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Xylooligosaccharides production by fungi cultivations in rice husk and their application as substrate for lactic acid bacteria growth

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ABSTRACT

We describe a three-steps bioprocess to convert rice husk as source of xylooligosaccharides and then used these prebiotics to grow lactic acid bacteria as a model to produce probiotics from vegetal sources. First, *Aspergillus brasiliensis* BLf and the recombinant *Aspergillus nidulans* XynC A773 strains were solid-state cultivated in rice husk to produce xylanases (234.7 ± 0.01 U·g^{-1} and 192 ± 0.03 U·g^{-1} substrate, respectively). These enzymatic preparations were directly applied to rice husk to hydrolyze its hemicellulosic structure and obtain xylooligosaccharides (37.25 mg XOS·g^{-1} substrate and 75.92 mg XOS·g^{-1} substrate, respectively). Prebiotic effect of XOS was tested by cultivating probiotic strains *Lactobacillus plantarum* BL011 and *Bifidobacterium lactis* B-12, which grew in this substrate. The results in this work showed that it is possible to produce xylooligosaccharides and then proceed to use these prebiotic sugars to grow probiotic bacteria using the same agro-residue, being possible their use in food related applications.

1. Introduction

Xylooligosaccharides (XOS) are xylose oligomers that can be produced by enzymatic hydrolysis of xylan. XOS contain from 2 to10 units of xylan, which are linked by β-(1,4) bonds, and are considered fibers having important nutraceutical properties (Milessi et al., 2015). Although XOS can be obtained via chemical synthesis or by enzymatic synthesis using pure substrates, one alternative for XOS production would be the enzymatic hydrolysis of biomass materials, making the process more cost-effective and sustainable (Moniz et al., 2014).

Rice (*Oryza sativa*) is one of the most produced and consumed cereals in the world, characterized as the main food for more than half of the world population. Rice husk is the abundant residue of rice processing, of which about 11% is composed by hemicellulose, in dry basis (Menezes et al., 2017; Saha et al., 2005; Hickert et al., 2013), with xylan being its main sugar polymer (Perez et al., 2005; Walter et al., 2008).

XOS are added-value functional food additives that can be produced from lignocellulosic biomass and the use of rice husk is an economical alternative for its production because this material has a very low or no cost at all, in many cases simply being discarded in the environment (Nara et al., 2014; Samanta et al., 2015). Xylanases (1.4-β-D-xylanases, EC 3.2.1.8) are the main *endo*-enzymes that hydrolyze randomly β-1,4 linkages of xylans, the main polysaccharide of hemicelluloses (Akin, 2008). Several microbial sources of xylanases, both bacterial and fungal, are used as enzymatic extracts to produce XOS. Some studies also reported on the use of recombinant microorganisms to overexpress genes for xylanase production. The use of a xylanase from *Bacillus subtilis* (XynA), overexpressed in *Escherichia coli*, was tested for XOS production and good results were obtained (Bragatto et al., 2013). Driss et al. (2014) studied the production of xylooligosaccharides obtained from corncobs using *Penicillium occitanis* xylanase. Zheng et al. (2014) characterized a thermostable xylanase from *Paenibacillus* sp. NF1 that could be applied in xylooligosaccharides preparations.

Scientific and commercial interest in oligosaccharides has increased in the last decades due to the identification of several health benefits these compounds can bring to hosts, both in human nutrition and animal feed preparations (Rastall, 2010). In this context, oligosaccharides, in general, show excellent effects and health benefits as prebiotic when consumed as part of the diet, improving the growth of probiotic microorganisms, such as *Bifidobacterium* and *Lactobacillus*, bacterial strains that are found in the human digestive system (Falck et al., 2013). Probiotic microorganisms live in the digestive system of humans and other animals, being responsible for several important mechanisms of nutrient absorption and overall health of the body (Adolfsson et al., 2004). It has been shown that XOS selectively stimulate the growth of

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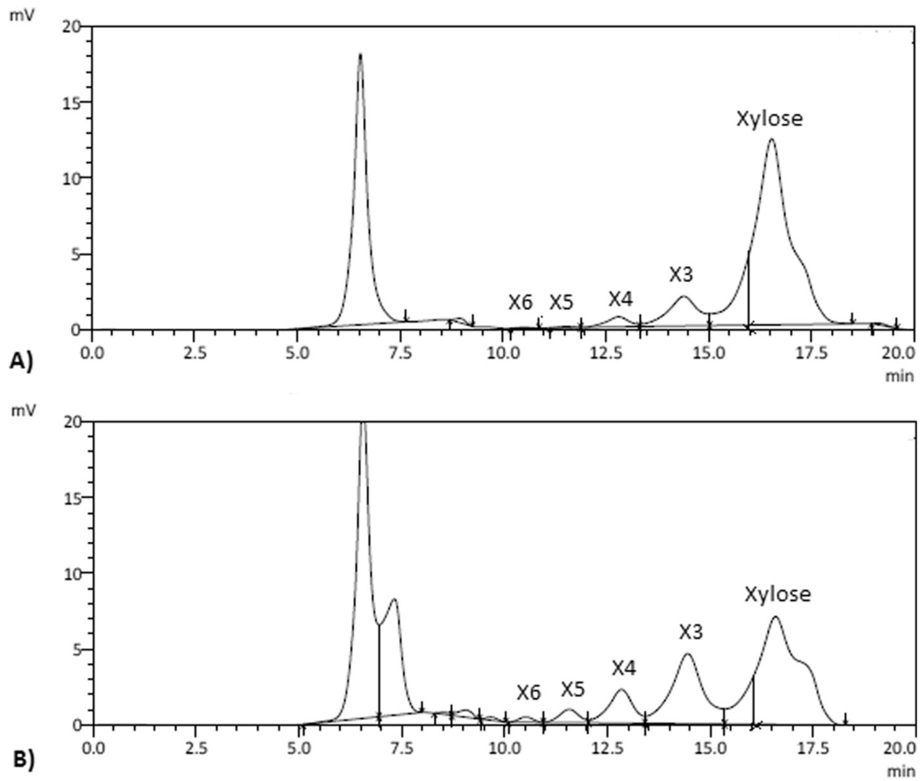

