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RESEARCH ARTICLE

Mining Xylose Isomerase Producing Microbes

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ABSTRACT

Xylose isomerase is an enzyme that catalyzes the interconversion of D-xylose and D-xylulose. An aerobic, rod-shaped, mesophilic, gram-positive xylitol producing bacterial isolates were isolated from different enriched substrates. 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 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*.

Key words: *Microbial strains; XLY3; Xylose isomerase; 2, 3, 5-Triphenyl tetrazolium dye; Xylitol*

INTRODUCTION

India is a tropical country, receives more than 12 h of sunshine a day, and the conversion of sunlight into biomass through photosynthesis is too high. 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 2.5×10^{21} Btu year^{-1} (Kumar *et al*., 2016). Hemicellulose is the second most important and abundant polysaccharide in nature. Bioconversion of lignocellulosic biomass (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 glucose-fructose syrups production (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*, produces xylitol (Rafiqul and Sakinah, 2013).

Biotechnological xylitol production is a potentially attractive replacement for the 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.

MATERIAL AND METHODS

Isolation and screening of xylose isomerase producing microbes

Different hemicelluloses containing substrates were placed in a 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 h. 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_2PO_4 buffer (pH 7.8), 0.1M $MgSO_4$, 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 chloride (0.1 %) in 1N NaOH was added and incubated at 30 °C for 1 min in the dark. The xylose isomerase producing isolates retained of pink color.

Genomic DNA isolation and microbial identification using 16S rRNA sequence

The total genomic DNA was isolated from the

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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 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-ACGGCTACCTTGTTACGACTT-3'). The PCR conditions 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 analyzed using NCBI BLAST analysis, and the 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 essential compound with several advantages like preventing dental cavities, having an 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).

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

Substrate	Name of the isolates
Pomegranate	POY
Papaya	PAY
Pear	PEY
Banana	BAY
Grapes	GRY
Apple	APY
Xylan	XYY
Xylose	XLYI,XLY2,XLY3
Arabinose	ARY
Carboxymethyl cellulose	CMCY1,CMCY2
Glucose	GLY

D-xylose utilization by microorganisms 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 xylose reductase (EC 1.1.1.21), to xylitol, whose further oxidation to D-xylulose occurs employing xylitol dehydrogenase (EC 1.1.1.9). Hence the present study focuses 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 the 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 colour on the colony (Figure 1).

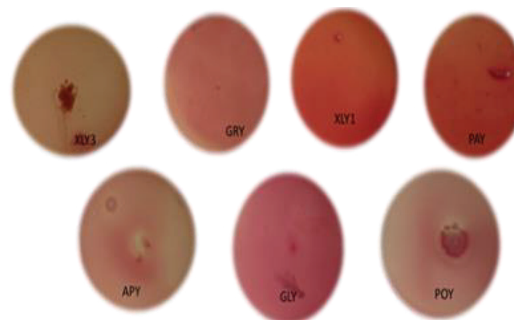

Figure 1.Positive cultures for xylose isomerase

The rose-red colour denotes the presence of xylulose formed by isomerization of xylose (Sapunova *et al*., 2004). The degree of pink colour intensity varies between microbial isolates. Sapunova *et al*. (2004) discuss that the intensity of rose-red colour depends on the capability of D-xylulose to oxidize colourless 2, 3, 5-triphenyl tetrazolium chloride in an alkaline medium to formazon with rose-red colour. Among the 7 positive 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.

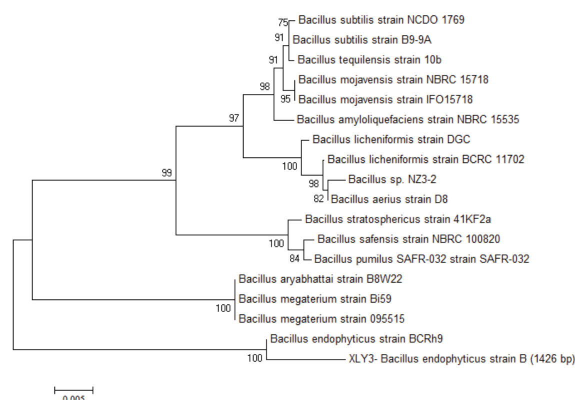

Figure 2. Phylogeny tree of Microbial isolate XLY3 based on 16SrRNA gene

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

CONCLUSION

Xylan-rich substrates can be used for the production of xylitol by the 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 through xylitol dehydrogenase. In the present study, *Bacillus endophyticus* XLY3 with high xylose isomerase was isolated. Xylose reductase and xylitol dehydrogenase in the microbe needs to be characterized. With further optimization of culture conditions, *B. endophyticus* XLY3 can effectively be used for xylitol production.

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