INTRODUCTION

Since Japanese researchers had demonstrated that specific non-digestible oligosaccharides were selectively fermented by bifidobacteria and had the capacity, upon feeding, in stimulating their growth in human faeces in the 1980’s, prebiotic oligosaccharides have attracted the interest of industrial as well as academic scientists (Mitsuoka et al., 1987; Roberfroid et al., 2010). Prebiotics is defined as ‘a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbials, thus conferring benefit(s) upon host health’ by International Scientific Association for Probiotics and Prebiotics (ISAPP)(6th meeting in Ontario, Canada, November 2008). Disaccharides, such as lactose and sucrose, and storage polysaccharides in plants, such as starch and fructans, are important resources for producing prebiotic oligosaccharides that are applied to the food and drink industry or towards investigating biological activities related to human health (Crittenden and Playne, 1996; Nauta and Schoterman, 2009). Oligosaccharides are categorized as prebiotics when they fulfill the following 3 criteria (Gibson and Roberfroid, 1995; Lomax and Calder, 2009): (1) resistance to hydrolysis or absorption in the upper gastrointestinal tract, (2) fermentation by intestinal microbials, or (3) selective stimulation of the growth and/or activity of beneficial intestinal microbials classified into prebiotics defined as ‘living non-pathogen-ic micro-organisms, which as food ingredients (supplements) also beneficially affect host’s health’. These microbials may be lactic acid bacteria, such as lactobacilli, lactococci and bifidobacteria, or yeasts such as Saccharomyces cerevisiae subsp. boulardii (Bongaerts et al., 2005, Roberfroid et al., 2010).

According to the definition of prebiotics, prebiotics appears to be merely food additives or nutraceuticals. However, prebiotics causes intestinal fermentation and the promotion of growth of beneficial gut microbials, these compound could improve host health through diverse direct or indirect biological activities, including the stimulation of host immunity and protection against pathogens, as well as facilitating mineral absorption by hosts (Gibson and Roberfroid, 1995; Lenoir-Wijckop et al., 2007). In addition to these general activities of prebiotics, some prebiotic oligosaccharides have other biological activities, such as anti-inflammatory and anticarcinogenic properties (Nurmi et al., 2005). Therefore, prebiotics has become a popular research topic in the biomedical fields recently (Roberfroid et al., 2010) and the potentiality of prebiotics as therapeutics is increased. For a simple example, for the treatment of hepatic encephalopathy, prebiotics associated with probiotics might be an alternative therapeutics to reduce ammonia production by urease-positive bacteria Sloga instead of antibiotics resulting in survival of ammonia-producing antibiotic-resistant bacteria (Bongaerts et al., 2005).

Subtle differences in structure, such as changes in size and...
the presence of branch(s) and differences in the complexity of the substituents, can cause significant changes in the probiotic properties of oligosaccharides (Kabel et al., 2002a; Kabel et al., 2002b; Holck et al., 2011). Most currently available probiotics are derived from disaccharides and simple polysaccharides. Therefore, to develop novel probiotics with other biological activities, which are not present in existing probiotics, an alternative resource of polysaccharides is required that supplies oligosaccharides with more diverse and complex structures. In this context, heteropolysaccharides, which form plant cell walls, are ideal substances to supply polysaccharides with more diverse and complex structures. The exact nature and relative abundance of each component of the plant cell wall, including xyloans and pectins, varies according to plant species, as well as the types of tissues and organs used (Ebringerova and Heinze, 2000; Bauer et al., 2006; Popper, 2008). Furthermore, within a plant, the composition of oligosaccharides varies dynamically according to developmental stages (Ebringerova and Heinze, 2000), as well as environmental factors, such as temperature and pathogens (Zablackis et al., 1995).

In this paper, we discuss the potential application of 2 classes of heteropolysaccharides of plant cell walls, xyloans and pectins, as substances to supply diverse polysaccharides. In addition, we review the general process used to produce oligosaccharides from polysaccharides, including the fragmentation of polysaccharides, as well as the separation and identification of resultant oligosaccharides. The bioassays used to select prebiotics among available oligosaccharides are not considered within the scope of this review.

