Abstract
Metabolism of complex carbohydrates by probiotics in human gastrointestinal (GI) tract is generally accepted to significantly improve the health and resistance to diseases for the host. Presence of sufficient amounts of carbohydrates that selectively can stimulate the growth and viability of these beneficial bacteria is imperative. Some probiotic species of bifidobacteria and lactobacilli have the ability to utilize xylooligosaccharides (XOS) and produce short chain fatty acids which is having beneficial effect on health. Current article reviews the results of several studies available on degradation and utilization of XOS by beneficial gut microbiota and discusses their future prospective as emergent prebiotic in synbiotic preparations with reference to biological effects observed on human and animal health.

Key words: xylooligosaccharides, prebiotic, Weissella, xylanase, immunomodulatory, gut microbiota

Introduction
Diet is an important determinant of disease risk associated with changing lifestyle in population of all age groups. Recent evidences support that functional food ingredients can have an impact on a number of gut-related diseases. In colon, probiotic bacteria and the saccharolytic microbiota utilize the carbohydrates that resist hydrolysis by human digestive enzymes and are not absorbed on transit through the small intestine. Non-digestible oligosaccharides (NDOs) which beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of probiotic bacteria in the colon are termed as prebiotics (1, 2). More refined definition of prebiotic states that a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits Gibson (3). To date, probiotics and prebiotics are predominantly used in food, and their application in medicine is rising significantly. Consumption of probiotic in combination with a suitable prebiotic (synbiotic) can result in synergistic effects, by improving the growth of the strain in the colon and by increasing autochthonous bacteria. Prebiotics possess remarkable functional and physiological attributes such as low energetic value, low sweetness, non-cariogenicity, and hypolipidaemic and hypocholesterolaeic properties (4). They are indigestible by human gastrointestinal enzymes and are not degraded by low gastric pH; however, they are fermented in the large bowel and enhance the population of beneficial microbes such as lactobacilli and bifidobacteria (5). Postulated health benefits of prebiotics include immunomodulation, inhibition of pathogens, stimulation of calcium absorption and effectiveness in colon cancer.

Some well known prebiotics are inulin, lactulose, fructooligosaccharide (FOS), Xylooligosaccharides as an Emerging Prebiotic
galactooligosaccharide (GOS), isomalto oligosaccharides and lactosucrose. Numerous researchers have demonstrated potential prebiotic effects of GOS and FOS on the growth of bifidobacteria and lactobacilli (6, 7, 8). Currently, xylooligosaccharides (XOS) is also an emerging prebiotic that are sugar oligomers made up of xylose units and obtained from bamboo shoots, fruits, vegetables, milk, and honey (9, 10).

Depending upon various xylan sources used for XOS production, the structures of XOS vary in degree of polymerization (DP), monomeric units, and types of linkages. Generally, XOS are mixtures of oligosaccharides formed by xylose residues linked through β-(1→4)-linkages (2, 4). The number of xylose residues involved in their formation can vary from 2 to 10 and they are known as xylobiose, xylotriose, and so on. XOS also exhibits a various bioactive properties such as reducing cholesterol, anti-microbial, anti-inflammatory, anti-allergy and improves mineral absorption (4, 11). In addition, XOS show favourable technological features including stability in wide range of pH, resistance to heat, ability to offer lower available energy and ability to achieve significant biological effects at low daily intakes which would be useful for food industry (9). Bifidobacteria possess a wide range of genes involved in carbohydrate catabolism (12) and have been described for their ability to grow on FOS and XOS (13, 14). Prebiotic effect of XOS has not yet been effectively exploited for LAB compared to FOS and GOS (15, 16).

**Production and purification of XOS** : XOS are obtained from xylan containing lignocellulosic materials (LCMs) by chemical methods, direct enzymatic hydrolysis of a susceptible substrate or a combination of chemical and enzymatic treatments (17, 18). The production of XOS with chemical methods can be accomplished by steam, diluted solutions of mineral acids, or alkaline solutions. However, extraction of xylan with steam or acid produces large amounts of monosaccharides and their dehydration products (18, 19).

