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Utilization of Agro-Industrial based Polysaccharides waste for Microbial Production of Prebiotics: A Review

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ABSTRACT: Prebiotics are non-digestible short-chain carbohydrates that could be used to selectively stimulate the growth of some groups of beneficial bacteria in the colon. There are a varied number of microorganisms (mainly bacteria and fungus) used for the production of prebiotics. The agricultural activities produce immense amount of waste matter which needs proper disposal for environmental safety and balance. It has been noted that majority of agricultural residue comprise of lignocellulose, proteins, polysaccharides and polyphenols which can be converted into commercially valuable products by the action of microbes and microbial enzymes. These valuable compounds can be in the form of prebiotics that can be consumed by humans for better health and lifestyle.

In recent times, the production of prebiotics from agro-industrial by-products is under examination. The agro-industrial residues majorly include polysaccharides which exhibit their potential as prebiotics. These polysaccharides can be utilized as a substrate by the human gut microbiota, converting it into prebiotics for improved health. Along with this, the use of agricultural by-products is advantageous as it is available in abundance at low cost. Currently, majority of agricultural waste is burnt or discarded in the landfill leading to large scale environmental pollution and barren lands. This waste can be valorized into commercially important products that can not only help in the economical growth, but also reduce environmental degradation. The present review article focuses on the production of prebiotics by microbes using agricultural residues as substrate.

Keywords: Prebiotics, *Lactobacillus* species, Gut microbiota, Polysaccharides, Agro-industrial.

INTRODUCTION

Prebiotics are non-digestible compounds that are produced by metabolic activity of microorganisms present in the gut which modulate the microbial composition and activity in the intestine leading to beneficial physiological effect on the host (Valcheva & Dieleman 2016; Chen *et al.*, 2020). For past 50 years, the agricultural practices have been intensified for feeding the growing human population. This up scaling in production of agricultural crops, livestock breeding, and industrial activities has resulted in accumulation of large amounts of waste matter (Tarkeshwar & Saini 2023). The balanced relationship between livestock and agricultural produce that existed earlier have been deteriorated since chemical fertilizers are introduced for enhanced production of fodder. The industrial residues generated from commercial factories along with increased agricultural produce have generated the problem of their proper disposal (Rajendran *et al.*, 2017). The lack of disposable options for the large amount of agricultural waste that is generated annually on a very large scale leads to deterioration of land and soil where it is discarded and has become one of the major problems related to environmental imbalance.

These agro-industrial wastes are generally comprised of polysaccharides, polyphenolic constituents, carbohydrates, etc. (Marinari *et al.*, 2000; Singh *et al.*, 2019). In order to solve the present environmental crisis caused by agro-industrial waste, the scientific communities are exploring new methods for converting the waste material into commercially valuable compounds. Some carbohydrates, such as fructooligosaccharides (FOS), are well known for their prebiotic effects (Vazquez-Olivo *et al.,* 2018). The production and evaluation of the prebiotic activity of other carbohydrates such as xylooligosaccharides (XOS), pecticoligosaccharides (POS), arabinose-xylooligosaccharides (AXOS), and iso-maltooligosaccharides (IMOS) have been reported (Lomax & Calder 2008).

The demand of prebiotics and functional foods has been rising globally, fostering the need for their efficient production. Prebiotics from agricultural residues can be obtained using chemical, physical and enzymatic methods. The physicochemical methods generally generate unwanted residual compounds as the reactions are carried out at high temperature and pressure. Other major drawback that exist with these method is corrosion of equipments and production of residual pollutants (Marim *et al.,* 2021). The enzymatic method makes use of milder conditions and is highly specific towards its substrate resulting in no residual compounds. The enzymatic reaction is also economic to maintain as it doesn't require specialized setup (Avila *et al.,* 2020). The production of high added-value biomolecules from agroindustrial residues with benefits for health and well-being is paramount to stimulate the market for more sustainable production.

Recently, there has been a rising demand for health supplements that are of natural origin. The polysaccharide-rich agricultural residues can prove to be a promising solution for the demand (Kaprelyants *et al.,* 2017). This agro industrial waste can prove to be the abundant and economic source for the derivation of carbohydrates and other healthy probiotic supplements (Sabina *et al.,* 2014).

AGRO-INDUSTRIAL WASTE MATERIALS

At the moment, waste is increasing in large quantities daily, which has a significant impact on environment health and human community. All industries operating in agriculture sector have a pressing demand of recycling and biotransformation of the generated waste (Bakar *et al.,* 2018). Agro–industries like sugar industries, coffee processing industries, etc. generate an humangous number of lignocellulosic wastes that is threat to the environment and require proper disposal, recycling and biotransformation (Tripathi *et al.,* 2022). Table 1 shows the utilization of different agro-industrial wastes and the microbes involved.

