NOVEL AND SIMPLE TEST PLATING FOR SCREENING RELATIVE TRANSFRUCTOSYLATION ACTIVITY OF FUNGI

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Fructooligosaccharides (FOS) have received particular attention recently because of their excellent biological and functional properties, namely, as prebiotic compounds that promote the growth of intestinal microflora. They are also low calorie non-carcinogenic sweeteners with numerous suggested health benefits. These include immune system activation, resistance to infections, synthesis of B-complex vitamins, calcium absorption. They can be used as a treatment for breast cancer, diarrhoea, and constipation.

Although FOS are present in trace amounts in fruits, vegetables and honey as natural products, its mass production is limited by seasonal restrictions and the inherent inefficiencies of these systems. Hence, microbial FOS production by fungi in bioreactors is more realistic.

Several microorganisms are reported to have transfructosylation activity due to fructosyltransferase (EC 2.4.1.9) and/or fructofuranosidase (EC 3.2.1.26) activities. However, the search for other fungi with higher transfructosylation activity is still a challenge.

So, a presumptive and indirect colorimetric plate assay for the evaluation of transfructosylation activity in fungi was developed by the simultaneous determination in the same plate of glucose and fructose released from sucrose. The method entailed the coupling of two dye systems, namely the glucose oxidase-peroxidase coupled reaction using phenol and 4-aminoantipyrine for determination of glucose; and the fructose dehydrogenase oxidation in the presence of a tetrazolium salt for determination of fructose. In order to have a standard assay, the fungi were grown on Czapek Dox (CD) agar. 1 disc of mycelium (8 mm diameter) was cut from the edge of each colony and then put in contact with CD agar plates. After incubation at 25 ºC for 72 h each assay plate was overlaid with soft agar containing the reagents. The presence of enzymes with transfructosylation activity was identified by the formation of pink (presence of glucose) and blue (presence of fructose) halos around the discs. In conclusion, the results showed that the method is suitable for screening a large number of fungi due to its simplicity, reproducibility and rapidity.

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