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Characterization of a novel invertase from Trichoderma sp., a producer of a wide range of fructooligosaccharides

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PURPOSE OF THE ABSTRACT
Functional foods are known as foods that not only provide essential nutrients but also have additional functions such as health-promoting benefits and disease prevention. There is currently a growing biotechnological interest in the market for a new, safer functional foods with beneficial properties, where bioactive oligosaccharides are gaining relevance over compounds such as dietary fibers, peptides, or unsaturated fatty acids. Bioactive oligosaccharides are considered as prebiotics where fructooligosacharides (FOS), galacto-oligosaccharides (GOS) and glucooligosacharides (GlcOS) are the most common ones. They selectively stimulate the growth and activity of health-promoting lactobacilli and bifidobacter in the gut as well as inhibit the growth of pathogenic microorganisms, improves immune and cardiovascular systems, mineral absorption and even mental health [1].
FOS are basically oligomers of fructose units with a glucose moiety attached to the terminal fructose. A sucrose terminal group is formed as a result of this bond. Based on the linkage pattern between monosaccharides, FOS can be included into three main series: levan type, where fructose units are linked by β-(2➝6) linkages (6F-FOS, e.g., 6-kestose), inulin-type with β-(2➝1) bond (1F-FOS, e.g., 1-kestose), and neoseries where a β-(2➝6) linkage connects fructose to the glucosyl moiety of sucrose (6G-FOS, e.g. neokestose).
Invertases or β- fructofuranosidases (EC 3.2.1.26) are biotechnologically important enzymes that catalyze the release of β-fructose from the non-reducing termini of various β-D-fructofuranoside substrates. They belong to the GH32 glycosyl hydrolase family (CAZy; http://www.cazy.org), which also includes invertases, inulinases and fructosyltransferases. These enzymes may also catalyze the synthesis of short-chain FOS, in which some fructosyl moieties are linked to a sucrose unit [1,2].
Trichoderma is the most prevalent culturable genus of the soil fungi. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. It is a filamentous soil fungus that functions as a biocontrol agent for a wide range of economically important aerial and soilborne plant pathogens. In this work we detected a sucrose hydrolyzing activity in a Trichoderma sp. and upon bioinformatical analysis, the enzyme responsible for this activity was identified. The GH32 family protein (possible invertase) TIGH32 was successfully heterologously expressed in Escherichia coli cultures, purified and characterized biochemically. By using different high-performance liquid chromatography (HPLC) techniques the enzyme's potential to produce FOS threw transfructosylation reactions was analyzed and some of the products formed identified. A structural model of TIGH32 was generated using AlphaFold 2 to provide more information about its structure- function determinants.
This study provides more insight into the enzymatic activity of the GH32 proteins and particular specificity of TIGH32 as a potential FOS producer.

FIGURES

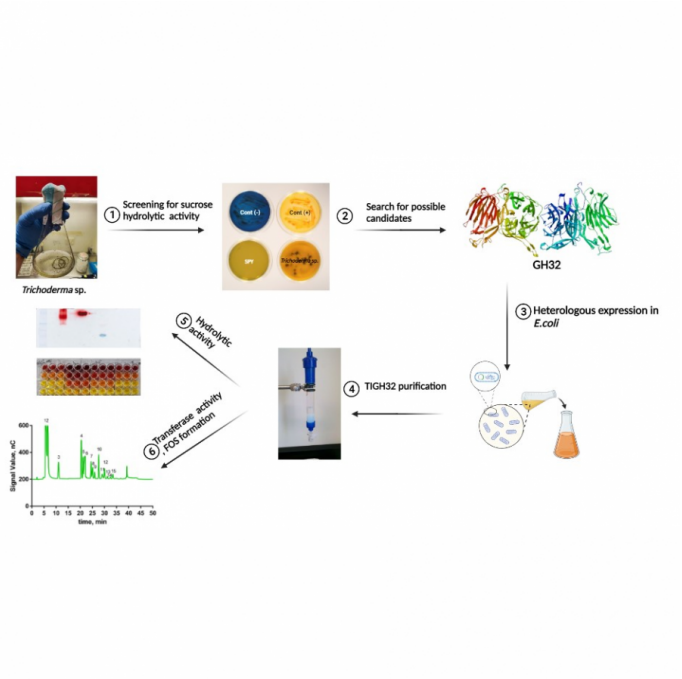

FIGURE 1

Experimental design

The work-flow of the experiments presented in this work.

FIGURE 2

KEYWORDS

fructooligosaccharides | transfructosylation | invertase | enzyme kinetics

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