RESEARCH PAPER

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Research Paper

Screening of novel yeast inulinases and further application to bioprocesses

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Inulin is a carbohydrate composed of linear chains of β-2,1-linked D-fructofuranose molecules terminated by a glucose residue through a sucrose-type linkage at the reducing end. Jerusalem artichoke (JA) is one of the most interesting materials among unconventional and renewable raw materials, with levels of inulin reaching 50–80% of dry matter. Inulin or inulin-rich materials can be actively hydrolyzed by microbial inulinases to produce glucose and fructose syrups that can be used in bioprocesses. In this study, several microbial strains were isolated and their ability to inulinase biosynthesis was evaluated. The novel yeast strain Talf1, identified as *Zygosaccharomyces bailii*, was the best inulinase producer, attaining 8.67 U/ml of inulinase activity when JA juice was used as the inducer substrate. *Z. bailii* strain Talf1 and/or its enzymatic crude extract were further applied for bioethanol production and biodesulfurization (BDS) processes, using inulin and JA juice as carbon source. In a consolidated bioprocessing for ethanol production from 200 g/l inulin, Z. *bailii* strain Talf1 was able to produce 67 g/l of ethanol. This ethanol yield was improved in a simultaneous saccharification and fermentation (SSF) process, with the ethanologenic yeast *Saccharomyces cerevisiae* CCMI 885 and the Talf1 inulinases, achieving a production of 78 g/l ethanol. However, the highest ethanol yield (~48%) was obtained in a SSF process from JA juice (~130 g/l fermentable sugars), where the *S. cerevisiae* produced 63 g/l ethanol. Relatively to the dibenzothiophene BDS tests, the *Gordonia alkanivorans* strain 1B achieved a desulfurization rate of 4.8 μM/h within a SSF process using Talf1 inulinases and JA juice, highlighting the potential of JA as a less expensive alternative carbon source. These results showed the high potential of *Z. bailii* strain Talf1 inulinases as a versatile tool for bioprocesses using inulin-rich materials.

Introduction

Fructans are one of the most abundant non-structural polysaccharides found in a wide range of plants. Inulin is a polydisperse fructan consisting of linear chains of β-2,1-linked D-fructofuranose molecules terminated by a glucose residue through a sucrose-type linkage at the reducing end [1]. Depending on plant source, inulin may have between 2 and 60 fructose monomers, or more [2,3]. Thus, inulin is of great interest as it is a relatively inexpensive and abundant substrate for the production of fructose syrups and bioethanol [4]. It is present as a reserve carbohydrate in the roots and tubers of several plants, such as Jerusalem artichoke (topinambur), chicory, dahlia and yacon [5]. The inulin content depends on the plant species, being chicory, Jerusalem artichoke and dahlia the major sources of inulin for industrial scale production.

This polymer can be actively hydrolyzed by microbial inulinases (fructofuranosyl hydrolases) to produce inulo-oligosaccharides (IOS), glucose and fructose as main products. In general, inulinases act using two mechanisms: exo-inulinases sequentially split-off the terminal β-(1,2) fructofuranosidic bonds, while endo-inulinases, lacking invertase activity, hydrolyse the internal linkages in inulin and release IOS, high added-value products [1,6,7]. The microbial exo-inulinases have been proposed as the most promising approach

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