**BIOLOGICAL ACTIVITIES OF PREBIOTICS**

**Selective stimulation of beneficial bacteria in the gut**

In the gut, the selective nature of prebiotics stimulates indigenous beneficial bacteria (Saulnier et al., 2009). For example, β-(2-1) fructans, one of prebiotics stimulates the growth of *Lactobacillus* and *Bifidobacterium* species (Kruse et al., 1999; Kleessen et al., 2001). In addition, β-(2-1) fructans is selectively fermented by *Lactobacillus* species (Kaplan and Hutkins, 2000) and *Bifidobacterium* species (Gibson and Roberfroid, 1995). These bacteria produce enzymes, such as intracellular fructosylyfructofuranosidase, which hydrolyzes the β-(2-1) glycosidic bond in β-(2-1) fructans. Such fermentation by beneficial bacterial leads to the production of short chain fatty acids (SCFA), such as butyrate. Recent studies have shown that other gut species, such as *Faecalibacterium prausnitzii*, also have the ability to degrade prebiotic oligosaccharides (Ramirez-Farias et al., 2009). Regarding selectivity of prebiotics, it is possible prebiotics have enzymes for hydrolysis and fermentation, as well as a mechanism of oligosaccharide uptake, such as transporters of specific oligosaccharides. By increasing the number of beneficial probiotics, prebiotics help outcompete pathogenic bacteria for nutrients and binding sites on the intestinal epithelium, thus suppressing the survival of the pathogenic strains (Lomax and Calder, 2009). Furthermore, prebiotics are able to produce antibacterial substances that inhibit the growth and survival of pathogens (Gibson and Wang, 1994). Through altering bacterial flora in the intestines, pathogenic bacteria become less abundant (Kruse et al., 1999; Kleessen et al., 2001).

The fermentation products of prebiotics, such as SCFAs, cause the acidification of the colonic environment, which is detrimental to some pathogenic bacteria (Blaut, 2002). Furthermore, this acidification process improves mucosal morphology by increasing mucus production, as well as decreasing pathogenic bacteria colonization and translocation (Barceló et al., 2000).

Ultimately, because of the stimulation of probiotic growth and fermentation, prebiotics might contribute to host health, particularly with respect to defense against pathogenic bacteria.

**Stimulation of host immunity**

An increase in the number of beneficial microbiota by prebiotics stimulates human immune cells. It has been suggested that bacteria might cross the host intestinal barrier onto the Peyer’s patch, and activate immune cells in that region. In contrast, it has been suggested that microbial substances, such as bacterial cell wall components, rather than micro-organisms, act as the stimulating factor (Berg, 1985; De Simone et al., 1987). Besides, prebiotics may influence the immune system directly. Although there is currently little evidence, the potential direct ligation of pattern recognition receptors on immune cells by prebiotic oligosaccharide may result in immunomodulation (Brown and Gordon, 2001; Herre et al., 2004; Roberfroid et al., 2010). Fermentation product of prebiotics, SCFAs, such as butyrate, might participate in the stimulation of host immunity. Butyrate bound to SCFA receptors, including GPR41 and GPR43 on leukocytes, particularly neutrophils, within the gut lymphoid tissues (GALT) may regulate leukocyte function in the immune system (Brown et al., 2003; Nilsson et al., 2003). Alternatively, SCFA might alter the signaling of epithelial cells to the mucosal immune system (Sanderson, 2007). Finally, butyrate alters epithelial cell gene expression, such as IL-8 and monocyte chemo-attractant protein 1. Regarding the gene expression participating in immunomodulating, some genes such as major histocompatibility complex class I and II, interferon and phosphatidulinositol metabolites participating in the intestinal immunomodulating were identified by DNA microarray analysis in mice fed with prebiotics manufactured from sucrose (Fukasawa et al., 2007).

