

## Research Article

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# Evaluation of rice husk as a substrate for the growth of *Lactobacillus* species

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### Abstract

The most produced and consumed cereal is Rice (*Oryza sativa*). Rice husk is composed of 11% hemicellulose with xylan being the main sugar subunit. Xylan is the most abundant carbohydrate in lignocellulosic biomass the precursor of Xylooligosaccharides (XOS), used as prebiotics. Toxic products and activity of pathogenic bacteria are decreased by xylooligosaccharides which enables the growth of probiotic bacteria. The present study was aimed to study the by-products of rice cultivation as a substrate for the growth of *Lactobacillus* species. The collected rice husk was weighed and dried in Hot Air Oven. After hot air drying the sample was weighed again and measured the dry weight. A two-step auto-hydrolysis method was used. The liquor contained xylooligosaccharides which were refined by filtration. The non-digestible oligosaccharides XOS were predominant in acid extract when sulfuric acid was 2% (w/w), temperature and time were 100°C and 0.5 hours respectively. The prebiotic efficacy of XOS was checked with *Lactobacillus* strains grown in MRS broth for in-vitro evaluation. The OD600 was high with the liquor extract of rice husk instead of other sugar. This study shows xylan from agriculture waste produces XOS, an emerging prebiotic with a promising low-cost strategy of production.

**Keywords:** Fermentation; *Lactobacillus*; Probiotics; Rice Husk; Substrate

### Introduction

A broad variety of non-starch, starch, polysaccharides, oligosaccharides, amino acids, proteins and dietary fibers in the diet that cannot be digested by the human small intestine are fermented by colonic microflora. As the primary end product of fermentation, oligosaccharides contain short-chain fatty acids (SCFA). SCFA varies according to the forms of oligosaccharides

and includes butyrate and propionate as well. To supply nutrition to the organism, these molecules are further metabolized and are used for essential physiological processes such as absorbance of calcium, metabolism of lipids and bowel function, decreasing the risk of colon cancer [1].

Plant cell wall contains lignin associated with hemicellulose which provides rigidity to the outer boundary of the cell. The cell wall

contains a greater quantity of these polysaccharides. These polysaccharides are soluble in alkali and partially soluble in water. Xylan, glucomannan, mannan, arabinoxylan, galacto-glucomannan, and glucuronoxylan are different polysaccharides in hemicellulose. Plants contain a higher amount of xylan which is necessary for the development of xylooligosaccharides (XOS), the recalcitrance of the cell wall is increased which defends the plants against pathogens. *Lactobacillus* and *Bifidobacterium* are major probiotics present in the gastrointestinal tract of a human. Fermentation of xylose composed of oligosaccharides and polysaccharides is done efficiently by *Bifidobacterium*. Lactic acid bacteria produced short-chain fatty acid by degradation and fermentation of non-digestible oligosaccharide and carbohydrates with enzymatic reactions. These fatty acids cause acidification of human intestines and provide metabolic energy to the host. *Staphylococcus*, *Escherichia coli*, and *Clostridium perfringens* are harmful bacteria and their multiplication is inhibited with XOS [2]. Utilizing lignocellulosic biomass

for fermented feedstock has reduced the raw material cost and helped to dispose of solid waste. Xylan is the structural component of dicot and monocot plants, composed of a backbone of d-xylose with d-galactose, glucuronic and acid residues. Xylan is heteropolysaccharide in nature, it is used in different products like xylitol and xylooligosaccharides, anti-oxidant, and biodegradable films. As a result of chemical hydrolysis hazardous byproducts are produced, therefore hydrolytic degradation of xylan has been preferred. Xylanase has a specific interest because it is directly involved in glycosidic bond cleavage and short XOS liberation. Enzymes with lower xylosidase or xylanase activity lead to the production of XOS rather than xylose production [3].

XOS can be produced alternatively by rice husks at no cost because it is usually discarded in the environment. The most produced and consumed cereal is Rice (*Oryza sativa*), which is the main food for half of 50% of the world population (Table 1).

**Table 1. Components of rice husk**

Components of Rice husk	g/100g of oven-dried weight
Starch	2.9
Cellulose	22.7
Xylan	18
Araban	1.9
Acetyl groups	1.7
Uronic acids	1.1
Ash	12.6
Lignin	34
Moisture	10

Husks of rice are composed of 11% hemicellulose with the main sugar subunit xylan as the main residue after rice processing. Rice husk is composed of xylan, glucan, lignin, arabinan and ash which serves as a value-added product for the fermentable

sugars [4]. Xylooligosaccharides are the sugar oligomers made up of the main backbone of xylose. Rice husk, bran, Corn cob, straws, and hardwood is typically lignocellulosic xylan comprising material, used for XOS production.

