RESEARCH ARTICLE

# Mining xylose isomerase producing microbes

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**ABSTRACT**

An aerobic, rod shaped, mesophilic, gram positive xylitol producing bacterial isolates were made from different enriched substrates. Xylose isomerase is an enzyme that catalyzes the interconversion of D-xylose and D-xylulose. The microbes producing xylulose from xylose via xylose isomerase enzyme were screened for the xylose isomerase activity based on 2, 3, 5-Triphenyl tetrazolium dye retention capacity. Among the fourteen isolates screened, only six isolates showed the positive results by retaining the dye of which XLY3 was the best. Phylogenetic and sequence analysis of 16SrRNA gene showed 98 % homology to *Bacillus endophyticus*.

Keywords: Microbial strains, XLY3, Xylose isomerase, 2, 3, 5-Triphenyl tetrazolium dye

## Introduction

India being a tropical country receives more than 12 h of sunshine a day, the conversion of sunlight into biomass through photosynthesis is too high, and we need to focus on the effective utilization of plant biomass for generating value-added bioproducts. The amount of solar energy received on earth's surface is $2.5 \times 10^{21}$ Btu year$^{-1}$ (Kumar et al., 2016). Hemicellulose is the second most important and abundant polysaccharide in nature. Bioconversion of LCB into sugar-derived fuels and other biomaterials has gained extensive research interest during the last few decades due to the feasibility of this sustainable and eco–friendly process (Kim, 2019).

Xylan is a group of hemicelluloses that are made from units of xylose and are found in plant cell walls and some algae (Saha, 2003). Xylose is one of the sugars present in xylan and can be utilized by its direct conversion to xylulose via xylose isomerase and by reduction to xylitol using xylose reductase. The xylose isomerase enzyme is used for large scale production of glucose–fructose syrups (Bhosale et al., 1996).

The microbiological process uses bacteria, fungi, yeast, and recombinant strains to produce xylitol from pure xylose or a hemicellulosic hydrolyzate. A few bacteria, such as *Enterobacter liquefaciens*, *Corynebacterium* sp., *Mycobacterium smegmatis*, *Bacillus* sp., and *Gluconobacter oxydans*, produce xylitol (Rafiqul and Sakinah, 2013).

Biotechnological xylitol production is a potentially attractive replacement for existing chemical process, as it occurs under milder process conditions and can be based on sugar mixtures derived from low-cost industrial and agri-wastes (Dasgupta et al., 2017). Compared to chemical processes microbial utilization of xylose will be cost effective. With this background, the present paper discusses the isolation and identification of xylose isomerase producing microbes.

## Materials and method

### Isolation and screeing of xylose isomerase producing microbes

Different hemicelluloses containing substrates were placed in compost pit for enrichment. From the enriched substrates, xylitol producing microbes were isolated by dilution plate technique on YPD (Yeast extract 0.5%, Peptone 0.5%, D-Glucose 2%, Agar, pH 5.5) and the plates were incubated at 30°C for 24 hours. The isolated microbes were screened for xylose isomerase activity (Sapunova *et al.* 2002). The cultures were spot inoculated in YPX medium (yeast extract 10g/l, peptone 20g/l, xylose 50g/l, agar 20g/l and pH 5.0) and grown for 3 days. These plates were incubated with reaction mixture (Water, 0.2M k, Na- $PO_4$ buffer (pH 7.8), 0.1M MgSO4, 0.1M D-xylose in 12: 5: 2:1 ratio) at 70°C for 10 min and washed with distilled water. The 2, 3, 5-triphenyl tetrazolium chlo- ride (0.1 %) in 1N NaOH was added and incubated at 30°C for 1 min in dark. The xylose isomersse producing isolates retained of pink colour.