The usual purity of commercial XOS lies in the range of 75% to 95%. Thus, to produce food-grade XOS, the autohydrolysis liquors have to be refined by removing both monosaccharides and nonsaccharide compounds to obtain a concentrate with an XOS content as high as possible (4). Solvent extraction and precipitation, adsorption and chromatographic separation are most commonly employed methods for the purification of XOS. Though expensive, ultrafiltration and nanofiltration are the most promising methods for refining and concentrating oligosaccharides.

**Degradation and utilization of XOS by bacteria** : The degradation and utilization of XOS are strain-specific and are also affected by the DP of oligomers present in XOS mixture (4). *Bifidobacterium* strains are found to efficiently ferment XOS and to produce metabolically active compounds such as short chain fatty acids (SCFA) (20, 21). Only some *Lactobacillus* strains have been found to utilize XOS. In a study conducted by Crittenden et al. (2), they reported that except *L. brevis* none of the tested *Lactobacilli* showed XOS utilization during fermentation. However, in another study Van Laere et al. (22) found that an *L. acidophilus* strain was able to ferment xylobiose, -triose, and -tetraose as revealed by high Performance Anion Exchange Chromatography (HPAEC) analysis of the samples.

Madhukumar and Muralikrishna (23) reported that XOSs from wheat bran are having more prebiotic activity than Bengal gram husk xylooligosaccharides as indicated by their prebiotic activity experiments, which may be due to their relatively higher arabinose content. Out of all the microorganisms tested *P. pentosaceus* NCDO 813 and *L. brevis* NDRI strain RTS utilized more effectively both WBO and BGO followed by *P. pentosaceus* ATCC 8081, *B. adolescentis* NDRI 236, *B. bifidum* NCDO 2715, *B. bifidum* ATCC 29521 and *L. plantarum* NDRI strain 184. Acetate was found to be the major SCFA produced as the end product of xylo-oligosaccharides fermentation.
Recently, we reported utilization of hydrolyzed birch wood xylan from novel probiotic isolates of genus *Weissella* (16, 24). Total six isolates of genus *Weissella*, belonging to either *W. confusa* or *W. cibaria* were checked for growth on hydrolyzed birch wood xylan by optical density measurements in microtiter plates and secondly in batch cultures also confirming concomitant decrease in pH. Out of six, four strains namely 85, 92, 145 and AV1 showed growth in hydrolyzed xylan with remarkable decrease in pH. Analysis of XOS before and after growth established consumption in the DP2 - DP5 range in the four XOS-fermenting strains. XOS were consumed simultaneously with glucose, while xylose was consumed after glucose depletion. The SCFA profile revealed that lactate and acetate were the major SCFA produced as the end product of xylo-oligosaccharides fermentation while propionate or butyrate production was below the detection level and could not be confirmed (16).

**Mechanism of XOS Degradation and Utilization**: The ability of bifidobacteria to metabolize XOS depends on the efficiency of their xylanolytic enzyme systems. Figure 1 demonstrates the degradation of various XOS and their end products. One xylosidase and a few arabinosidases have been purified and characterized from bifidobacteria. They are α-D-xylosidase from *B. breve* K- 110 and arabinosidases from *B. adolescentis* DSM20083 (22) and arabinosidase from *B. breve* (25). Ohara et al. (26) cultivated strains of *Leuconostoc lactis* SHO-47 and *L. lactis* SHO-54 with a hydrolyzed birch wood xylan along with *Lc. lactis* IO-1 and found that xylosidase enzyme of these strains is

