MICROBIAL UTILIZATION OF MUNICIPAL SOLID WASTE (MSW) FOR THE PRODUCTION OF XYLITOL: A HIGHLY VALUABLE PRODUCT

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Abstract: Huge amount of waste is generated everyday as a result of various household, commercial and industrial activities. However, inadequate management leads not only to pollution but also economic losses and health hazard globally. India, the second largest nation alone generates huge organic waste which is increasing at the rate of 1.3% annually. A major fraction of this waste comprises organic matter (51%), recyclables (17.5 %) and 31% inert waste. Organic industrial waste can be used for the production of commercially viable products including xylitol which has been approved as natural sugar free sweetener. In the present work soil samples were collected from different locations of Himachal and resulting microbial isolates were screened for their ability to produce xylose reductase enzyme. Total 45 isolates found positive for intracellular enzyme and only one produced extracellular enzyme. Fungal isolate (XYLFV-11) with highest xylose reductase activity (301 U/ml) growing as purplish-orange colored colony was characterized morphologically and microscopically. Xylitol production was recorded from both fungal and bacterial isolates. The potential utility of the hyper fungal isolate for the production of xylitol will be tested at large scale so as to use it in various applications for the welfare of mankind and society.

Keywords: hyper fungal isolate, waste, xylose reductase, xylitol.

Introduction: Various household, commercial and industrial activities generate a huge amount of waste every day. However, lack of proper management has resulted in deterioration of environment, economic losses and health hazards globally. India, second largest nation, is generating about 960 million tones of solid waste annually during industrial, municipal and agricultural processes. A major fraction of this waste comprises organic matter (51%), besides recyclables (17.5 %) and 31% inert waste which is increasing at the rate of 1.3% annually. Agricultural sources are one of the biggest contributors of organic waste as 350 million tones are organic wastes contributed from agricultural sources alone. Management of this huge amount of waste is big global issue hence advancements in solid waste management is necessary for safe guarding of environment, including not only recycling of waste [2] [10] [11] but also use of organic waste in generation of some commercial products like xylitol. Xylitol is a natural sweetener alternative [8]. In 1983, *"Joint Expert Committee on Food Additives"* an advisory committee appointed by FDA and WHO referred xylitol as extremely safe for consumption after analysis of all aspects related to xylitol consumption [9] [14].

Chemically xylitol is a poly-alcohol with molecular formula $C_5H_{12}O_5$ and molecular weight 152.15 g·mol–1 [7] [12]. Xylitol has an advantage of having traditional sweetener *i.e.* sucrose as its sweetening power is equal to that of sucrose, but with less calories. Xylitol has been reported as a safe alternative for sweetener specially for diabetic patients as it's metabolism is independent to insulin hence referred as safer alternatives for diabetic patients [4] [6]. Apart from this xylitol also has anti-cariogenic property and has been reported to prevent inflammation and infection of middle ear. On the basis of diverse application of xylitol, it is considered to be one among top 12 market value products [1]. In the present work soil samples were collected from different locations of Himachal Pradesh *i.e.* Kangra, Shimla, Mandi and Solan for isolation and isolates were screened for xylose reductase production.

Materials and Methods

Collection of soil samples: Soil samples were collected from different locations *i.e.* Mandi, Kangra, Shimla and Solan in month of July-August. Samples were primarily collected from the waste disposal area near fruit juice counter, gardens and forest area.

Soil enrichment: Before isolation, soil samples were enriched with xylose syrup for 72 hours. This would increase the population of microbes utilizing xylose.

Isolation and primary screening: Isolation and primary screening of isolates were done simultaneously. Primary screening of isolates were done on the basis of their ability to utilize xylose. After 72 hours of enrichment, soil samples were serially diluted and inoculated on different medium for bacteria and fungi. As for bacteria minimal medium (64g/l Na2HPO4-7H2O, 15g/l KH_2PO_4, 2.5g/l NaCl and 5.0g/l NH_4Cl) while for fungi modified YMEA (4g/l Yeast extract, 10g/l malt extract and 4g/l carbon source). Both the medium were supplemented with xylose and 2% agar was added for solid medium.

Secondary screening: In secondary screening enzyme activity (xylose reductase "XR") was determined for all the isolates. For this purpose different production medium was used for bacteria

and fungi. Bacterial isolates were inoculated on medium (10g/l Meat extract, 5g/l carbon source, 5g/l Sodium chloride, 1g/l peptone, pH 7.0) and incubated at 30° C for 3 days, while fungal isolates were inoculated on (3 g yeast extract, 3 g K_2HPO_4, 1 g $MgSO_4.7H_2O$ and 30 g D-xylose at pH 5.0; by Yokoyama et al. 1995) and incubated at 27 °C for 5 days. After respective incubation period, both cell pallet and supernatant was tested for enzyme activity.

