

Extraction, purification and analysis of thermal stability of xylose isomerase

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Abstract

Thermostable enzymes are the enzymes which active even at high temperatures, such enzymes are industrially as well as biochemically very important. Xylose isomerase (EC 5.3.1.5) is one such enzyme with suitable commercial applications. It is heat stable and does not require expensive cofactors such as NAD or ATP for activity. The microorganisms producing this enzyme were isolated from hot water spring near 'Surat'. The organisms were isolated and purified by using different screening methods. The isolated organisms were then subjected to optimum growth conditions for enzyme production. This enzyme was then assayed for its thermal stability at elevated temperatures by using DNSA.

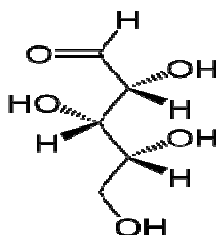
Keywords: Thermostable, xylose isomerase, xylulose, DNSA

INTRODUCTION

Sugars are major sources of energy in living organisms. These are polyhydroxy derivatives of aldehydes and ketones.

Physical Properties of Xylose

It is white coloured sugar amorphous in nature. Sugar is water soluble. Molecular formula: $C_5H_{10}O_5$. Appearance: monoclinic needles or prisms, colorless. Structural formula of xylose is as follows [1]



Chemical Properties: It is reducing, pentose sugar.

Chemical formula: $HOCH_2(CH(OH))_3CHO$.

Molar mass: 150.13 g/mol

Density: 1.525 g/cm³ (20 °C)

Melting point: 144-145 °C

Xylose is the main building block for hemicellulose which comprises about 30% of plant matter. Xylose is otherwise pervasive, being found in the embryos of most edible plants. It was first isolated from wood by Koch in 1881. Xylose is also the first saccharide added to the serine or threonine in the proteoglycan and so it is the first

saccharide in biosynthetic pathways of most anionic polysaccharide such as (heparin) sulfate and chondroitin sulfate. In animal medicine, xylose is used to test for malabsorption by administration in water to the patient after (fasting). If xylose is detected in (blood) or (urine), within the next few hours it will be absorbed by the intestines. Organic redox reaction of xylose by catalytic hydrogenation produces the non-cariogenic sugar substitute xylitol.

Xylose is utilized by various pathways as follows

The oxo-reductive pathway

This pathway is also called the Xylose Reductase-Xylitol Dehydrogenase or XR-XDH pathway which results in the formation of D-xylulose-5-phosphate which is an intermediate of the pentose phosphate pathway [2].

The isomerase pathway

This pathway results in the conversion of D-xylose into D-xylulose. D-xylulose is then phosphorylated to D-xylulose-5-phosphate as in the oxidoreductive pathway.

Dahms pathway

The Dahms pathway starts as the Weimberg pathway. One organism that can naturally ferment D-xylose to ethanol is the yeast *Pichia stipitis*.

Enzymes

Enzymes are biological catalysts which accelerate the various biological reactions without participating in it.

Xylose Isomerase (XI)

Xylose isomerase is an enzyme synthesized by microorganisms, active at high temperatures and shows two types of activities as follows [3].

Received: Dec 02, 2011; Revised: Dec 28, 2011; Accepted: Jan 17, 2012.

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1. In vivo Xylose $\xrightarrow{\text{Xylose isomerase}}$ Xylulose
2. In vitro Glucose $\xrightarrow{\text{Xylose isomerase}}$ Fructose.

Physical Properties

Generally enzyme activity is affected by physical as well as chemical environmental factors viz. Temperature, pH, substrate concentration, amount of enzyme, etc. As enzymes are being protein in nature their structure is also an important factor affecting their activity & stability. More the complex & highly folded structure, more is activity & stability. Xylose Isomerase is a thermostable enzyme, showing optimum activity at temperature range of 60°C-97°C [4].

