Prebiotics production by *Aspergillus ibericus*

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Introduction: Great efforts searching for new and high-quality food ingredients have been developed to meet consumers' needs and commercial trends. The high demand for prebiotics is driving the search for new sources of fructo-oligosaccharides (FOS) producers and for FOS with differentiated functionalities. The present work explores the possibility of using a new isolated strain from Portuguese grapes, named *Aspergillus ibericus* MUM 03.49, as a FOS producer.

Methods: FOS were produced in a one-step bioprocess using the whole cells of the microorganism. The temperature of fermentation and initial pH were optimized in shaken flasks to yield a maximal FOS production, through a central composite experimental design. The process was further scaled-up to a 2 L bioreactor. FOS were analyzed by HPLC.

The produced FOS prebiotic potential was studied and compared with the ones produced by *Aureobasidium pullulans* and with a commercially available FOS mixture. The fermentability of these FOS mixtures was evaluated using a number of probiotic bacteria (*bifidobacteria* and *lactobacilli*). Finally, the hydrolytic resistance of the different FOS mixtures to the simulated harsh conditions of the digestive system was assessed *in vitro*.

Results: A temperature of 37ºC and a pH 6.2 were set by experimental design as the optimal fermentation conditions to produce FOS by *A. ibericus*. The model (R^2 = 0.918) predicted a yield of 0.56 that was experimentally confirmed (0.53 ± 0.03 $g_{FOS}.g_{initial\ sucrose}^{-1}$). A yield of 0.64 ± 0.02 $g_{FOS}.g_{initial\ sucrose}^{-1}$ was obtained in the bioreactor, at 38 h, with a content of 118 ± 4 $g.L^{-1}$ in FOS and a purity of 56 ± 3%. FOS were identified as 1-kestose, nystose, and 1^F-fructofuranosylnystose.

Overall, probiotics preferred the FOS synthesized by *A. Ibericus*, followed by *A. pullulans* and lastly the commercial FOS. All mixtures were resistant to salivary fluid. Kestose and nystose were slightly hydrolyzed in the presence of gastric and intestinal fluid.

Discussion: The FOS mixture produced was able to stimulate the growth of probiotic strains and was simultaneously resistant to hydrolysis along the simulated harsh conditions of the gastrointestinal system, thus suggesting that it could reach the large intestine intact. Additionally, the fermentation of the prebiotic mixture was found to be both substrate and strain specific, rather than based on the species or genera. The probiotic strains tested preferentially metabolized the FOS mixture synthesized by *Aspergillus*, followed by the one from *A. pullulans* and finally the commercial FOS. The one-step fermentation process using the whole cells proved to be efficient, fast and economic. The results gathered in this study highlight the possibility of using the fungus *A. ibericus* as a prebiotic producer at a large scale since high yields of FOS were achieved as compared to other microorganisms described in the literature.