Screening of fungal sources of β-galactosidase with potential for the synthesis of prebiotics

Silvério, SC, Rodrigues, LR, Teixeira, JA

CEB - Centre of Biological Engineering, Universidade do Minho, 4710-057 Braga, Portugal
Group: B.Factory; BIOSYSTEMS | Line: Industrial and Food Biotechnology and Bioengineering

β-Galactosidases (EC 3.2.1.23), also known as lactases, are a family of enzymes able to catalyse two different types of reactions, namely hydrolysis and transgalactosylation. The hydrolytic activity is commonly applied in the food industries to reduce the lactose content of dairy products, preventing lactose crystallization problems and increasing sweetness, flavour and solubility. On the other hand, transgalactosylation reactions have been explored in the synthesis of lactose-based prebiotics, such as galacto-oligosaccharides (GOS), lactosucrose [1] and lactulose [2], with potential application in the pharmaceutical and food industry. These prebiotics are enzymatically produced through the hydrolysis of lactose and further transfer of a galactosyl residue to a suitable acceptor, i.e. fructose for the disaccharide lactulose; sucrose for the trisaccharide lactosucrose; and lactose for GOS. The sources of β-galactosidase are extensively distributed in nature, namely in microorganisms, plants and animal organs. Nevertheless, β-galactosidases from microbial sources exhibit higher industrial relevance mainly due to their easy handling, great catalytic activity and high production yields. In this study, fifty fungal strains obtained from MUM (Micoteca of University of Minho, Portugal) and from DIA-UAC (Food Research Department, Autonomous University of Coahuila, Mexico) were screened for β-galactosidase production. A chromogenic test performed in agar plates supplemented with the substrate X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) was used in the screening study. Twelve promising fungal strains were identified and further validated as effective β-galactosidase producers under submerged fermentation (28 ºC, 150 rpm) using a culture medium composed of lactose, peptone, yeast extract and salts (KH_2PO_4, Na_2HPO_4 and $MgSO_4$). Under these conditions, only eight fungi (*Aspergillus brasiliensis*, *Aspergillus restrictus*, *Aspergillus uvarum*, *Penicillium brevicompactum*, *Penicillium italicum*, *Penicillium spinulosum, Mucor sp*. and *Trametes versicolor*) were able to consume lactose and produce β-galactosidase. The crude extract enzymes were characterized regarding their optimal pH and temperature. Additionally, their ability to synthesize lactose-based prebiotics was evaluated by incubating the crude enzymes with suitable mixtures of substrates (fructose + lactose or sucrose + lactose) at 37 ºC. Lactulose and GOS were produced by all the crude β-galactosidases when mixtures of fructose and lactose were used. However, the best results were obtained for β-galactosidases from *A. restrictus* and *A. uvarum*. When lactose and sucrose were used as substrates, GOS were the only lactose-based prebiotics obtained. Additionally, other type of prebiotic was synthesized in these conditions, namely fructo-oligosaccharides (FOS), suggesting also the presence of β-fructofuranosidase activity in the enzymatic extract. Overall, the eight fungi can be interesting biocatalysts for the prebiotic synthesis.

[1] Silvério, SC; Macedo, EA; Teixeira, JA; Rodrigues, LR, Perspectives on the biotechnological production and potential applications of lactosucrose: a review, *Journal of Functional Foods* 19, 74-90, 2015.

[2] Silvério, SC; Macedo, EA; Teixeira, JA; Rodrigues, LR, Biocatalytic approaches using lactulose: end product compared with substrate, *Comprehensive Reviews in Food Science and Food Safety* 15, 878-896, 2016.