Chemical Properties

Two types of Xylose Isomerases are found. Type I enzymes are shorter than type II by about 50 amino acids at their N-terminus. It requires Mg⁺⁺, Co⁺⁺ & Mn⁺⁺ for significant & stabilized activity [5]. Thermal stability of an enzyme is due to high proline contents. Mutations in Proline residues affect stability & activity of the enzyme.

Micro organisms synthesizing Xylose Isomerase

Fungi – *Aspergillus awamori*

Bacteria– *Thermoanaerobacterium thermosulfurigenes*, *Thermotoga neapolitana*, *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus fermenti*, *Micromonospora coerulea*, *Nocardia asteroides*, *Oceanobacillus iheyensis*, *Paracolobacterium aerogenoides*, *Rhizobium loti*, *Ruminococcus flavefaciens*, *Salmonella typhimurium*, *Sarcina*, *Tetragenococcus halophilus*, etc.

MATERIALS AND METHODS

Sample collection

Sample was collected from hot water spring near 'Surat'. Hot spring water is very good source of thermophilic organism.

Enrichment

Enrichment of flora of hot spring water was done in mineral broth containing 1% Xylose. Broth was incubated at 55°C for 24 hours in shaking water bath [6].

Isolation of Xylose utilizing micro-organism

Loopful from enriched broth was inoculated in sterile saline & serial dilutions were prepared. Loopful from 10³ dilutions was streaked on nutrient agar plate containing 1% xylose, to get maximum isolation. Plates were incubated at 55°C for 24 hours

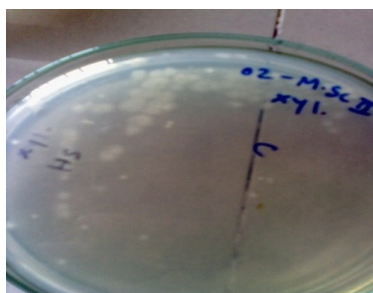


Fig 1. Growth of xylose utilizing organism on nutrient agar plate with 1% xylose.

Production of enzyme

For the production of enzyme, selected organism was inoculated in Nutrient broth containing 1% Xylose and Glucose separately [7]. Broth was incubated at 55°C for 24 hours.

Extraction of intracellular enzyme

Incubated broth containing 1% xylose was centrifuged at 10,000 rpm for 10 min. Supernatant was discarded. Pellet was resuspended in sterile saline and cells were broken using sterile glass beads and after centrifugation supernatant containing enzyme were collected [8].

Extraction of extracellular enzyme

24hrs old broth inoculated with the isolated culture containing 1% glucose was centrifuged at 10,000 rpm for 10min. Supernatant was collected which contains enzyme.

Purification of enzyme

Purification of enzyme was done by passing enzyme samples through Glass-wool and then followed by Dialysis [8].

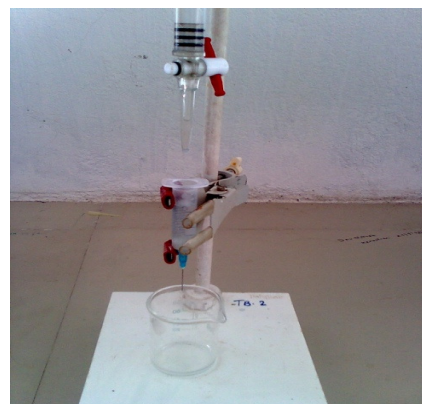


Fig 2. Extra Cellular Enzyme



Fig 3. Intra Cellular Enzyme

Detection of Protein by Biuret test

After the enrichment of culture, production of protein by organism was detected by Biuret test [9].

Biuret Reagent

300 mL of 10% (w/v) sodium hydroxide copper sulfate pentahydrate and 1.2% sodium potassium tartarate were mixed by continuous stirring. Then diluted to 1 lit. For this, 2ml broth was centrifuged in micro-centrifuge at 5000 rpm for 10 min. Supernatant was used as sample and mixed with 5 ml Biuret reagent and incubated at room temperature for 15 minutes and observed for Bluish Violet colour [10].