Xylose reductase assay: XR reduces xylose into xylitol using NADPH as cofactor. XR activity of all isolates was determined by using method given by Yokoyama *et al.,* in 1995. While reduction of xylose into xylitol, equivalent amount of NADH/NADPH will be oxidized. The amount of NADH/NADPH oxidized was estimated at 340nm and enzyme activity of isolates was calculated using following formula:

$$Activity = \frac{1000 \times TV \times \Delta OD}{\varepsilon \times V \times CF} \quad (1)$$

Activity: Volumetric Activity (U/L)
TV : Total volume in cuvette (1000 µL)
V : Volume of cell extract used (50 µL)
E : Molar extinction coefficient for NADPH (6.22 L/mmol for a path length of 1.0 cm)
CF : Dilution of cell extract

One unit (U) of xylose reductase activity is defined as "*The amount of enzyme required to produce 1.0 µmole of xylitol in one minute*". Or
"*Rate of decrease of 1µmol of NADPH per min*".

Characterization: Isolate showing best XR activity was selected for further study and characterized morphologically and microscopically using lacto-phenol blue staining. Stained culture was observed under microscope at 40x and 100x.

Results and Discussion: Soil samples were processed for isolation after enrichment of 72 hours with xylose. Initially 128 isolates (43 bacterial and 85 fungal) were found that can utilize xylose. In secondary screening for enzyme activity of 128 isolates, only 45 isolates including 8 bacterial (XYLBV-1-8) and 37 fungal isolates (XYLFV-1-37) were producing xylose reductase. Enzyme activity was determined as per mentioned in equation (1). Determined activity of isolates was as given in table I below:

Table I: Enzyme activity of isolates (bacterial & fungal)

Isolate	Enzyme Activity (U/ml)	Nature	Isolate	Enzyme Activity (U/ml)	Nature
Bacterial Isolate			**Fungal Isolate**		
XYLBV-1	23.98	Intracellular	XYLFV-17	52.92	Intracellular
XYLBV-2	28.23	Intracellular	XYLFV-18	05.66	Intracellular
XYLBV-3	17.49	Intracellular	XYLFV-19	05.14	Intracellular
XYLBV-4	20.06	Intracellular	XYLFV-20	01.41	Intracellular
XYLBV-5	**24.63**	**Extracellular**	XYLFV-17	03.79	Intracellular
XYLBV-6	28.16	Intracellular	XYLFV-18	00.64	Intracellular
XYLBV-7	08.55	Intracellular	XYLFV-19	14.92	Intracellular
XYLBV-8	11.7	Intracellular	XYLFV-20	04.18	Intracellular
Fungal Isolate			XYLFV-21	03.79	Intracellular
XYLFV-1	11.25	Intracellular	XYLFV-22	15.3	Intracellular
XYLFV-2	25.85	Intracellular	XYLFV-23	03.41	Intracellular
XYLFV-3	14.08	Intracellular	XYLFV-24	11.7	Intracellular
XYLFV-4	35.88	Intracellular	XYLFV-25	04.37	Intracellular
XYLFV-5	16.98	Intracellular	XYLFV-26	06.17	Intracellular
XYLFV-6	27.2	Intracellular	XYLFV-27	08.94	Intracellular
XYLFV-7	17.1	Intracellular	XYLFV-28	11.32	Intracellular
XYLFV-8	26.36	Intracellular	XYLFV-29	13.63	Intracellular
XYLFV-9	18.71	Intracellular	XYLFV-30	10.61	Intracellular
XYLFV-10	12.41	Intracellular	XYLFV-31	14.66	Intracellular
XYLFV-11	**301.01**	**Intracellular**	XYLFV-32	05.22	Intracellular
XYLFV-12	03.47	Intracellular	XYLFV-33	26.11	Intracellular
XYLFV-13	06.75	Intracellular	XYLFV-34	52.92	Intracellular
XYLFV-14	09.32	Intracellular	XYLFV-35	05.66	Intracellular
XYLFV-15	04.12	Intracellular	XYLFV-36	05.14	Intracellular
XYLFV-16	01.29	Intracellular	XYLFV-37	01.41	Intracellular

As given in table among 45 isolates, all the isolates except 1 bacterial isolate XYLBV- 5 have intracellular xylose reductase while XYLBV-5 has extracellular XR (24.63 U/ml). All fungal isolates have intracellular XR

enzyme and XYLFV-11 was showing maximum XR enzyme activity *i.e.* 301.01 U/ml.

Characterization: As mentioned earlier, XYLFV-11 was showing maximum activity among all 45 isolates, hence selected for further research work and characterized morphologically and microscopically. Fig. 1 and table II shows the spores formation in XYLFV-11 and characteristics of XYLFV-11.