Xylooligosaccharide is included in a list of promising prebiotic as a nonviable food component associated with modulation of the host microbiota. Corn cob is used as animal feed contains xylose and xylitol (oligosaccharides) [3]. Anaerobic respiration carried out in the gut are:

- Saccharolytic Fermentation
- Proteolytic Fermentation

Proteolytic fermentation includes phenolic, amines, and ammonia compounds as end products all of which are toxic compounds. Saccharolytic fermentation occurs mainly in the proximal part of the colon. Microorganisms preferentially ferment carbohydrate and when carbohydrates are depleted they twist to protein fermentation. SCFA, acetate, propionate, and butyrate have contributed to colonic health as end products of carbohydrate metabolism. The main site for the saccharolytic fermentation is the proximal colon and the distal colon has more proteolytic metabolism due to the depletion of carbohydrates [5]. Short-chain fatty acids can decrease pH, enhance the process of mineral absorption, and provide a source of energy to epithelial cells of colons, furthermore, butyrate shows anti-carcinogenic and anti-inflammatory activity. Acetate is the key substrate in the process of cholesterol formation, while propionate inhibits synthesis. Lipid metabolism requires acetate and propionate [6].

Lignocellulosic biomass is the richest source of xylan a carbohydrate which is the precursor of xylooligosaccharides. Gastrointestinal disorders are prevented when xylooligosaccharides are used as prebiotic, providing health benefits to livestock and humans. FOS and inulin do not exhibit temperature and acid stability as XOS, making these oligosaccharides as potential prebiotic. Nutraceutical that can be obtained from lignocellulosic biomass shows a niche among prebiotic [7]. Oligosaccharides are carbohydrates consist

of 2-10 branched or linear monosaccharides connected with glycosidic linkages which have the  $\alpha$ - $\beta$  configuration. As a prebiotic oligosaccharide has many health benefits. XOS comprise of 2 to 10 residues number of xylose. Polymerization degree for xylooligosaccharide up to 20 is referred by scientists, thus branched XOS is obtained as a result of the hydrolysis of xylan. XOS is suitable for diabetic patients because it is low-calorie sugar which shows no elevation of blood glucose. Cryto-protective and antioxidant properties of XOS makes this sugar a dietary supplement and drug adjuvant [8].

Lignocellulosic material is a source of promising raw materials and agriculture residues have main features like ready availability, abundance, renewability, and low costs which make it a promising raw material. Agriculture residues of corn cob and other agro residues have been used while rice straw is the most substantial residue worldwide for research purposes and biorefineries. Asia is known as the leading region for rice production and 90% of wheat straw left over after harvesting of the rice. The structural composition of rice husk is 34 % Glucan, 15 % Xylan, 17% Lignin, 11% ash, and 2.2% Arabinan. Nondigestible oligosaccharides (NDO) and Fermentable sugar can be produced by using it as raw material [9]. The increasing application of XOS in cosmetics, pharmaceutical, and medical contributed emerging modern technologies for the production of XOS. The treatment of xylan rich material in aqueous media is an alternative method for XOS production due to fast growth and high selling price of XOS in the functional food market. The advantages of using the water as the only chemical include low by-product generation reduced operational cost and limited corrosion. XOS should be highly pure for food application and it has higher prices than other NDO. Membrane technologies

have been developed for the XOS as food constituents from undesired compounds in autohydrolysis liquors. Endo-1-4-xylanases enzymes are used for further reduction of the degree of polymerization. The rice husk derived XOS is used for the in vitro growth of different species of *Bifidobacteria* [10]. This work is aimed for the production of NDOs using water extraction methods and acid extraction method from rice husk which is considered as renewable resource for the production of healthy material.

## Materials and Methods

### Feedstock

Rice husks (the vegetative part of rice) were collected from agronomy farms of The University of Agriculture. The rice husks were milled, sieved, air dried and placed in screw-capped glass jar. The rice husks were placed at room temperature for 1 hour and weighed using the weight balance. The sample was placed on filter paper and air-dried. Weighed 05 gram of sample was taken and dried in Hot Air Oven at 105°C for 5 hours. After hot air drying the sample was weighed again and measured the drying loss. Rice husks were cooled to room temperature by placing in controlled temperature chamber for 30 minutes. The rice husks were weighed again.

