TITLE: OPTIMIZATION OF THE β-D-FRUTOFURANOSIDASE PRODUCTION BY *Aspergillus thermomutatus* UNDER SUBMERGED FERMENTATION

AUTHORS TODERO, L. M.[1]; JORGE, J. A.[2]; GUIMARÃES, L. H. S[2].

INSTITUTION: [1]UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" – INSTITUTO DE QUÍMICA (RUA PROF. FRANCISCO DEGNI, 55, CEP 14800-060, ARARAQUARA – SP, BRASIL); [2]UNIVERSIDADE DE SÃO PAULO – FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO (AV. BANDEIRANTES, 3900, CEP 14040-901, RIBEIRÃO PRETO - SP – BRAZIL)

ABSTRACT:
β-D-fructofuranosidase (EC 3.2.1.26) or invertase is a hydrolase that catalyzes the breakdown of sucrose generating an equimolar mixture of D-glucose and D-fructose, which is called inverted sugar. Moreover, at high concentration of sucrose, it has transfructosylating activity t to produce fructooligosaccharides (FOS). FOS are oligosaccharides formed by a fructose chain attached to a terminal glucose by a β-glycosidic linkage, with a general formula of GFn. Due to β bond, these sugars are not hydrolysed in the human organism. So they become free of calories; presents prebiotic power; stimulate the bacteria of the intestinal tract; and can be used as substitute for sucrose. Therefore, the objective of this study was optimize the invertase production by the filamentous fungus *Aspergillus thermomutatus* under submerged fermentation. The fungus was cultured under different conditions, varying the culture medium, as additional sources of carbon and culture time. Both, intracellular and extracellular enzyme activities were quantified according to Miller (1959) using DNS and the protein in the crude extract by the Bradford method (1976). Five different culture media (Khanna, Vogel, YPD, SR, Adams) were tested and Khanna medium (2.95 $U.mg^{-1}$) was selected for the following steps. Ten different additional carbon sources were tested (glucose, frutose, sugarcane bagasse, sucrose,peel and weaves cassava, rye flour, oatmeal flour, inulin, starch and without additional carbon source). The highest intracellular and extracellular activities, were obtained with inulin (5.01 $U.mg^{-1}$) and sucrose (16.04 $U.mg^{-1}$). This laster was selected for the next test. The concentration of sucrose in culture was varied (0% - 2.5%), in which the concentration of 1% presented the highest extracellular activity. Using Khanna medium with 1% sucrose, the influence of the fermentation time was evaluated (24h - 120h), obtaining the highest activity at 120 h. FOS production was qualitatively analyzed by Thin Layer Chromatography (TLC), using extracellular crude extract, varying the concentration of 2% - 50% sucrose as substrate. It was observed the formation of FOS using concentration sucrose with above 20%. Considering these preliminary studies, it is possible to infer that the β-D-fructofuranosidase produced by the fungus *A. thermomutatus* has transfructosylating activity and can have promising application for the production of FOS.

Keywords: β-D-fructofuranosidase, fructooligosacharide, *Aspergillus thermomutatus*, submerged fermetation.

Development agency: CAPES, FAPESP