Table II: Characteristics of fungal sample

Characters	Description
Color	Purplish orange, become white on sporulation
Body	Mycelium made of hypae
Hyphae	Septate and branched
Reproduction	Asexual
Type of spore	Aplanospores

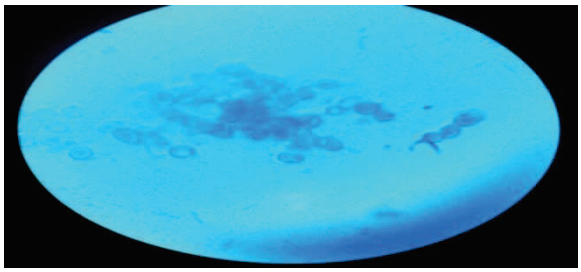

Fig. 1: LCB stained slide of XYLFV-11, showing spores at 100x

In earlier work, yeast have been extensively used for xylitol production but bacteria and filamentous fungi is somewhat remain untouched as some of the filamentous fungi *Petromyces albertensis, Penicillium, Aspergillus, Rhizopus, Glicoladium, Byssochlamyz, Myrothecium, and Neurospora* have been reported with very low yield [3] [5]. Among the yeast *i.e. Candida, Pichia* and *Saccharomyces* have been used successfully for xylitol production [15].

Summary: As waste material is one of the matters of concern globally, need to be addressed for the health and environmental sustainability. But waste management is required revenue and resources investment; this is one of the reasons that lead to waste left unaddressed by industries. Microbial route would be advantageous way to deal with waste as it can generate commercial products along with waste degradation. Xylitol is one of the dominant commercial products can be generated from organic waste. Xylitol is not only a low calorie sweetener but also anticariogenic. For production of xylitol from waste, 128 isolates were isolated from soil samples (after enrichment) that can utilize xylose. In secondary screening only 45 isolates (8 bacterial and 37 fungal) were producing xylose reductase. Among 45 one of the fungal isolate XYLFV-11 showing maximum activity which was selected for further use and characterized.

References:

1. A. Koutinas, R. Wang, G. M. Campbell and C. Webb (eds) B. Kamm, P. R. Gruber and M. Kamm, A Whole Crop Biorefinery System: A Closed System for the Manufacture of Non-food Products from Cereals, in Biorefineries-Industrial Processes and Products: Status Quo and Future Directions, Wiley-VCH Verlag GmbH, Weinheim, Germany. doi: 10.1002/9783527619849.ch8, 2008.
2. A. Pappu, M. Saxena and S. R. Asolekar, Solid wastes generation in India and their recycling potential in building materials, Build Environ, Vol 42, 2007, 2311–2320.
3. C. Chiang and S. G. Knight, Metabolism of D-xylose by moulds, Nature, Vol 188, 1960, 79-81. (doi:10.1038/188079a0)
4. C. F. Huang, Y. F. Jiang, G. L. Guo and W. S. Hwang, Development of a yeast strain for xylitol production without hydrolysate detoxification as part of the integration of co-product generation within the lignocellulosic ethanol process, Bioresour. Technol. Vol 102, issue 3, 2011, 3322–3329.
5. J. S. Dahiya, Xylitol production by *Petromyces albertensis* grown on medium containing D-xylose, Can J Microbiol, Vol 37, issue 1, 1991, 14-18.
6. J. Yoshitake, H. Obiwa and M. Shimamurs, Production of polyalcohol by *Corynebacterium* sp. I. production of pentitol from aldopentose. Agricultural and Biological Chemistry, Vol 35, 1971, 905-911.
7. K. K. Mäkinen, Dietary prevention of dental caries by xylitol-clinical effectiveness and safety, J Appl Nutr, Vol 44, 1992, 16–28.
8. K K Mäkinen, The rocky road of xylitol to its clinical application. J. Dent. Res., Vol 79, 2000, 1352–1355.
9. Life Sciences Research Office, Health aspects of sugar alcohols and lactose. Report prepared for the Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, D.C., under contract No. FDA 223-83-2020 by the Life Sciences Research Office, Federation of American Societies for Experimental Biology (FASEB), Bethesda, Md., 1986.
10. National Environmental Engineering Research Institute (NEERI), Air Quality Assessment, Emissions Inventory and Source Apportionment Studies: Mumbai. New Delhi: Central Pollution Control Board (CPCB), 2010.

11. R. K. Annepu, Sustainable Solid Waste Management in India, M.Sc. project report, Waste-to-Energy Research and Technology Council (WTERT), Columbia University, 2012, pp-189.
12. R. Ylikahri, (Eds) Chichester CO, Mrak EM and Stewart GF, Metabolic and nutritional aspects of xylitol. In: Advances in Food Research, Academic press, New York, 25, 1979, 159–180.
13. S. I. Yokoyama, T. Suzuki, K. Kawai, H. Horitsu and K. Takamizawa, Purification, Characterization and Structure Analysis of NADPH-Dependent D-Xylose Reductase from Candida tropicalis, J. Ferm. Bioeng., Vol. 79, Issue 3, 1995: 217-223.
14. WHO/FAO Evaluation of certain food additives and contaminants, Twenty-seventh Report of the joint FAO/WHO Expert Committee on Food Additives, Geneva, WHO Technical Report Series No .696, 1983.
15. X. Chen, Z. H. Jiang, S. Chen and W. Qin, Microbial and Bioconversion Production of D-xylitol and Its Detection and Application, Int J Biol Sci, Vol 6, Issue 7, 2010, 834-844.

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