**Prevention of pathogen adherence to the epithelial membrane of the gut**

Some oligosaccharides bind to receptors on pathogenic bacteria and prevent them from attaching to the same sugar on the epithelial membrane of the gut (Ouwehand et al., 2005; Shoaf-Sweeney and Hutkins, 2009). For example, galactooligosaccharide reduces the adherence of enteropathogenic *E. coli* to cultured cells, such as HeP cells (Shoaf et al., 2006).

**Promotion of mineral absorption in the gut**

Prebiotics promote host absorption of minerals, such as Ca\(^{2+}\) and Mg\(^{2+}\) ion. For example, β-(2-1) fructans promotes the absorption of Ca\(^{2+}\) by the colon mucosa in human (Abrams et al., 2007). For Ca\(^{2+}\) absorption, SCFA contributed to lower luminal pH in the large intestine which, in turn, elicits a modification of Ca\(^{2+}\) speciation and hence solubility in the luminal phase so that its passive diffusion is improved (Lopez et al., 2000). Besides, prebiotics may directly modulate transcellular active Ca\(^{2+}\) transport by increasing expression of the carrier protein, calbindin D9K in the cecum and colorectum (Taka-
saki et al., 2000). This promoting effect of prebiotics might be useful in the prevention of osteoporosis (Scholz-Ahrens et al., 2001).

Furthermore, other indirect biological activities of prebiotics have been reported. For instance, the fermentation products of prebiotics appear to have anti-inflammatory and anticarcinogenic properties on colonic cancer cells and the enteroocyte-like cell line Caco-2 (Nurmi et al., 2005; Ewaschuk et al., 2006). Using laboratory animal models, the effects of prebiotics on inflammation were studied. Prebiotic treatment decreased proinflammatory cytokine, IL-1β, but increased immunomodulatory molecules, TGF-β in cecal tissues of HLA-B27 transgenic rats, which develop spontaneous colitis under specific pathogen-free conditions (Hoentjen et al., 2005). Prebiotics in food reduced the both numbers of cells stained immuno-histochemically with monoclonal antibodies to CCR4 and mast cells, and the edema formation rate in the duodenum of ovalbumin-induced food allergy model Nc/jic mice (Fujitani et al., 2007).

**EXISTING PREBIOTIC OLIGOSACCHARIDES**

Prebiotic oligosaccharides derived from disaccharides

Many prebiotics that are currently applied to the food industry are mostly derived from disaccharides. These disaccharides are converted to prebiotic oligosaccharides that have a small DP (degree of polymerization) and a simple structure following treatment with enzymes.

**Galacto-oligosaccharides (GOS)** are produced from lactose by β-galactosidase mediated hydrolysis and polymerization of β-linked sugars (Nauta and Schoterman, 2009). GOS is one of the most commonly produced prebiotic oligosaccharides. β-Galactosidase reacts with lactose to form an oligosaccharide, and liberates a glucose molecule. The successive transgalactosylations with lactose, or previously formed oligosaccharides, as a donor results in the production of mixtures of GOS with DP up to 8 (Nauta and Schoterman, 2009) (Fig. 1A). Interestingly, GOS are similar to oligosaccharides found in human milk, which also appear to have prebiotic activities (Sharon and Ofek, 2000).

**Galacto-fructose** is a disaccharide made through the isomerization of lactose (Montgomery and Hudson, 1929). During the isomerization process, the glucose unit of lactose is converted into fructose. Since the β-(1-4) glycosidic linkage connecting to galacto-fructose disaccharide is not cleaved by human intestinal enzymes, galacto-fructose disaccharide reaches the colon intact.

**Lactosucrose** is a non-reducing trisaccharide (Gal β-(1-4) Glc α-(1-2) Fru) produced from sucrose and lactose. The fructopuranosyl residue in sucrose is transferred to a glucose moiety of lactose by β-fructofuranosidase (Fujita et al., 1992).

**Glycosyl sucrose** is a trisaccharide made from sucrose and maltose by cyclomaltooltrextrin glucanotransferase (Crittenden and Playne, 1996).