### Water extraction

Extractive free rice husk were obtained by extraction using Soxhlet apparatus with water extraction for 24 hours followed by 95% SIGMA ALORICH Ethanol for 24 hours. Weighed the sample and packed the rice husk in filter paper pouch for the extraction and placed in extractor of Soxhlet apparatus. A 6% w/w rice husk extract solution was made in preheated deionized water. This mixture was placed in water bath at 50°C for 1 hours, 60°C for 3 hours, 70°C for 5 hour, 100°C for 0.5 hour and after interval of 15 minutes again placed the extract at the temperature of 100°C for 15 minutes. The extract was

manually agitated for 10 minutes and filtered using the filter paper.

### Acid extraction

A 10ml aliquot of clear water extract of rice husk with 1% H<sub>2</sub>SO<sub>4</sub> and deionized water was prepared. The total volume of aliquot is 25 ml. H<sub>2</sub>SO<sub>4</sub> catalyst was used to convert the liquid product of xylan into xylooligosaccharides. This mixture was added to autoclave-able tubes and incubated at 100°C for 1 hour. After incubation the mixture is quickly cooled in ice-bath at room temperature. The extract was neutralized by using solid CaCO<sub>3</sub> suspension, after waiting for 1 hour at normal room temperature. The CaCO<sub>3</sub> was allowed for the precipitation.

### Determination of XOS

The above clear liquid was filtered and placed in ice-bath. The autoclave-able jar was placed in autoclave condition at 121°C for 0.5 hour. The clear supernatant was collected which is rich source of xylooligosaccharides [11-13]. Lactic acid bacteria *Lactobacillus* species were procured from the Probiotic laboratory of The Institute of the Microbiology, University of Agriculture. Glycerol stock culture was preserved and suspension was used for inoculating the MRS broth [10].

### Fermentation of XOS

Aliquot of rice husk were prepared. The neutralized extract of rice husks had added to the MRS medium which had glucose concentration equal to 0.5% (w/v). The overnight culture suspension of *Lactobacillus* had been inoculated in medium and incubated at 37°C for 24 hours. *Lactobacillus* was grown in MRS broth with 20 ml of rice husk clear filtrate and then incubated for 24 hours. The spectrophotometer was used at 600nm to check the absorbance of culture after 24 hours. After the inoculation of *Lactobacillus* and XOS, fermentation was carried out at 37°C temperature. Acid production will be detected by checking the pH with a pH-meter.

The spectrophotometer was used for the detection of bacterial growth at 600 nm [4, 14].

## Results

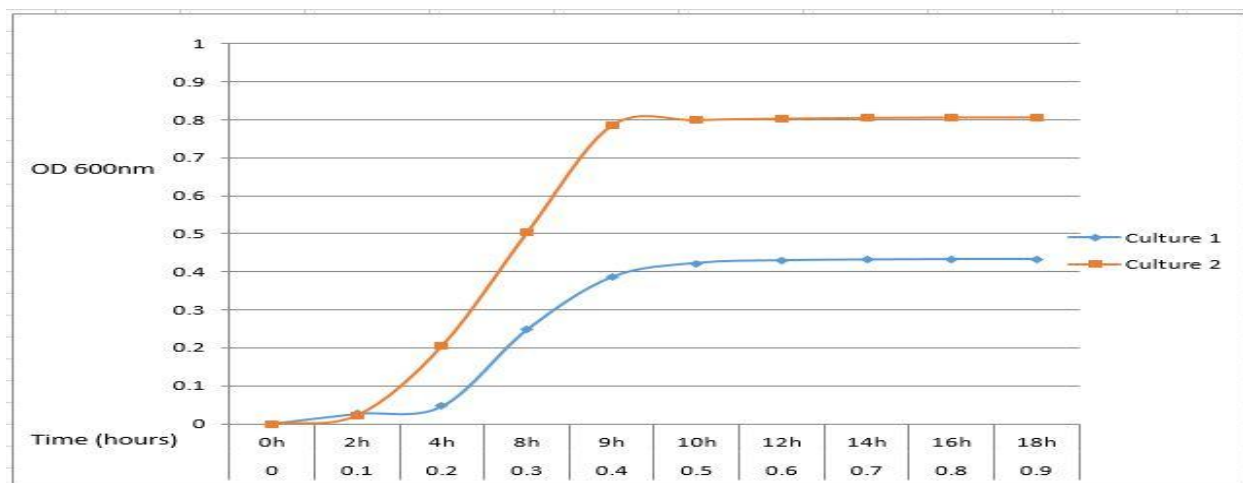
### Quantification of Xylooligosahharide