UTILITY OF AGRO-INDUSTRIAL WASTE

Agricultural by-products are extensively studied for biofuel (Wu *et al.,* 2017; Brienzo *et al.,* 2009), enzyme (Oberoi *et al.,* 2011a; Dhillon *et al.,* 2011), and cattle feed production (Oberoi *et al.,* 2011b). Biofuels produced using agro-residue is a promising alternative to fossil fuels. The total crop residues and waste crops are estimated to produce 491 billion liters of bioethanol per year (Kim & Dale 2004). The production of bioethanol from agricultural wastes is majorly carried out by enzymatic hydrolysis. These enzymes are mainly obtained from microbial source and apart from bioethanol, they are utilized for production of other valuable compounds as well (Laufenberg *et al.,* 2003). Agro-industrial wastes are rich substrates for the growth of micro-organisms. The residues like rice/wheat straw, bran, corncobs, bagasse etc. are natural source of carbon that can be utilized by the microbes for their growth, and in return synthesize valuable enzymes which can be extracted for commercial applications (Jecu, 2000; Idres *et al.,* 2021). A variety of agricultural wastes have been utilized for the production of different enzymes by microbes through solid state fermentation (Salim *et al.,* 2017). Agro-industrial waste can also be employed for the generation of various food preservation and flavoring compounds. A variety of flavouring agents like vanilin are also synthesized through microbial bioconversion. The production of vanilin using industrial residues open gateways for the production of other relevant chemicals through valorization of waste products. Vanilin is an important compound of food industry. Apart from it, other compounds like ferulic acid, ascorbic acid etc. have also been reported to be produced from agricultural waste like wheat/rice straw, corncob etc. The use of ascorbic acid in food industry is well known as a preservative and have been utilized for that purpose from centuries (Ayala-Zavala *et al.,* 2009). The waste obtained from citrus fruits is capable of inhibiting the growth of microbes which overall reduces the food spoilage without altering the food properties. It is currently utilized for the preservation of cheese. Agro-industrial residue can also be utilized for the production of biodegradable plastics. Agricultural by-products like sugar beet pulp, apple pomace and berry pomace contain high quantity of pectin which are potential source of prebiotic called as pectin oligosaccharides (POS) (Martinez *et al.,* 2010; Munoz *et al.,* 2012).

Prebiotics. Prebiotics are important active ingredients within any food material that remains resistant to degradation by the human digestive enzymes (Nobre *et al.,* 2018). A plethora of microbes has been utilized for prebiotic production. Prebiotics possess positive effect on the host by allowing a selective group bacterium to actively thrive and simulate within the colon keeping the gut of the host healthy (Gupta *et al.,* 2016). Prebiotics acts as asubstrate for the growth of probiotic bacteria which overall impact the composition of bacterial communities as well as their metabolic activities. This improves health by restoring balance to the host's microbiota (Bindels *et al.,* 2015; Hutkins *et al.,* 2016). According to FAO, "a prebiotic may be a non-viable food component that confers health benefits on the host as related to modulation of microbiota". To qualify as a prebiotic, a substrate must fulfill three criteria issued by FAO:

(1) Component: not an organism or drug; a substance which will be characterized chemically and, in most cases, these are going to be food-grade.

(2) Health benefits: measurable and unrelated to the component's absorption within the bloodstream when acting alone.

(3) Modulation: Deliver changes in the composition or activities of the target host's microbiota, such as fermentation, receptor blockage, or others.

Therefore, a prebiotic can be a fiber, but not all fibers are prebiotic. Following that, efforts were made to broaden the location of action of prebiotics to include the skin, mouth, and feminine genital tract in addition to its primary action site, i.e., the gastrointestinal tract. According to Samanta *et al.* (2007). An ideal prebiotic should have the following criteria:

(1) Beneficial gut microflora selective fermentation (observed in *in-vitro* or *in-vivo* experiments).

(2) A shift in gut microflora homeostasis toward the beneficial side increases population or metabolic activity.

(3) Protects the host's health and well-being (increases productivity or product quality in animals).

(4) Derived from plants or produced by microorganisms or their enzymes.

(5) Maintains structural integrity while passing through different parts of the gastrointestinal tract.

(6) There are no residue issues.

(7) Ideally to be used as a food/feed additive.

(8) Compatibility with other ingredients in food or feed.