**Prebiotic oligosaccharides derived from plant storage polysaccharides**

Both starch, which is insoluble polyglucan formed in the plastid, and fructans, which is a soluble polyfructan formed in the vacuole as a storage compound (Heldt, 2005), comprise other reliable sources of prebiotics that are currently available to the food and health industries. The glucose molecules in starch are connected to each other by α-(1-4) and α-(1-6) linkages. In fructans, especially 1-kestose type, the fructose residue of sucrose is connected with other fructoses by β-(2-1) linkages (Heldt, 2005). These simple structured storage polysaccharides are fragmented and modified into the following prebiotic oligosaccharides by enzymatic processes.

**Isomalto-oligosaccharides** (IMO) are produced from starch through a two-stage enzymatic process (Crittenden and Playne, 1996). Starch is converted to malto-oligosaccharides by the sequential treatment of α-amylase and β-amylase. Then, α-glucosidase catalyzes the transglycosylation to convert the α-(1-4) linkage into a α-(1-6) linkage. While most IMO contain only α-(1-6) linkages with a DP range of 2 to 6, trisaccharide, panose (Glu α-(1-6) Glu α-(1-4) Glu) contains both α-(1-4) and α-(1-6) linkages.

**Gentio-oligosaccharides** contain α-(1-6) linked glucose with DP of 2 to 6. They are produced from glucose syrup, which is made from the hydrolysis of starch by enzymatic transglucosylation (Playne and Crittenden, 1996; Wichenchot et al., 2009).

**β(2-1) Fructans** such as inulin (Fig. 1B) and oligofructose, which are also known as short-chain fructo-oligosaccharides, are fructose polymers that originate from sucrose. β-(2-1) Fructans play several important roles towards enhancing the tolerance of plants against cold and drought. Inulin is a linear molecule, which contains β-(2-1) fructosyl-fructose linkages with a terminal glucose (De LeenHeer and Hoebregs, 1994). In particular, inulins found in chicory contain β-(2-1) linked fructoses with DP ranges from 2 to 60. In contrast, oligofructoses derived from inulin by enzymatic hydrolysis contain fructoses with DP from 2 to 7 (De LeenHeer and Hoebregs, 1994).

Plant cell wall polysaccharides

The minor discrepancy in the structures of oligosaccharides results in significant changes in the properties of prebiotic oligosaccharides (Kabel et al., 2002a; Kabel et al., 2002b; Holck et al., 2011). Therefore, to develop novel prebiotics, alternative sources of polysaccharides are necessary, from which oligosaccharides with diverse structures are derived. In this study, we provide a comprehensive overview of prebiotic oligosaccharides derived from plant storage polysaccharides.
context, hetero-polysaccharide, which forms the plant cell wall, might be of interest.

The plant cell wall contains cells that are unique in the plant. They perform many essential biological roles, such as the regulation of cell expansion, control of tissue attachment, ion exchange, and defense against pathogenic microbes (Popper, 2008). The plant cell wall is composed of various types of polysaccharides and proteins, as well as lignin, a polymer of phenylpropane derivatives such as cumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. However, polysaccharides constitute the greater part of the plant cell wall. These polysaccharides are often classified into (1) cellulose, which is composed of β-(1-4) linked glucan chains organized in more or less crystalline microfibrils, (2) hemicelluloses, and (3) pectin (Vázquez et al., 2000; Scheller and Ulvskov, 2010). Both hemicellulose and pectin together constitute a matrix in which cellulose microfibrils are embedded (Harholt et al., 2010). However, the nature of each cell wall component, including these polysaccharides, varies dynamically according to plant species and the types of organs, as well as the stage of plant development and various environmental factors (Zablackis et al., 1995; Ebringerova and Heinze, 2000; Bauer et al., 2006; Popper, 2008).

