution was used for enzymatic assays.

Xylitol Extraction

The fermentation broth was centrifuged at 6000 rpm for 15 minutes to separate cells. The supernatant was filtered through 0.45µm membrane (Millipore, USA). Further, permeate was extracted against ethyl acetate (1:3). The aqueous fraction was analyzed for xylitol presence using Fourier Transform Infrared Spectroscopy and High Performance Liquid Chromatography. (Datas not shown)

Enzyme assay

Xylose reductase activity was determinedspectrophotometrically at 340 nm at room temperature.[5]One unit of XR was defined as amount of enzyme required to produceone µmol of xylitol in one minute.[6]Specific activities were expressed asU/mg of protein based on protein determinations according to the Lowry's methodusing bovine serum albumin as the standard.[7]

Determination of Kinetic parameters and specific activity

Values of the yields for growth and xylitol formation were calculated Kinetics parameters for xylose reductase were obtained by keeping the constant xylose concentration by varying co-substrate (NADPH) concentration (0.1 – 1.5 mM). All of the measurements were performed at optimal pH and temperature. K_m and V_m values were estimated from double reciprocal plot. The experiments were performed in duplicates and average values are taken.

Results & Discussion

Growth of Microorganism

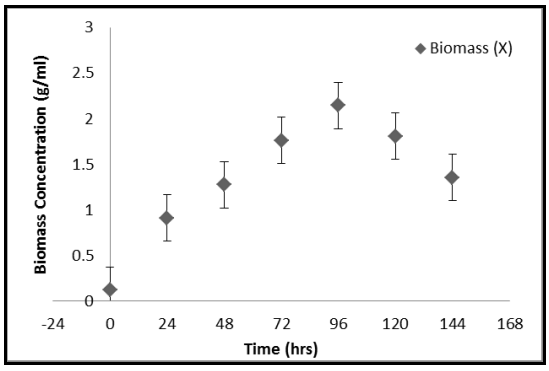

Fig.1 Growth Pattern of Candida parapsilosis strain BKR1

The microorganism was identified based on molecular techniques and bioinformatics tools. The conserved sequence was deposited in NCBI database. The identification revealed that the organism belongs to *Candida sp.*, and named as *Candida parapsilosis strain BKR1*.[8]The growth of the microorganism is plotted as graph between time and biomass concentration.(Fig.1). Experiments were conducted in triplicates. Microbial growth rate depletes from the 4th day from inoculation. The crude enzyme was extracted from 48 and 72 hours culture.

Calculation of Kinetic Parameters & Specific Activity for Xylose Reductase Enzyme

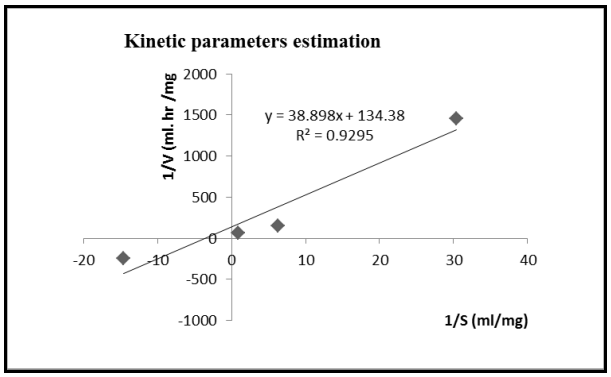

Fig.2 Estimation of Kinetic Parameters of Xylose Reductase obtained from Candida parapsilosis strain BKR1

Kinetics of Xylose reductase from *Candida parapsilosis strain BKR1* was investigated at various concentrations (0.1-1.5 mM) of NADPH as co-substrate and constant xyloseconcentration using spectrophotometry at 340 nm. The values obtained for kinetic parameters MichelisMenten constant (K_m) and maximal reaction velocity (V_m) for xylose reductase of *Candida parapsilosis strain BKR1,* showed the following characteristics: the apparent K_m& V_m values were found to be 48.23 mM and 0.560 U/ml respectively. The specific activity of the xylose reductase was calculated to be 0.465 U/mg of protein. (Fig.2) The decrease in the Km values represents the increased substrate affinity and increase in Vm reveals the highest enzyme activity. From the earlier investigations of Sirisansaneeyakul et al., the specific activity of Xylose Reductase from *Candida mogii* was found to be 0.12 U/mg of Protein.[9] It is much interesting to denote the xylitol production capability increases by enhancing specific activity of Xylose reductase. According to Cortez et al., if the enzyme molecules are partially disrupted in structure, the affinity for the substrate decreases and that of coenzyme increase.[10] Hence it is important to consider the kinetic constants may be affected for purified enzymes. It is noteworthy to discuss the findings of Guo et al., that the *candida sp.*, isolated from them could yield a specific activity 0.59 U/mg of protein.[11]The cofactor NADPH assures better xylitol yield compared to another cofactor NADH. According to Furlan et al., the oxygen limitation leads to the increase in specific xylitol production rate possibly due to the action of xylose reductase enzyme.[12] According to Bruinenberg et al., NADPH is mainly regenerated by the enzymes glucose 6 phosphate and 6 phosphogluconate dehydrogenase in almost all xylose consuming yeast.[13] Thus oxygen limiting period allows the yeast to create imbalance in NAD^+/NADH redox system and xylose reduction is fully dependent on NADPH. Ultimately the NADH/ NADPH concentration increases, which favours the xylitol accumulation.

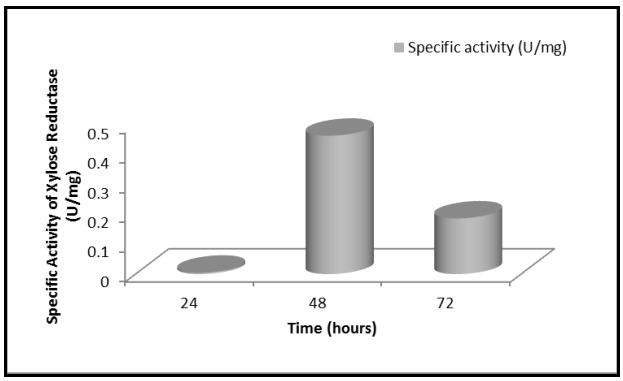

Fig.3 Comparision of specific activities of Xylose Reductase during different time period from Candida parapsilosis strain BKR1

However, the enzyme was extracted from 72 hours culture and the specific activity was compared with 48 hours enzyme extracts. It was found that the activity was reduced in the crude enzyme extracted from 72 hours culture. (Fig 3)

Conclusion

The Candida parapsilosis strain BKR1 was effective in converting the xylose from xylitol under the optimized culture conditions. The primary enzyme responsible for the conversion is xylose reductase. Based on the present investigation, the specific activity of xylose reductase is high during the 2nd day from the inoculation. The yield of xylitol is also high during the second and third day from the inoculation. The study paves the way to understand the role of Xylose reductase in xylitol production. The value of kinetic constants reveals that it has the commercial significance at the cost of cheaper raw material source. Further exploration on the commercial production of xylitol will lead to satiate the xylitol demand in present market.

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