Based on the UV Spectrophotometer absorbance at OD value of 600 nm the component of Rice husk XOS was unambiguous result of XOS originating from lignocellulosic biomass of rice husk. Although XOS was produced with autohydrolysis of many agricultural byproducts. From the results it can be concluded that RH-XOS contain a very high proportion (~84%) of partially O-acetylated XOS with structural features typical of oligosaccharides derived from the methyl-

glucuronoxylan-type hemicelluloses, some carrying single side chains. In Vitro Ferment ability of Purified XOS and accumulation of Lactate and SCFA in the Fermentation Media. Two broths sample were put under the UV spectrophotometer to check the absorbance value. I used culture of two samples for the reading in the spectrophotometer. One sample which was not inoculated with the rice husk extract had the showed turbidity too late and the growth was not in dense state. The second sample which contain the rice husk has highest turbidity rate within small duration of time and the OD value was high for the broth culture with rice husk extract. The values for the OD values are in (Table 2 & Fig. 1) [1].

**Table 2. OD values of broth cultures**

OD value at 600nm	Time	Culture 1	Culture 2
0	0h	0	0
0.1	2h	0.026	0.023
0.2	4h	0.046	0.206
0.3	8h	0.247	0.503
0.4	9h	0.385	0.785
0.5	10h	0.422	0.799
0.6	12h	0.429	0.803
0.7	14h	0.431	0.805
0.8	16h	0.432	0.806
0.9	18h	0.432	0.806

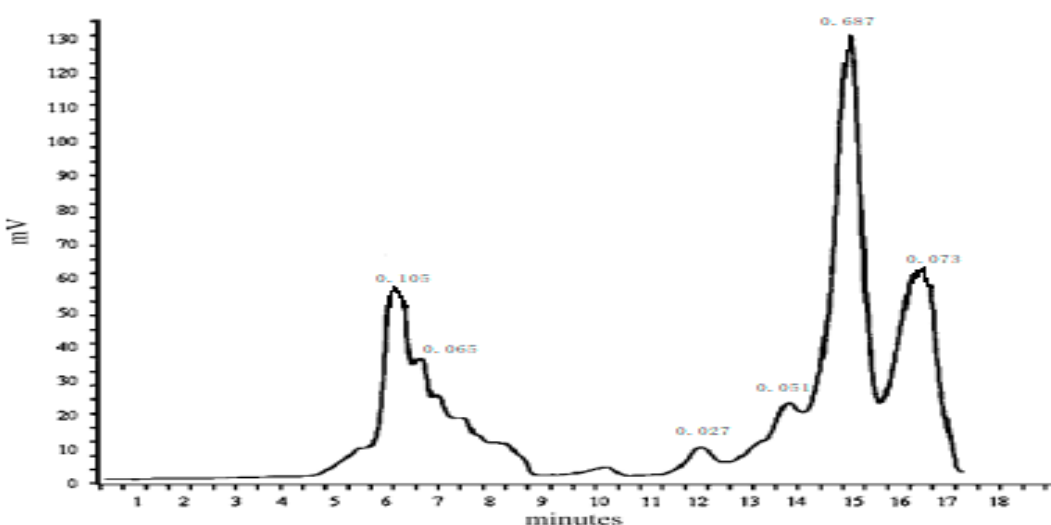


**Figure 1. Growth curve**

### Quantification of Xylooligosaccharides

The aliquot obtained at the end of extraction process is filled in the xylooligosaccharide column of HPLC, the curve represented different components in the rice husk extract. The highest peak curve of the xylooligosaccharides and arabino-

xylooligosaccharides. Reducing sugar were the component which is represented by the peak. All water extract and hydrolyzate were filtered through 0.2 mm syringes prior to HPLC analysis. An Altech HPLC system was equipped with an evaporative light scattering detector, a column heater and pump (Fig. 2).



**Figure 2.** HPLC chromatogram of rice husk extract

A Rezex Pb2 column equipped with guard column was used to separate the monosaccharides and the oligosaccharides. The separation condition were 50 ml injection and HPLC grade water as the mobile phase 0.6 ml minimum flow rate 60 column temperature and 25 minute retention time. The vast majority of efforts are channeled on the isolation of *Lactobacillus* and their exploitation for providing health benefits. The aim of this study was the isolation and identification of xylooligosaccharides from rice husk for evaluation of growth of *Lactobacillus*, These isolates were cultured on MRS broth and sub-cultured when required. Isolates that appear to be gram positive, rod-shaped, non-spore formers, catalase negative and facultative anaerobes confirmed their belonging to genus *Lactobacillus* [15]. It was analysed that xylose is the source xylitol which was used