**Xylans**

Hemicelluloses comprise several different polymers, including xylans, xyloglucans, and (gluco)mannans, which are characterized by a backbone of β-(1-4) linked sugars with an equatorial linkage configuration (Bauer et al., 2006; Scheller and Ulvskov, 2010). Among these compounds, xylans are major hemicellulosic polysaccharides in the secondary cell walls of dicots and in all types of cell walls of grasses (Faik, 2010). The content of xylans is 25-35% of the dry mass of woody tissue of dicots and lignified tissue of monocots. In cereal grains, the content of xylans is up to 50% (Moure et al., 2006). In higher plants, xylans are partially acetylated in the native state (Wende and Fry, 1997). Even if diverse types of xylans (such as those listed below) are present in plants, certain types are common to certain plant families (Ebringerova and Heinze, 2000).

**4-O-methyl glucuronoxylan (GX)** contains a single side chain of 2-linked-4-O-methyl-α-D-glucopyranosyl uronic acid units (MeGlcA) (Fig. 2). GX is common in the wood of dicots (Ebringerova and Heinze, 2000). The ratio of Xyln to MeGlcA in GX isolated from different woods is 4-16:1 (Timell, 1964).

**Arabino(glucurono)xylan (AGX)** contains a single side chain of 2-O-linked-α-D-glucopyranosyl uronic acid (GlcA) and/or its 4-O-methyl derivative (MeGlcA), in addition to 3-O-linked-α-L-arabinofuranosyl units. AGX is common in gymnosperms and in the supporting tissues of monocots (Ebringerova and Heinze, 2000). Water-soluble AGX contains terminating β-D-xylopyranosyl residue and disaccharide side chains composed of 2-O-β-D-xylopyranosyl-α-L-arabinofuranose next to the single Araf and MeGlcA side chain. This disaccharide is usually esterified by ferulic acid at position O-5 of the Araf unit.

**Neutral arabinoxylan (AX)** has xylofuranosyl residues that are substituted at position 3 and/or at both positions 2 and 3 of Xylp by α-L-arabinofuranosyl units (Fig. 2). In contrast, water insoluble AX contains a relatively low amount of Araf units, which are mainly positioned on mono-substituted Xyln residues. AX is one of the major types of xylans in grasses, and is mainly found in the starch endosperm of cereal grains (Ebringerova et al., 2005; Faik, 2010). Especially, in sorghum, gluconor(gluco)arabinoxylan (GAX) comprises the structural features of AX, and contains disaccharide moieties composed of 2-O-α-L-arabinofuranosyl-L-arabinofuranose linked at position O-3 to the xylan backbone (Fig. 2) (Ebringerova and Heinze, 2000). In particular, grasses have type II walls that are rich in GAXs (Ebringerova et al., 2005).

**Heteroxylan (HX)** is the most structurally diverse xylan. The
HX backbone is highly branched with Xylp-, Araf-, and Galp-containing monosaccharide, disaccharide, and trisaccharide side chains, as well as phenolic acid, which is mainly ferulic acid esterified to Araf units. HX is commonly found in cereals and seeds (Wilkie, 1979).

**Pectins**

Pectin is a hetero-polysaccharide that contains a high content of GlcA units. Various types of pectins, such as homogalacturonan (HG), xylogalacturonan (XGA), rhamnogalacturonan I (RG I), and rhamnogalacturonan II (RG II), are present in the plant cell wall (Buchholt et al., 2004; Harholt et al., 2010). These types of pectins are not separate polymers, but are covalently linked together. While the ratio in the amounts of these pectins is variable, HG is usually the most abundant type. The content of HG type is up to 65% of total pectin, while GR I type constitute 20-35% (Mohnen, 2008). XGA and RG II are minor components, with each type constituting less than 10% (Zandleven et al., 2007; Mohnen, 2008).

**Homogalacturonan (HG)** is a linear homologous chain of α-(1-4) linked GlcAs (Fig. 3). GlcA residues in the galacturonan backbone are substituted at various positions with sugar moieties, such as Xylp and apiofuranose (Harholt et al., 2010). In the pure galacturonan backbone, GlcA is further modified by methyl esterification at the C-6 carboxyl position and by O-acetylation at the O-2 or O-3 position.

