



Production, purification and characterization of an extracellular inulinase from *Kluyveromyces marxianus* var. *bulgaricus*

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The yeast *Kluyveromyces marxianus* var. *bulgaricus* produced large amounts of extracellular inulinase activity when grown on inulin, sucrose, fructose and glucose as carbon source. This protein has been purified to homogeneity by using successive DEAE-Trisacryl Plus and Superose 6HR 10/30 columns. The purified enzyme showed a relative molecular weight of 57 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and 77 kDa by gel filtration in Superose 6 HR 10/30. Analysis by SDS-PAGE showed a unique polypeptide band with Coomassie Blue stain and nondenaturing PAGE of the purified enzyme obtained from media with different carbon sources showed the band, too, when stained for glucose oxidase activity. The optimal hydrolysis temperature for sucrose, raffinose and inulin was 55 °C and the optimal pH for sucrose was 4.75. The apparent K_m values for sucrose, raffinose and inulin are 4.58, 7.41 and 86.9 mg/ml, respectively. Thin layer chromatography showed that inulinase from *K. marxianus* var. *bulgaricus* was capable of hydrolyzing different substrates (sucrose, raffinose and inulin), releasing monosaccharides and oligosaccharides. The results obtained suggest the hypothesis that enzyme production was constitutive. *Journal of Industrial Microbiology & Biotechnology* (2000) 25, 63–69.

Keywords: inulin; inulinase; *Kluyveromyces marxianus* var. *bulgaricus*; extracellular enzyme

Introduction

The extracellular glycoprotein, inulinase, catalyzes hydrolysis of the polyfructoside inulin outside the cell wall. The enzyme also hydrolyses sucrose, both within and outside the cell wall [23]. For a microorganism to utilize inulin as its carbon and energy source, production of an extracellular inulinase is required to hydrolyse the fructan into fermentable monosaccharides. Fructans are polyfructose molecules that can be subdivided into two categories, the inulin and the levan type. The inulin fructans are found as carbohydrate reserves in several plants, including chicory, dandelion, dahlia, and Jerusalem artichoke. They consist of a linear chain of (2-1)-linked fructose molecules and, depending on the plant source, may have between 5 and 45 fructose monomers [2]. The levan fructans produced by many bacterial species consist of branched molecules with predominantly (2-6)-linkages branched at C1 [10,11]. Inulinases have been purified from yeast [7,21,25,31], molds [9,27] and bacteria [1,3,8,29,30] and have potential uses in the preparation of fructose syrups from inulin and invert sugar in the food industry [19,28]. The results of the study on the production and purification of an extracellular inulinase by *Kluyveromyces marxianus* var. *bulgaricus* are presented.

Materials and methods

Strain

K. marxianus var. *bulgaricus* ATCC 16045 was obtained from the Bioengineering Laboratory, Campinas State University, São Paulo,

Brazil. The strain was maintained on solid medium at 4 °C. The medium composition (g/l) was comprised of the following: Difco yeast extract 10.0; Difco Bacto-peptone 20.0; glucose 20.0 and Difco agar 20.0. Cells were harvested from 18 slants and used to inoculate liquid medium.

Culture media

Liquid media with 1% (w/v) glucose, sucrose or fructose were obtained from Reagen and raffinose, inulin from dahlia tubers and, inulin from Jerusalem artichoke were obtained from Sigma. Media were supplemented with 1% yeast extract and the pH was adjusted to 3.0 with orthophosphoric acid.

Culture conditions

The yeast was inoculated into 250 ml Erlenmeyer flasks containing 50 ml of medium incubated at 30 °C for 48 h in a temperature-controlled shaker (New Brunswick Scientific, USA) operated at 200 rpm. The culture was pelleted by centrifugation at 10,000 ×g for 10 min.

Fractionation of cultures for inulinase activity assays

Supernatant enzyme: The organism was grown at 30 °C in a continuous laboratory fermenter with a working volume of 600 ml, aeration of 0.2 vvm, pH 3.0 and sucrose as carbon source. One hundred milliliters of culture was harvested by centrifugation at 4 °C (10 min, 10,000 ×g). The supernatant was used as a source of extracellular enzyme.

Cell wall enzyme: The cell pellet obtained after centrifugation was suspended and incubated for 1 h at 30 °C in 10 ml of enzyme

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