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Xylooligosaccharides (XOS) as Emerging Prebiotics: Its Production from Lignocellulosic Material

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Abstract

Prebiotics are non-digestible food supplements (oligosaccharides) which play an important role in stimulating the growth of beneficial bacteria especially Lactobacilli and Bifidobacteria in the colon of the host. Xylooligosaccharides (XOS) are more effective than other oligosaccharides such as fructo-oligosaccharides as dietary supplements. Chemical methods are preferred to produce XOS mixtures with a wide DP range, while enzymatic methods are preferred in the food or pharmaceutical industries to reduce formation of degradation products. With the growing importance of making fuels fromlignocellulosic biomass (LCM) and the increasing demand for XOS, more opportunities are emerging to utilize xylan-rich component generated in biorefinery into high-value products such as XOS that could further lower the cost of LCM derived biofuels.

Keywords

Xylooligosaccharides, Prebiotics, Xylanase

1. Introduction

The food industry today is looking for the new products possessing the functional groups helping to solve global healthcare problems. Particularly natural biologically active food supplements are preferred as prebiotics/therapeutic agents by the customers. Prebiotics are substrates which are selectively utilized by host microbial community conferring a good health. These prebiotics play a major role in preventing number of widespread diseases related to cardiovascular systems, gastro-intestinal tracks, oncology and the endocrine systems [1]. Due to the increased demand for such dietary/food supplement and consumers

awareness, researchers are facing the problem of raw materials for these supplements production.

In recent years, Xylooligosaccharides (XOS) have received great attention as prebiotic due to their multi-dimentional beneficial effect especially in preventing gastrointestinal disorders [2]. In addition, they are moderately sweet, and stable over a wide range of pH and temperatures and have organoleptic characteristics suitable for incorporation into foods. XOS are oligomers consisting of 2 - 7 xylose units linked through β-(1,4) linkages. The XOS also have a branched structure decorated with the substituents such as acetyl groups, uronic acids and arabinose units that explain a variety of their physiological effect [3]. The commercial importance of these non-digestible oligosaccharides is based on their beneficial properties such as prebiotic activities. Such prebiotics stimulate the growth and/or activity of one or a limited number of bacteria in the colon (*Bifidobacterium* and *Lactobacilli*) by suppressing the activity of entero-putrefactive and pathogenic organism and also facilitate the absorption of nutrients. Therefore, XOS containing diets were considered to be beneficial in improving gastrointestinal health. Furthermore, the XOS seemed to be more efficient than the fructooligosaccharides in dietary supplementation. Apart from prebiotics and bulking agents, it is also employed in cosmetics as stabilizers, immune-stimulating agents, and antioxidant and in pharmaceutical [4].

Biorefinery concept is based on the desire to maximize and utility and value of biomass by its conversion to multiple products and/or energy streams. Biomass should be upgraded to fuels by utilizimg each of the components such as cellulose to ethanol and hemicelluloses to furfural, xylitol or XOS. Lignocellulosic material (LCM) found in nature and several residue streams generated from activities such as agriculture, forestry and municipal waste treatment, is the most abundant source of biomass on the earth. Due to the food verses fuel debate, the LCM has received a great attention as a raw material to produce value added chemicals [5]. Presently, abundantly available LCM is being exploited for production of second generation biofuels (ethanol) and chemicals. The LCM bioconversion to ethanol is unprofitable due to high cost of LCM pretreatment and enzyme production. In addition, hemicellulose (second largest component) and lignin components remain underutilized with no much value addition making the entire LCM bioconversion process uncompetitive. Hence there is a need to integrate the processes that would produce high value products from hemicelluloses and lignin. XOS is high value product that can be produced from hemicellulose component of LCM such as wheat straw, rice straw, sugarcane bagasse, corncob etc. In India these materials are available in large amount with cheaper cost. The market price of XOS varies from US$ 25 - 50/kg depending on its purity that prompts the researchers to think about developing the process for XOS production from biomass.

2. XOS Production by Chemical Methods

The XOS are produced by chemical, auto-hydrolytic [6], enzymatic [7] [8] or

combination of both the methods. The raw materials for XOS production are the hard woods (birchwoods and beechwoods), corn cobs, bagasse and rice hulls etc. The cheap agricultural residues are also being considered and have been extensively studied due to the increasing demand of XOS as fast-growing functional food supplement. The thermo-chemical XOS production includes the use of steam, dilute mineral acids or dilute alkaline solutions [9]. The auto-hydrolytic method is accomplished by hydronium-catalyzed degradation of xylan with steam or water to produce XOS in single step. The auto-hydrolytic reaction takes place at acidic pH due to the acetic acid generated by partial cleavage acetyl groups present in the plant cell wall. Auto-hydrolytic treatment produce significant amount XOS attached with acetyl or uronic acid groups which make them more soluble water [10]. Although auto-hydrolysis requires no corrosive chemicals for extraction and degradation of xylan, it requires temperature and pressure higher than acid or alkali treatment. XOS can be produced by acid or alkali hydrolysis and the degree of polymerization (DP) of XOS depends on acid concentration, temperature and reaction time. Dilute sulphuric acid (<0.5 M), strong alkali solutions like KOH, NaOH, $Ca(OH)_2$ or ammonia are usually used for XOS production [6] and the impurities generated were removed by adsorption chromatography and membrane separation. Chemical or auto-hydrolytic methods produce undesired by-products such as hydroxymethylfurfural (HMF) and furfural with no control over on DP [11] which adds to the cost of downstream processing [12]. In addition, these methods require robust equipment that can be operated at higher temperatures and pressures and also resistant to acids and alkali [13].