Many studies suggests that prebiotic intake plays a crucial role within the human system, and regulates metabolism of lipid and minerals. Prebiotics can even protect against colon cancer, cardiovascular diseases and metabolic syndromes (Lam *et al.,* 2013). The demand for prebiotics have been hiked over the recent times as consumers demands are switching towards consumption of more healthier products (Slavin *et al.,* 2013).

IMPORTANT SOURCE OF PREBIOTICS AND THEIR PRODUCTION

Pectic Oligosaccharides (POS). Agricultural by-products that are rich in pectin are a potential source of pectic oligosaccharide (POS) prebiotics. Pectin is a complex polysaccharide that is found in the cell wall of higher plants (Chen *et al.,* 2013). Pectin comprises a family of acidic polymers, known as homogalacturonan (HG) and rhamnogalacturonan (RG) with several neutral sugars/polymers such as arabinans, galactans, and arabinogalactans (attached as side chains) (Fig. 1) (Obro *et al.,* 2004; Strasser & Amando 2001). POS promotes the growth and activity of *Lactobacilli* and *Bifidobacateria* in the human gut and reduces the possibility of occurrence of pathogenic microbe (Balden *et al.,* 2003; Manderson *et al.,* 2005). The extraction of POS from pectin rich agriculture waste is a promising approach towards production of prebiotic and proper utilization of naturally available residual waste (Westphal *et al.,* 2010). Short-chain fatty acids (SCFA) are produced by the digestion of POS prebiotics, which exerts several health effects like inhibition of pathogenic bacteria, relief of constipation, reduction in blood glucose levels, improvement in mineral absorption, decreased incidence of colonic cancer, and modulation of the immune system (Gullon *et al.,* 2013; Mussatto & Mancilha 2007). POS can also act as an antibacterial agent, flowering inducer, and phytoalexin elicitor (Iwasaki *et al.,* 1998).

Fructooligosaccharides (FOS). The applications and health advantages of FOS are well established. FOSs are not used as a source of energy in the body and are rarely hydrolyzed by digestive enzymes providing low content of calorie. This provide FOS an advantage that it can be safely included as the product in the diet of any diabetic patient. Apart from this it also plays a vital role in reducing the triglycerides, cholesterol and phospholipid content and enhances the mineral absorption in the intestines. It is also used commercially in chewing gums and dental products (Yun, 1996). FOS is non-carcinogenic and conventionally it was produced by the action of enzymes β-fructofuranosidase (FFase; 3.2.1.26) and fructosyltransferase (FTase; 2.4.1.9) on sucrose to yield FOS. Its production is a two-step process in which a hydrolyzing enzyme is synthesized which is later used for biotransformation of the substrate into FOS (Ganaie *et al.,* 2014). For the commercial production of FOS high concentration of sucrose is necessary (Dominguez *et al.,* 2013). A plethora of fungal strains have been reported for the production of FOS by enzymatic degradation of sucrose rich industrial residue. The most common fungi include *Aspergillus niger, Rhizopus stolonifer, Penicillium citreonigrum, Aspergillus oryzae* etc. The structures of various macromolecular moieties have been depicted in Fig. 2.

Xylooligosaccharides (XOS). Among the prebiotics, XOS holds a significant position due to its multifaceted effects on human health and its potential to play a key role in the treatment of different gastrointestinal disorders. The production of XOS is mostly carried out using lignocellulosic compounds which are available in abundance throughout the world.

XOS is sugar oligomers comprised of xylose units through β-(1-4) xylosidic linkages, *viz*., xylobiose (2 monomers), xylo-triose (3 monomers), xylotetrose (4 monomers), xylopentose (5 monomers), xylohexose (6 monomers) (Kumar & Satyanarayana 2011). Xylose residues range from 2 to 10 and side groups such as acetyl groups, α-D-glucopyranosyl uronic acid or its 4-O-methyl derivative, and arabinofuranosyl residues are commonly found (Fig. 3) (Aachary & Prapulla 2010). The XOS is heat resistant and remains stable in acidic Ph (Moura *et al.,* 2006). XOS is non-carcinogenic and regulates insulin secretion from the pancreas, additionally to the extent of mineral absorption from the intestine. Owing to multiple advantages of XOS, its incorporation in the diet can lead to proper health and functionality of human gut (Di Bartolomeo *et al.,* 2013). XOS is found to be safe for human consumption when taken in range of 7-10% without exhibiting any side effects (Hsu *et al.,* 2004). Human studies, on the other hand, have shown that XOS has benefits when consumed in amounts ranging from 2 to 5 g per day (Kobayashi *et al.,* 1991). Xylooligosaccharides (XOS) which showed significant prebiotic effects on *Bifidobacteria* and *Lactobacilli* are produced from agro-residues and hardwood such as mahogany and mango sawdust by using a purified xylanase from *Clostridium* strain BOH3 (Rajagopalan *et al.*, 2017). XOS poses beneficial effects on the human body which include immune stimulation and increased absorption of minerals. This opens the door for the wider applicability of XOS in diversified products.