Fig 4. Detection of Protein

Thermostability assay

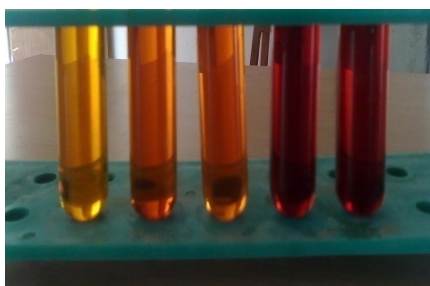
This assay was carried out by DNSA [9]. Purified enzyme solution was mixed with its substrate i.e 1% Xylose solution for intracellular enzyme activity and 1% Glucose solution for extracellular enzyme activity. It was allowed to react for 5 minutes. DNSA reagent was added to enzyme substrate mixture and incubated at desire period of time. After the completion of incubation period, the optical density was noted down at 540 nm [11].

Table 1. Intracellular enzyme activity

Temperature in °C	O.D at 540 nm.
4	0.09
R.T. (30)	0.20
50	0.22
65	0.87
95	0.75

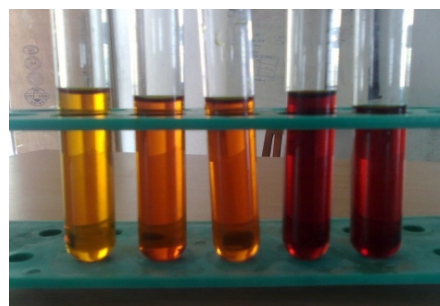
Table 2. Extracellular enzyme activity

Temperature in °C	O.D at 540 nm.
4	0.19
R.T. (30)	0.30
50	0.30
65	0.62
95	1.86



Maximum activity was observed at 65°C.

Fig 5. Thermo stability assay for intracellular enzyme (Temperature range: 0°C-95°C from left to right)



Maximum activity was observed at 95°C.

Fig 6. Thermo stability assay for extracellular enzyme (Temperature range: 0°C-95°C from left to right)

RESULTS

Both the purified enzymes were found to be catalyse, the conversion of xylose with different temperature optima. Maximum activity for intra cellular enzyme was observed at 65°C whereas for extra cellular enzyme maximum activity was observed at 95°C.

DISCUSSION

Xylose isomerase is a thermostable enzyme which converts Xylose into Xylulose in vivo [12]. The enzyme extracellularly acts as thermostable [2]. Syrup products lie in the discovery of glucose-isomerizing enzymes. Historically, four different types of enzymes have been termed glucose isomerases. Although the affinity of this enzyme was 160 times lower for glucose than for xylose [1], it was sufficient for the enzyme to be commercially significant. Production of the enzyme required xylose in the growth medium and was enhanced in the presence of arsenate. Later, a xylose isomerase activity, which was independent of xylose, was found in *Escherichia intermedia*. The enzyme was a phosphoglucose isomerase (EC 5.3.1.9), which can isomerize the unphosphorylated sugar only in the presence of arsenate. Takasaki and Tanabe isolated from *Bacillus megaterium* glucose isomerase (EC 5.3.1.18) which was NAD linked and was specific for glucose. A similar glucose isomerase, which catalyzed the isomerization of both glucose and mannose to fructose, was isolated from *Paracolobacterium aerogenoides*. The glucose isomerases produced by heterolactic acid bacteria require xylose as an inducer and are relatively unstable at higher temperatures. Of these glucose isomerizing. The potential for using sugar substitutes produced from starch was proposed by several workers. Enzymatic glucose isomerization was first accomplished on an industrial scale in 1967 by Clinton Corn Processing Co. in the Unit1974. The demand for HFCS in the food industry increased, and by 1980 practically all major starch-processing companies in the western world were resorting to GI technology. Today, the enzyme commands the biggest market in the food industry. Immobilized Glucose isomerase is also commercially available.

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