Fig. 1. Chromatogram of the obtained XOS by the application of the enzymatic extracts of (A) *Aspergillus brasiliensis* BLf1 and (B) *Aspergillus nidulans* XynC A773 over rice husk at their maximum concentrations; (×3) xylotriose; (×4) xylotretaose; (×5) xylopentose; (×6) xylohexose.

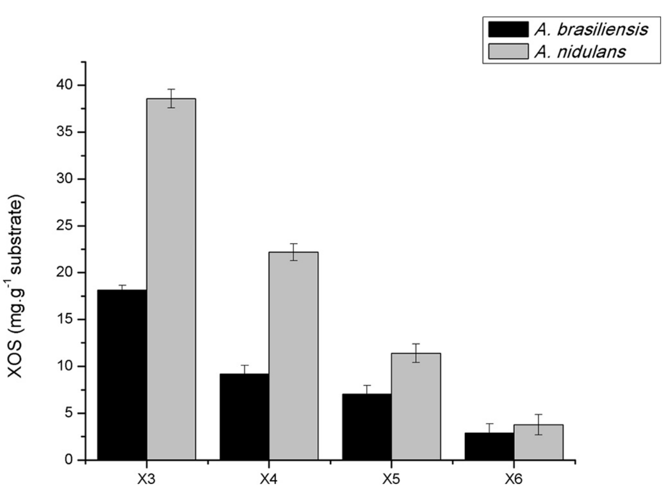

Fig. 2. Comparison of XOS liberated from rice husk by *Aspergillus brasiliensis* BLf1 (black) and *Aspergillus nidulans* XynC A773 (grey) enzymatic extracts. Results are the mean of duplicate kinetics.

these beneficial bacteria in the colon, thus possessing prebiotic properties and could be used in foods as functional additives (Mäkeläinen et al., 2010; Falck et al., 2013; Reddy and Krishnan, 2016; Jagtap et al., 2017). The efficient and complete metabolization of XOS by these bacteria requires several enzymes, including β-xylosidase, α-glucuronidase, α-L-arabinofuranosidase, and acetyl xylan esterase, thus the ability of probiotic microorganisms to metabolize XOS must be tested (Zeng et al., 2007). In some countries, such as Japan, XOS have been approved for Food for Specified Health Uses (FOSHU), as ingredients for food to improve gastrointestinal conditions (Mäkeläinen et al., 2009).

Considering the potential market demand for XOS to be applied in the food industry, and the growing interest in the health benefits of probiotic bacteria, the present work aimed at studying the possibility to produce xylooligosaccharides using rice husk as substrate and their subsequent use as prebiotics. We postulated a three-steps approach: first, we produced xylanases by culturing *Aspergillus brasiliensis* BLf1 and the recombinant strain *Aspergillus nidulans* XynC A773, separately, as solid-state cultivations (SSC) in rice husk; then, enzymatic supernatants of fungi SSC were applied to the same substrate in order to liberate XOS, which were recovered. Finally, the prebiotic effect of XOS was evaluated by cultivating the probiotic strains of *Lactobacillus plantarum* BL011 and *Bifidobacterium lactics* B-12 in media containing these sugars.

2. Materials and methods

2.1. Agro-industrial residue and other materials

The rice husk used in this work was provided by Extremo-Sul Company (Camaquã, RS, Brazil, geo-coordinates 30° 51″ 04′ S; 51° 48″ 44′ W). The material was milled in a knife mill until mean particle size of 1 mm in diameter was achieved. The processed material was stored in sealed plastic bags and kept at room temperature until experimentation, without any further modifications. The chemical composition of rice husk was published in a previous work (Menezes et al., 2017). All chemicals used in this research were of analytical grade and were purchased from Sigma-Aldrich (São Paulo, Brazil; St Louis, USA).

2.2. Microorganisms

The strain *Aspergillus brasiliensis* BLf1 was isolated from soil samples of the environment by our group and the complete isolation, identification, and characterization of this strain are described elsewhere (Menezes et al., 2017). The recombinant strain *Aspergillus nidulans* XynC A773 was provided by the National Laboratory of Bioethanol Science