**Rhamnogalacturonan I (RG I)** is a branched oligosaccharide with a backbone composed of disaccharide (α-(1-4)-D-GlcA-α-(1-2)-L-Rha) repeats (Fig. 3). Therefore, RG I is the only type of pectin that is not built on a pure galacturonan backbone. About 20-80% of the rhamnose residues in the RG I backbone are substituted with side chains, such as β-(1-4) galactan, branched arabinan, and/or arabinogalactan. In contrast to RG II, the structure of the side chains of RG I vary depending on plant species (Harholt et al., 2010). In particular, in the side chains of RG I of sugar beet, arabinose residues substituted with ferulic acid are rich. While GlcA residues in the pure galacturonan backbone are modified by both methylation and acetylation, GlcA residues in the RG I backbone are only acetylated at the O-3 position (Komalavilas and Mort, 1989; Ishii, 1997).

In xylogalacturonan (XGA), a single Xylp is attached to the O-3 position of some GlcA residues in the HG backbone, and additional Xylp residues are attached to the first Xylp with β-(1-4) linkage (Zandleven et al., 2006) (Fig. 3). XGA is abundantly present in reproductive tissues, and is also found in other tissues, such as leaves (Zandleven et al., 2007). In aquatic angiosperms, including *Lemma* and *Spirodela*, D-apiofuranose is attached to the O-2 or O-3 position of GlcA residues in the HG backbone (Hart and Kindel, 1970; Longland et al., 1989).

In rhamnogalacturonan II (RG II), a cluster of complex side chains is attached to the O-2 or O-3 position of GlcA residues in the HG backbone (Fig. 3). The side chains are composed of 12 different glycosyl residues that are linked together by at least 22 different glycosidic bonds (Harholt et al., 2010). These glycosyl residues, such as 2-O-methyl-L-fucose and L-arabinose acid, and glycosidic linkages, such as α-(1-3)-xylofuranose, are rare and unique in plant polysaccharides. Despite its

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**Fig 3.** Schematic illustration of four types of pectins found in plant cell walls. HG: Homogalacturonan, RG I: Rhamnogalacturonan I, XGA: Xylogalacturonan, RG II: Rhamnogalacturonan II, Kdo: 3-Deoxy-D-manno-2-octulosonic acid, DHA: 3-Deoxy-D-lyxo-2-heptulosonic acid, OAc: Acetylation, OMe: Methylation.
complexity, the structure of RG II is highly conserved among vascular plants (Matsunaga et al., 2004).

OLIGOSACCHARIDES DERIVED FROM XYLANS AND PECTINS

Reports about the oligosaccharides derived from complex heteropolysaccharides, xylans, and pectins remain limited. The properties of XOS (oligosaccharides derived from xylans), particularly with respect to fermentation by probiotic bacteria, have been found to significantly vary according to structure. The velocity of fermentation of acetylated XOS and XOS with substituents in its structure, such as 4-O-MeGlcA, was slower than non-substituted linear and branched XOS (Kabel et al., 2002a). The fermentation product also varied depending on the structure of XOS. While acetate and lactate were major fermentation products of both non-substituted linear and branched XOS, substituted XOS produced more propionate and butyrate and less lactate (Kabel et al., 2002b). Other properties of XOS such as the activities according to the structure remain to be inquired further. Acidic XOS consists of a linear polymer of β-(1-4) linked xylose branched with glucuronic acid showed suppressive effect on contact hypersensitivity in mice (Yoshino et al., 2006). On the other hands, XOS including glycosylated daidzein, which produced by cultured cells of Catharanthus roseus and Aspergillus sp. β-xyllosidase showed superoxide-radical scavenging antioxidant activity (Shimoda et al., 2011).

With respect to biological activities, prebiotic oligosaccharides derived from xylans and pectins appear to be involved in a range of activities that are different from those of classical prebiotics (Moure et al., 2006). For example, XOS exhibited activities, such as healing effect against atopic dermatitis (Izumi et al., 2004), enhancement of collagen production (Azumi and Ikemizu, 2004), and the prevention of type II diabetes (Monsan et al., 2004). Other novel effects of pectic oligosaccharides (POS) include reports of the stimulation of urinary excretion of toxic metals (Zhao et al., 2008) and the suppression of liver lipid accumulation rates (Yamaguchi et al., 1994).