***Genomic DNA isolation and microbial identification using 16S rRNA sequence***

The total genomic DNA was isolated from the xylose isomerase producing best isolate XLY3 ac- cording to Bust and Grab protocol (Harju *et al*., 2004). The genomic DNA from the isolate XLY3 was amplified using 16S rRNA gene specific primers 27 F (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492 R (5’-ACGGCTACCTTGTTACGACTT-3’). The PCR condi-tions adopted was as follows: 95°C for 5 min; 30 cycles of 94 °C for 1min, 55°C for 1 min, and 72°C for 90 s; and 72°C for 10 min (Weisberg *et al*., 1991). The PCR products were sequenced at Eurofins, Bangalore. The sequence was analysed using NCBI BLAST analysis and phylogenetic tree was constructed using Mega 4.0.

**Results and discussion**

Xylan, a hemicelluloses group of polymer is used for the production of several bioproducts, of which xylitol is an important compound with sever- al advantages like preventing dental cavities, having anti-ketonic and anti-infection effect. Xylitol is a sugar alcohol and contains 40% fewer calories than sucrose. Xylitol is produced by catalytic hydrogenation of xylose, which is costly and needs harsh reaction conditions. Biological xylitol production is more cost effective, since no expensive catalysts are needed and the reaction takes place at ambient temperatures (Mueller *et al*., 2011).

| S.No | Substrate | Name of the isolates |
|---|---|---|
| 1. | Pomegranate | POY |
| 2 | Papaya | PAY |
| 3 | Pear | PEY |
| 4 | Banana | BAY |
| 5 | Grapes | GRY |
| 6 | Apple | APY |
| 7 | Xylan | XYY |
| 8 | Xylose | XLYI,XLY2,XLY3 |
| 9 | Arabinose | ARY |
| 10 | Carboxymethyl cellulose | CMCY1,CMCY2 |
| 11 | Glucose | GLY |