3. XOS Production by Enzymatic Methods

XOS production with the use of enzymes is the more environment-friendly approach since it does not require high temperature, pressure and noxious chemicals. In addition, it does not produce by-products making the downstream process easy to recover the XOS [14]. The most common method of XOS production includes the recovery of pure xylan from LCM followed by its hydrolysis by xylanases such as endo-1,4-β-xylanases (EC 3.2.1.8) and endo-1,3-β-xylanases (EC 3.2.1.32) [11]. Although the enzymatic approach of producing XOS is environment-friendly, the approach is not cost effective and not easy to perform since it depends on both xylan extraction and its enzymatic hydrolysis. Xylan is present as xylan-lignin complex in LCM which is not accessible to enzymes and hence LCM needs pretreatment to increase the enzyme accessibility [11]. In addition, the process economics does not work due to low yields of xylan during extraction and the high cost of commercially available xylanases [15].

Endoxylanase preparations with low exo-xylanase and β-xylosidase activities are preferred for production of XOS [16] [17]. The other prerequisite for XOS production is the use of endoxylanases which do not produce xylose. Xylanases have been grouped in to mainly GH10 and GH11 glycoside hydrolase families in

addition to GH5, GH7, GH8 and GH43 based on the structural and sequence similarities. The GH10 xylanases act on xylosidic linkages from non-reducing end of the substituted residue producing XOS with smaller chain length while GH11 produce larger chain length arabinoxylooligosaccharides (AXOS) due to hindrances from substituent groups present in xylan [18]. GH11 endoxylanases of *Trichoderma viride* and *Neocallimastix patriciarum* produced AXOS from wheat aleurone rich fraction which were found to possess antioxidant activity [19].

Xylanases from extremophiles have been used for producing XOS with DP more than 2. The xylanase produced by engineered *Pichia pastoris* was found to be suitable generating XOS with xylotriose as a major end product [16]. Kumar *et al*., [20] described xylanases produced by several extremophilic bacteria which have potential in XOS production. The endoxylanase produced by *Streptomyces matensis* was reported to be the most suitable for XOS production from birchwood xylan with xylobiose and xylotriose as major endproducts. This enzyme hydrolyzed xylotetraose and xylopentaose to produce xylobiose and xylotriose through transglycosylation [21]. Xylanase A from *Schizophyllum commune* belonging to GH11 family was reported to have no activity on xylobiose but low activity on xylotriose and xylotetraose. However it cleaved xylopentaose and xyloheptaose rapidly to produce xylobiose and xylotriose [22]. Recently, both XOS and AXOS were produced from insoluble arabinoxylan fraction from pretreated wheat bran by endoxylanases (GH10) from *Geobacillus stearothermophilus* and *Rhodothermus marinus* [23]. The same group reported the endoxylanase (GH11) from *Thermomyces manugenosus* and *Neocallimastix patricianum* which produced XOS and AXOS. Xylanase preparation (1%) from *Bacillus subtilis* was used for hydrolysis of insoluble dietary fiber of wheat bran yielding XOS which contain xylobiose, xylotriose, xylotetraose and also xylose [24]. XOS were produced from corn xylan using both free and immobilized endo-xylanases of *Bacillus halodurans*. It was observed that immobilized endoxylanase proved to be more efficient than the free enzyme. While free enzyme produced XOS with higher DP (>4), the immobilized enzyme produced XOS with lower DP [25]. Amorim *et al*. [26] demonstrated the XOS production from brewers spent grain (BSG) by direct fermentation using *Trchoderma* strains in single step process. The oligosaccharides produced were identified as AXOS with DP varying from 2 - 5.

There are no reports in the literature for the production of industrially important enzymes and other biological products from *P. hubeiensis* and hence it remained still unexplored. *P. hubeiensis* designated as NCIM 3574 was first isolated from sandal wood in our laboratory followed by its identification in 2008 by National Collection of Yeast Cultures (NCYC) using 26 rDNA D1/D2 sequencing and standard taxonomic tests. We were the first to report cellulase free xylanase and production by this yeast [27] followed by its application to produce xylooligosaccharides and xylose from agrowaste materials [28]. Two xylanases

(PhX20 and PhX33) from *P. hubeinsis* NCIM 3574 were purified which produced XOS with lower chain length (X3-X7) XOS [29]. We also purified novel *β*-xylosidase from this yeast strain showing heavy metal and ethanol resistant. From the search of customized SWISSPROT database, it was revealed that SWISSPROT does not contain any entries similar to the purified enzyme [30]. *P. hubeiensis* NCIM 3574 produced significant levels of cellulase free xylanase (2480 IU/g DSS) in solid state fermentation (SSF) using wheat bran and xylan. It also produced high levels of *β*-xylosidase (198 IU/g DSS) when grown in SSF using ground nut oil cake and xylan (unpublished data). The high catalytic performance and the properties such as heavy metal and ethanol tolerance qualify the enzymes for use not only in biofuel production but also in XOS production.

4. Conclusion and Future Perspectives

As it has already been reviewed, XOS are novel emerging prebiotics that are widely used in cosmetic, agriculture, pharmaceutical and others and largely in food industry. The useful properties and health benefits of XOS justify their cost effective large scale production to be used as food supplements. XOS can be combined with probiotics to develop new functional foods having potential to reduce gastrointestinal disorders, obesity, diabetes and cancer leading to improved health. Cost effective large scale XOS production technologies will be possible only by selecting cheaply available substrates such as LCM, suitable xylanases followed by downstream processes to recover high purity XOS. From our research experience, corn cobs could be suitable raw material for XOS production. In addition, *P. hubeiensis* xylanase can be a potential source of xylanase to produce XOS with required DP without any further downstream processing.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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