FRAGMENTATION, SEPARATION, AND IDENTIFICATION OF NOVEL OLIGOSACCHARIDES FROM PLANT CELL WALL POLYSACCHARIDES

In the final section of this paper, we briefly review the general processes to obtain new oligosaccharides from complex plant cell wall heteropolysaccharides.

Fragmentation

The fragmentation of polysaccharides that are present in the plant cell wall into oligosaccharides is a prerequisite for the separation and identification of novel oligosaccharides derived from xylans and pectins. There are several methods to fragment polysaccharides, including: 1) chemical treatments, 2) enzymatic hydrolysis, and 3) a combination of chemical and enzymatic methods (Moure et al., 2006). Chemical treatments typically involve autohydrolysis with water or steam, diluted solutions of mineral acids, or alkaline solutions (Aachawat and Prapulla, 2011). For example, the autohydrolysis of xylan containing lignocellulosic materials (LCM) can cause cleavage of the hemicellulosic chain via the hydrolytic reaction of hydronium ions, which mainly produce oligosaccharides as a soluble material, and both cellulose and lignin as solid materials (Gullón et al., 2008). Most oligosaccharides produced by the autohydrolysis reaction tend to have DP ranging from 3 to 10 (Moure et al., 2006). The reaction conditions may also affect the molecular distribution of oligosaccharides (Nabarlatz et al., 2005). Harsh reaction conditions decrease DP and increase the decomposition of oligosaccharides. Therefore, the production of XOS from LCMs may be carried out by combined chemical-enzymatic treatments (Yuan et al., 2004). Treatment to LCMs with some alkalis, such as NaOH, KOH, and ammonium, may produce xylan or soluble xylan fragments. Once soluble forms of xylan are isolated, enzymatic reduction efficiently yields XOS (Hiroyuki et al., 1995).