Table 1. List of yeast isolates obtained from Manikaran enrichment samples

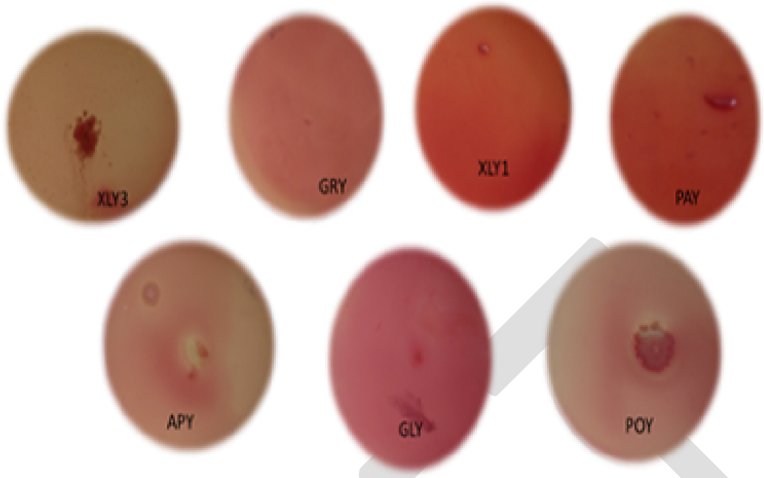


Figure 1.Positive cultures for xylose isomerase

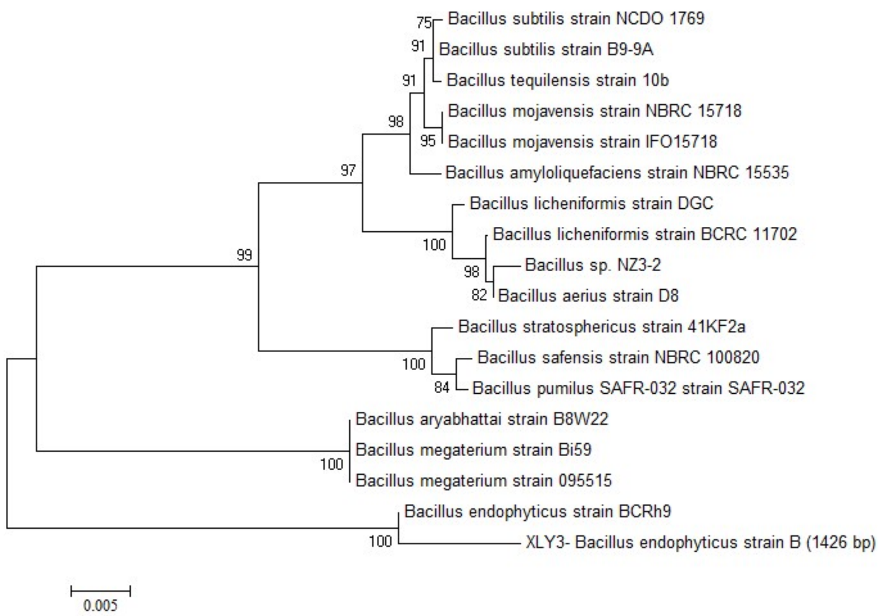


Figure 2. Phylogeny tree of Microbial isolate XLY3 based on 16SrRNA gene

D-xylose utilization by micro-organisms is possible both by its direct conversion to D-xylulose under the action of xylose isomerase (EC 5.3.1.5) and by reduction, with the involvement of xy- lose reductase (EC 1.1.1.21), to xylitol, whose further oxidation to D-xylulose occurs by means of xylitol de- hydrogenase (EC 1.1.1.9). Hence the present study fo- cuses on xylose isomerase producing microbes from various substrates.

The xylan rich substrates namely Pomegranate, Papaya, Pear, Banana, Grapes and Apple, Xylan, Xylose, Arabinose, Carboxymethyl cellulose and Glucose were enriched in compost pit and microbes were isolated from the enriched substrates. Totally 14 isolates were made (Table 1). Among the 14 isolates, 6 were positive for xylose isomerase activity. On reaction with tetrazolium chloride dye, the positive isolates retained the rose red color on the colony (Figure 1). The rose red color denotes the presence of xylulose formed by isomerisation of xylose (Sapunova *et al*., 2004). The degree of pink color intensity varies between microbial isolates. Sapunova *et al*. 2004 discuss that the Intensity of rose red color depends on the capability of D-xylulose to oxidize colorless 2, 3, 5-triphenyl tetrazolium chloride in an alkaline medi- um to formazon with rose red color. Among the 7 pos- itive isolates (XLY3, GRY, XLY1, PAY, APY, GLY and POY), XLY3 showed maximum retention of pink colour due to high xylose isomerase activity. Hence it is selected for further studies.

The part of the DNA most commonly used for taxonomic purposes for bacteria is the 16S rRNA gene (Weisberg *et al*., 1991). The amplification of 16S rRNA gene from XLY3 isolate resulted in 1500 bp fragment and was sequenced. The NCBI-BLAST analysis of XLY3 16S rRNA sequence showed 98 % homology to *Bacillus endophyticus strain BCRh9* and the phylogenetic tree was constructed on the aligned datasets using neighbor joining (NJ) method (Figure 2). Xylose isomerase producing bacteria had been reported from the genus *Clostridium, Arthrobacter, Streptomyces and Bacillus* respectively. Kwon *et al*. (1989) reported the production of xylose isomerase by Alkalophilic *Bacillus.*

**Conclusion**

Xylan rich substrates can be used for the pro- duction of xylitol by biological method. The microbial conversion of xylan to xylitol is cost effective. D-xylose utilization by microorganisms is possible both by its direct conversion to D-xylulose under the action of xylose isomerase and by reduction, with the involvement of xylose reductase to xylitol, whose further oxidation to D-xylulose occurs by means of xylitol dehydrogenase. In the present study, *Bacillus endophyticus* XLY3 with high xylose isomerase was isolated. Xylose reductase and xylitol dehydrogenase in the microbe need to be characterised. With further optimisation of culture conditions *Bacillus* *endophyticus* XLY3 can effectively be used for xylitol production.

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