### Table 1. Studies related to XOS degradation by LAB and Bifidobacteria.

<table>
<thead>
<tr>
<th>Strain able to ferment XOS</th>
<th>Source and xylooligosaccharides (XOS) type</th>
<th>References</th>
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<tbody>
<tr>
<td><em>B. adolescentis</em>, <em>B. longum</em>, and <em>B. infantis</em></td>
<td>A mixture of xylose, xylobiose, xylotriose, and other saccharides</td>
<td>Okazaki et al. (47)</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>Commercial XOS (DP 2-4)</td>
<td>Hopkins et al. (48)</td>
</tr>
<tr>
<td><em>L. brevis</em> PEL1, <em>B. adolescentis</em> VTT E-991436, <em>B. angulatum</em> ATCC 27535</td>
<td>Commercial xylan</td>
<td>Crittenden et al. (2)</td>
</tr>
<tr>
<td><em>L. acidophilus</em> (swine faeces isolate), <em>B. adolescentis</em> ATCC 15703, <em>B. longum</em> ATCC 15707</td>
<td>Plant cell wall polysaccharides-XOS</td>
<td>Van Laere et al. (22)</td>
</tr>
<tr>
<td><em>Leu. lactis</em> SHO-47, <em>Lc. lactis</em> SHO-54, <em>Lc. lactis</em> IO-1</td>
<td>Hydrolyzed birch wood xylan</td>
<td>Ohara et al. (26)</td>
</tr>
<tr>
<td><em>B. adolescentis</em> CECT 5781, <em>B. longum</em> CECT 4503, <em>B. infantis</em> CECT 4551, <em>B. breve</em> CECT 4839</td>
<td>XOS obtained from rice husk liquor</td>
<td>Gullon et al. (49)</td>
</tr>
<tr>
<td>isolates of <em>Weissella cibaria</em> and <em>W. confusa</em></td>
<td>Hydrolyzed birch wood xylan</td>
<td>Patel et al. (16)</td>
</tr>
</tbody>
</table>

Abbreviation: B.- Bifidobacterium; L.-Lactobacillus; Leu.-Leuconostoc, Lc.- Lactococcus, W.-Weissella
localized in their cytoplasm. There are no studies reported on the xylan or XOS fermenting ability of the genus *Weissella* and *Pediococcus*.

In another experimental approach it was found that xylanase activity was higher in the culture broth inoculated with wheat bran oligosaccharides compared to that of Bengal gram husk oligosaccharides; this might be due to the presence of higher DP xylooligosaccharides in the earlier substrate (23). In contrast to that, cell-associated β-xylosidase activity was detected in the XOS fermenting strains of *Weissella* (16). Analysis of genomic data suggests this activity to be linked to genes encoding glycoside hydrolases from family 3, 8 or 43. No endo-β-xylanase activity was detectable.

**Biological Properties of XOS as prebiotic and their applications:** In general, XOS have been reported to possess antimicrobial activity (27, 28); immunomodulatory activity (30, 31, 32); anti-inflammatory activity (33, 34, 35); and anti-cancer activity (36). The other health beneficiary effects of XOS include antioxidant activity (37), blood- and skin-related effects; anti-allergy, anti-hyperlipidemic effects (17) and applications in cosmetics. Besides biological effects concerning human health, XOS have been employed for phyto-pharmaceutical and feed applications. These properties are mainly attributed to acidic oligosaccharides containing uronic substituents, which can be produced from hardwoods by a combination of enzymatic and/or chemical treatments. There is no any side effect or toxic effect has been reported for XOS during clinical applications still date. The dose of XOS is also not standardized, however like other prebiotic oligosaccharides it is incorporated in milli gram quantities during food or therapeutic application.

*Fig.1. Degradation of Xylan and Xylooligosaccharides in microorganisms with their end products*

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xylan-type polysaccharides which differ greatly in the type, proportions, and distribution of glycosyl side-chains decorating the â-1,4-D-xylan backbone (4).

b. Antimicrobial activity: Fooks and Gibson (28) reported that L. plantarum 0407 and Lactobacillus pentoos 905 combined with FOS, inulin, XOS, and mixtures of inulin: FOS and FOS:XOS were effective in inhibiting growth of E. coli and Salmonella enteritidis. Acidic XOS were produced from birch wood xylan and were tested against Gram-positive and Gram-negative bacteria, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Proteus mirabilis and Helicobacter pylori (27). In that, aldopentauronic acid proved more active against Gram positive bacteria and H. pylori.