Mannanooligosaccharides (MOS). It is the mannan derived oligosaccharide that comprise of linear mannan containing only mannopyranosyl units linked by β-1,4 linkages (Fig. 4). MOS contributes to better health of human gut by allowing proliferation of normal bacterial flora and inhibiting the growth of any pathogenic organism (Patel & Goyal 2012). Moreover, MOS has also been proved to possess immune-pharmacological, therapeutic, and biomedical properties which makes it an important compound that can be incorporated in the food, feed, and pharmaceutical fields for better health and development (Ferreira *et al.*, 2012; Srivastava *et al.*, 2017). To derive mannanoligosaccharides from agricultural waste, enzymatic hydrolysis of linear chains into smaller oligosaccharide and of side groups are necessary. The enzyme β-Mannanases breaks the glycoside bonds of the mannan polymer chain producing mannanooligosaccharides. β- Mannosidase releases mannose units by attacking the terminal linkage of oligosaccharides or through cleavage of mannobiose. Glucopyranose units are often far away from glucomannan and galactoglucomannan by β-glucosidases. β mannanases are produced commercially by bacteria and fungi through solid state and submerged fermentation process. This enzyme is produced extracellularly once the microbe is allowed to grow on mannan rich substrate under optimum parameters like temperature, pH, and carbon source, either in submerged or solid-state fermentation (Srivastava & Kapoor 2017). Several studies have been published that describe the isolation of -mannanase from fungal or bacterial strains, optimization assays to increase yields or enzyme activities, and even cloning of -mannanase genes in different hosts to facilitate enzyme expression (Vera *et al.*, 2016). The main objective of those studies is to use the β-mannanase for mannan hydrolysis and obtain MOS thanks to its potential application as a prebiotic and proven efficiency as a supplement in the food, feed, and medical fields.

MOS selectively enhance the growth of *Bifidobacterium* and *Lactobacillus* in the gut. The *Lactobacillus* can indirectly stimulate growth of enterocytes which exerts a trophic action (Moreno *et al.*, 2017). The supplementation of MOS in the regular diet promotes weight loss in obese individuals by reducing the uptake through GI tract (Blibech *et al.*, 2011). Additionally, MOS is also known to decrease incidence of diarrhoea once included in the regulate diet. These non-digestable oligosaccharides when added with soluble fibres alter the mineral uptake process in the intestine enhancing bone and overall health of the individual (Jian *et al.*, 2013).

Galactooligosaccharides (GOS).

Galactooligosaccharides are galactose-containing oligosaccharides with β (1–3) and β (1–4) bonds between the monomers (Fig. 5). GOS is a collective term for a group of carbohydrate containing oligo-galactose with some amounts of glucose and lactose. GOS passes undigested into the large intestine where it enhances the bowel mass and promote growth of selective microbes like *Saccharomyces*, *Lactobacillus* and *Bifidiobacteria*. GOS are prebiotics comprising of plant sugars that are linked in chains. GOS are known to be an effective prebiotic that prevent food allergies, obesity, constipation and eczema (Fanaro *et al.*, 2005). GOS can be naturally occuring, as in milk, or can be derived by enzymatic hydrolysis of lactose by β-glucosidases. The dairy industrial waste like whey is a rich source of lactose and is utilized for the production of GOS. GOS is also known to be present in human milk protecting the infants from gastrointestinal pathogenic bacteria.