for the procurement of compounds containing ester and ether groups. These compounds were used in the formation of thermoplastics used in capsulation, coatings, and water-solubilized films. The process of auto-hydrolysis was used for the depolymerization of xylan. Lignocellulosic material in an aqueous condition was heated and auto-ionization of water produces hydronium ions which cause the depolymerization and deacetylation of xylan. Depolymerization of xylan was the source of xylooligomers and xylose, in addition to deacetylation, xylan produces acetic acids which increase the concentration of hydronium ions in the medium. The hydrolyzate contained some other compounds such as dehydration products, lignin, and monosaccharides, so purification is required. The high degree of polymerization and a low degree of

polymerization compounds result from auto-hydrolysis. The breakdown of oligosaccharides into monomeric sugars in an autoclave at 121°C for 4 hours. Chemical or

enzymatic methods are used to convert XOS to xylose and hydrolyzate of this xylose can be used to produce xylitol by fermentation (Table 3) [16].

**Table 3. Quantity of component of rice husk extract**

Component	Quantity	Percentage	Ratio
<b>Xylooligosaccharides</b>	0.682	68%	17: 25
<b>Arabinose</b>	0.105	10.5%	21: 200
<b>Glucose</b>	0.073	7.3%	73: 1000
<b>Xylanase</b>	0.065	6.5%	13: 200
<b>Phenolics</b>	0.052	5%	1: 20
<b>Unknown (others)</b>	0.027	2.7%	27: 1000

### Discussion

Rice straw are the cheapest source of XOS and due to their excess availability and low cost it is used for the production of non-digestible oligosaccharide. Temperature and the acid concentration of acid used for the research had effected the yield. The major monosaccharide produced from the rice husk are the xylose, which is the basic subunit of XOS. XOS is the most prominent oligosaccharide in the rice straw. The present examination was directed for the isolation of a prebiotic xylooligosaccharide from rice husk for the growth of *Lactobacillus* species. *Lactobacillus* species were procured and identified. For the isolation of bacteria sample was inoculated on selective media. MRS agar media was selective for isolation of *Lactobacillus*.

The XOS produced from this research is used for the nourishment of probiotics while the other NDO were by product. The consumption of discarded rice husk as a prebiotic can provide nutrients to gut microbiota of the gastrointestinal tract of humans and improve immunity by increasing the population of probiotics. Xylooligosaccharides which are obtained from rice husks are the cheapest source of prebiotics. The lignocellulosic material is necessary as the raw material of non-

digestible oligosaccharides sugars and considered safe for human consumption. Dilute acid extraction and water extraction methods are used for the production of non-digestible oligosaccharides (NDO). These NDOs obtained from agro residues are utilized for increasing the income of the farmer, reducing pollution in the environment, and refining human health.

### Conclusion

In our basic nutrition and clinical supplementation requirements, probiotics are an essential microbe with a functional attribute. These bacterial strains have demonstrated favorable responses to the treatment of various bacterial diseases such as diarrhoea, IBS, and food allergies. In addition, these probiotics have contributed to the prevention and treatment of diseases such as diabetes, obesity, and cancer. In general, probiotic supplementation related to milk products is often used in non-milk-fermented food products. In the process of testing new probiotic strains, these supplementations provide a replacement and profitable source. Many studies have been active in discovering certain extraordinary roles of various probiotic strains in current therapeutic and nutritional evaluations. The research is based on the recovery of xylan from the lignocellulosic biomass of rice husk. Mainly

the recovery of xylan from the agro residues which is converted to XOS and helped out in growth of probiotics. Sodium hydroxide is better for yielding the higher content of prebiotics. Steam is preferred over incubation for increase growth of xylan. The alkali is used for extracting the the xylan because it can eliminate the reducing sugar. FTIR is used for showing the pattern of xylan. Acid hydrolysis is used for the production of XOS from xylan. XOS can influence the growth of probiotics and XOS can be included in the synbiotic module. Economic methods are used for the production of XOS from agro residues which will cast a positive impact on the health of human as well as animals.

#### Authors' Declaration

The study was conducted according to the guidelines of the Declaration and approved by the Ethics Committee of the Institute of Microbiology, University of Agriculture, Faisalabad 38000, Pakistan.

#### Authors' contributions

Conceived and designed the experiments: M Ashraf & A Palwasha, Performed the experiments: A Palwasha & A Akram, Analyzed the data: A Waheed, M Ashraf & A Akram, Contributed materials/ analysis/ tools: N Fatima, ZMNM Awan, Wrote the paper: HU Rahman, W Khursheed, N Fatima & S Gul.

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