Beneficial colonic bacteria such as LAB and bifidobacteria produce carbohydrate degrading enzymes which ferment the NDOs like XOS and produce SCFA such as acetate, propionate and butyrate which provide metabolic energy for the host and help in the acidification of the bowel (11, 38). Acidification can affect the balance of the bacterial species, bacterial metabolic activity and product formation. Probiotic bacteria also shown to exhibit pronounced antibacterial activity against human enteropathogenic bacterial strains (39). Acetate is mainly metabolized in human muscle, kidney, heart and brain, whereas propionate acts as a possible gluconeogenic precursor suppressing the cholesterol synthesis (40). Butyrate is known to have prodifferentiation, anti-proliferation and anti-angiogenic effects on colonocytes (41). Moreover, decrease in pH due to production of SCFA as a result of fermentation correlates with the population growth of the beneficial microbes and in turn inhibits the growth of the undesirable pathogenic bacteria (40, 41).

c. Effect on gastrointestinal related problems: According to Moure et al. (10), administration of 0.12 g of XOS per kg of body weight to male Japanese adults was resulted in beneficial effect viz. help to restrain the growth of pathogenic bacteria, to retard disorders caused by imbalanced fermentation in colon, and to avoid intestinal disorders such as constipation, inflammatory bowel disease, diarrhoea, and gastritis. Furthermore, XOS intake has been found to be highly effective for the reduction of severe constipation in pregnant women without adverse effects, and nutritional infant formulas containing XOS have been claimed to have synergistic effects all along the intestinal tract, improving gut barrier maturation.

In comparison with other prebiotic oligosaccharides, the slower fermentation of branched XOS led to higher butyric acid production, which may result in even more advantageous effects, whereas the presence of feruloyl substituents may promote the growth of beneficial bacteria. XOS is used in preparation of micro/nano particles and hydrogels for drug delivery in the treatment and prevention of GT disorders (9).

d. Antioxidant, antidiabetic and cholesterol reduction activity: FOS and XOS supplementations significantly increased the activity of antioxidant enzymes – catalase and glutathione reductase – in the blood of diabetic rats (5). FOS and XOS exerted encouraging influences in diabetic rats by significantly improving body weight and reducing hyperglycaemia and cholesterol. Further, the characteristic diabetic complications such as severe glucosuria, proteinuria and advanced glycation end products in renal tissue, diabetic nephropathy, and blood creatinine and urea concentrations were markedly reduced (5). Previously, Imaizumi et al., (42) also reported improved growth retardation, hyperphagia, polydipsia and elevation of serum glucose, TAG and cholesterol in diabetic rats through XOS.

e. Synergistic or prebiotic effects: XOS have been reported to stimulate growth of bifidobacterium in the intestine and thus, they are incorporated in infant foods. Nutritional formula for infants found to own synergistic effects all over the intestine tract and led to improve gut barrier maturation (4). Recently several researchers
have deposited patents on the synergistic effects of probiotic microorganisms together with and prebiotic XOS in synbiotic preparations (43).

f. Immunomodulatory and miscellaneous effects: Recent clinical study established that addition of XOS to feed can increase growth performance, enhance endocrine metabolism, and improve immune function in broiler chickens (44). Furthermore, growth regulatory activities of XOS in aquaculture (45) and poultry (46) were also documented. There were significant differences in the relative weight gain rate and daily weight gain rate as compared with the control in fishes suggesting the usefulness of XOS as a feed additive in the diets of fishes. However, XOS had little influence on the overall bacterial community profile. Other biological effects of XOS alone or as active components of pharmaceutical preparations, cosmetics, exhibit a range of biological activities different from the prebiotic effects related to gut modulation.

Conclusion

XOS have immense prospective as agents to sustain and improve a balanced intestinal microflora for enhanced health and well-being. Available experimental evidences support the hypothesis that XOS and other prebiotics can offer an opportunity to prevent or alleviate gastrointestinal disorders. Even though encouraging results have been obtained for other prebiotics in preliminary clinical trials, the data on XOS are limited. More investigations are required to further elucidate the mechanisms involved in biological effects demonstrated. The properties of XOS offer a new dimension for the development of functional foods. One approach that may be encouraged for future research is the combination of prebiotics and probiotics (as synbiotics). Opportunities exist in exploring the improved knowledge of the symbiotic relationships between colonic microbiota, XOS, and whole body physiopathology.

Reference

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Xylooligosaccharides as an Emerging Prebiotic


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