Polysaccharide degrading enzymes are also useful tools for the fragmentation of xylan and pectin. The application of enzymes is much more specific, and under less drastic conditions than the chemical fragmentation of polysaccharides. However, degrading enzymes must be completely pure, or at least free from undesirable activity (Bauer et al., 2006). Many degrading enzymes with properties to digest specific polysaccharides and/or sites in polysaccharides have been purified from various microbial sources, such as plant pathogens and parasites (Shin et al., 2009). Alternatively, genes encoding such enzymes isolated from Aspergillus nidulans, Neurospora crassa, and Bacillus licheniformis are expressed in suitable hosts, such as Pichia pastoris (Bauer et al., 2006; Holck et al., 2011). In nature, xylanolytic enzyme systems consist of endo-1,4-β-D-xylanase, which is an enzyme that degrades the xylan backbone into XOS. In turn, exo-β-D-xylanidase is a debranching enzyme that degrades XOS into xyloses, and esterase (Shin et al., 2009). To prepare XOS, the enzyme complex should have low exo-xylanase activity. High exo-xylanase activity results in the suppression of XOS production, through the accumulation of high amount of xyloses (Vázquez et al., 2002). For the fragmentation of pectins, single enzymatic treatments are carried out. For example, for the preparation of POS derived from citrus pectin, endo-polygalacturonase isolated from Aspergillus pulvinulents is treated solely in the enzyme membrane reactor (Olano-Martin et al., 2001). Recently, the sequential enzymatic fragmentation method was developed, which uses various enzymes to produce sugar beet POS with a defined DP (Holck et al., 2011). Through treatment with Pectin lyase, the first POS is released from both HG and RG I type pectins. Through treatment with side chain degrading enzymes, such as β-galactosidase, β-galactanase, α-L-arabinofuranosidase, and α-arabinanase, the second POS originates from the side chain of RG I, with polysaccharides of the RG I backbone without side chains also being produced concurrently. Finally, through treatment with RG I lyase, the final POS derived from the RG I backbone is produced. However, enzymatic fragmentation of the backbone of highly branched RG I has only limited success, as the presence of side chains typically prevents enzymes from digesting adjacent regions of the backbone (Mutter et al., 1998). In this case, an appropriate chemical method to generate RG I fragments, such as the selective depolymerization of the methyl-esterified RG I backbone by β-elimination, must be combined with enzy-
Separation
A variety of compounds, such as monosaccharides, oligosaccharides, and diverse forms of polysaccharides, as well as inorganic materials and proteins, might be produced after fragmentation processes (Vegas et al., 2005). Thus, the purification of oligosaccharides might prove challenging, requiring multiple stages of processing to obtain high quality prebiotic oligosaccharides. Several approaches have been used to separate oligosaccharides, including: 1) solvent extraction, 2) chromatographic separation, 3) absorption, and 4) membrane technology. Solvent extraction, which is also termed as liquid-liquid extraction, is a technique used to separate mixed compounds based on their different solubility in different immiscible phases (Swennen et al., 2005). Typical solvents for this method are water and organic solvents. Solvent extraction can be used to separate desired oligosaccharides from unwanted non-saccharide components or volatile fractions. The separation of oligosaccharides has also been conducted using chromatographic methods, such as a variety of column, size-exclusion, and ion-exchange chromatography methods which provide a reasonably good separation of oligosaccharides with advantages of scales from micrograms to kilograms and relatively low cost (Kabel et al., 2002b). Often, combined multiple separation steps are required to acquire desirable level of oligosaccharides (Katapodis et al., 2003). Size-exclusion chromatography (SEC) is a method in which mixed compounds, including oligosaccharides in solution, are separated based on size or molecular weight (Mehrlaender et al., 2002). Ion-exchange chromatography is based on charge-charge interactions between oligosaccharides and the charges immobilized on resins (Kuhn and Maugeri, 2010). Alternatively, absorption has been utilized to separate oligosaccharides that contain solvents (Vázquez et al., 2000). Typical adsorbents in this method include activated charcoal, acid clay, bentonite, and porous synthetic materials. Membrane technology showed the most interesting method to separate oligosaccharides. Nano-filtration through a ceramic membrane along with a mass cutoff of 1 kDa, facilitates the simultaneous concentration and purification of oligosaccharides (Vegas et al., 2006).

Identification
Both NMR and mass spectrometric studies now have a central role in the elucidation of oligosaccharides, particularly XOS structures (Jones et al., 1994). NMR together with conventional MS and MS/MS data provide sufficient structural information about oligosaccharides (Housell et al., 1997). For example, one- and two-dimensional NMR techniques can provide information for the identification of individual sugar residues, anomeric configuration, interglycosidic linkages, sequencing, and the site of appended moiety. NMR experiments have also revealed the complete structures of oligosaccharides and polysaccharides isolated from milled aspen wood (Telemans et al., 2000). The first 2 polysaccharides were identified as O-acetyl-(4-O-methylglucurono) xylose, and the third fraction was found to be acetylated XOS. Tandem mass spectrometry with ESI has been used to elucidate the structure of underivatized neutral and acidic oligosaccharides. Preliminary structures of about 30 neutral oligosaccharides have been characterized using matrix-assisted laser desorption/ionization (MALDI) and infrared multi-photon dissociation tandem mass spectrometry (MS) (Li et al., 2011).

CONCLUSION
Without doubt, diverse types of xylans and pectins that are found in plant cell walls are potential resources for the development of novel prebiotic oligosaccharides. The potential of xylans and pectins as resources could be developed by the appropriate modification of heteropolysaccharide structures through region-selective chemical and enzymatic modification (Ebringrova and Heinze, 2000). Recent progress towards understanding the biosynthesis of plant cell wall components at the gene and genomics levels (Faik, 2010; Harbolt et al., 2010; Scheller and Ulvskov, 2010) might further expand on the potential applications of these polysaccharides via transcriptic techniques. However, it is necessary to advance existing methods of fragmentation, separation, and isolation of oligosaccharides from the plant cell wall component to realize the optimal potential of these polysaccharides.

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