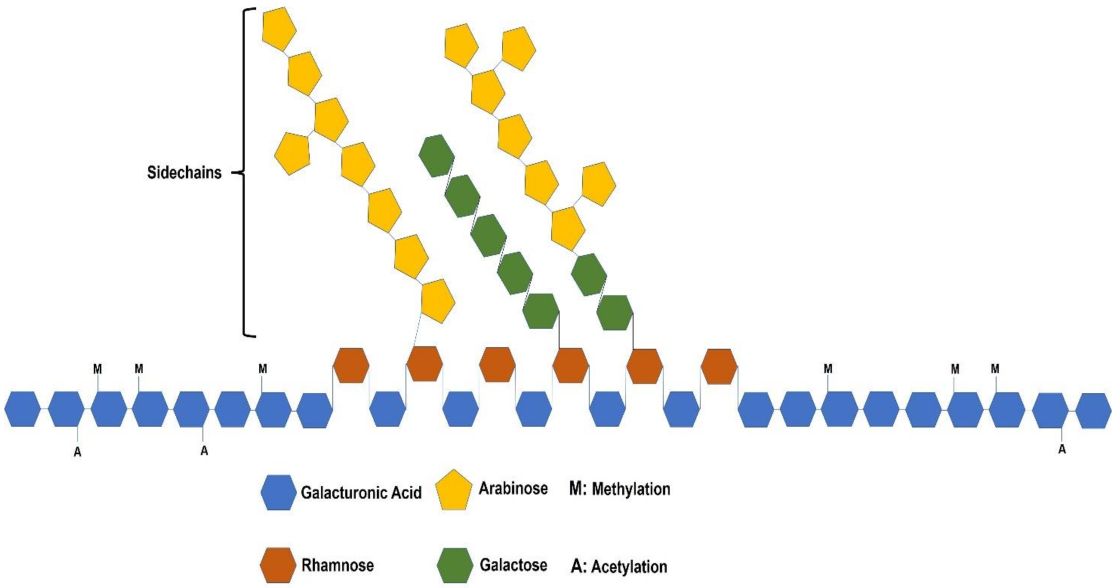

Fig. 1. Schematic structure of a pectic-oligosaccharide.

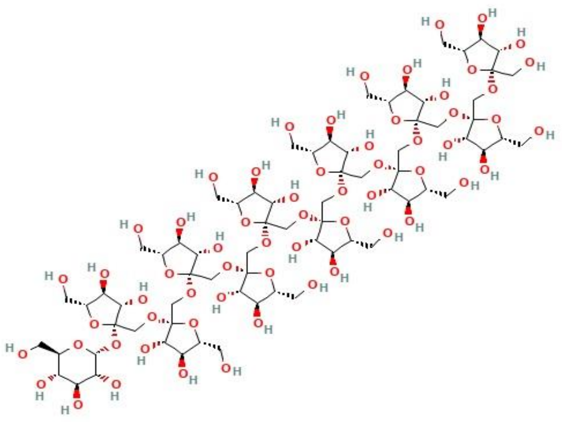

Fig. 2. Schematic structure of a fructo-oligosaccharide.

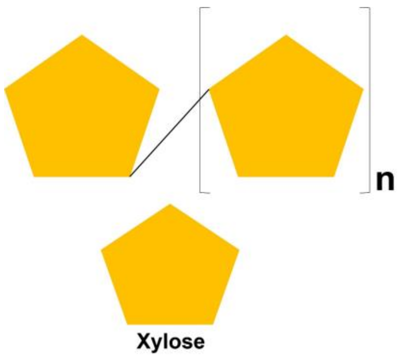

Fig. 3. Schematic structure of a xylo-oligosaccharide.

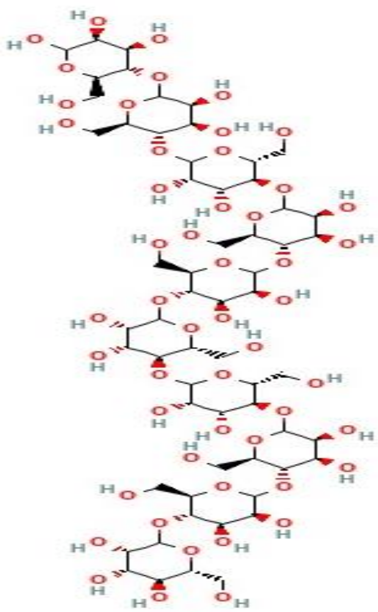

Fig. 4. Schematic structure of a mannan-oligosaccharide.

CONCLUSIONS

The administration of prebiotics promotes beneficial effects on human health. These have been used as complementary treatments for intestinal diseases since they are capable of competing with pathogenic microorganisms in addition to activating the cells of the immune system. It is also important to consider that the unbalance or lack of these microorganisms has been linked to the risk of suffering from obesity, diabetes, and some types of cancer. Nowadays, we know the effects of certain species of bacteria that have beneficial effects on human health and that the balance of these can influence the maintenance of homeostasis. This type of microorganism, therefore, should be included in diets frequently to achieve it.

FUTURE SCOPE

The present study underlines the important oligosaccharides that can be derived from the agricultural residues and can be extensively used as probiotics for improvement of human health. Apart from this, the valorization of the agricultural residues into commercially valuable prebiotics can enhance socio-economic state of the country as well as reduce environmental pollution that is rising due to accumulation agricultural by-products. The future scope of this study lies in the commercialization of current methodologies for prebiotic production for enhanced economy. Further research can also lead to development of other commercially valuable compounds that can be derived from the agricultural wastes.

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