PRODUCTION AND PROPERTIES OF INULINASE FROM *Aspergillus niger*

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SUMMARY

A thermostable inulinase was identified in a strain of *A. niger*. The highest activity was observed at 50°C (50 uLg⁻¹) and 77% and 34% of this was retained at 60°C and 85°C, respectively. pH stability, the effect of thermal stabilizers such as Propylene glycol (10%) and Sorbitol (10%) and effects of different cations were investigated. It was found that the activity was completely inhibited by Ag⁺ and Hg²⁺, while Na⁺ had an activator effect.

INTRODUCTION

Inulin occurs as an energy reserve in various plants particularly in Jerusalem artichoke, chicory and dahlia (Fleming and Groot Wassink, 1979). Amongst these, especially Jerusalem artichoke merits further consideration for food and non-food applications of inulin and its derivatives (Fuchs, 1990). Using a thermostable inulinase facilitates the control of microbial contamination and the use of high substrate concentrations where viscosity is a persisting constraint. Many strains of *Aspergillus* (Norman and Zittan, 1982; Peters et al., 1983, Dersycke and Vandamme, 1994), *Penicillium* (Shiomi and Donders, 1988), *Fusarium* (Gupta et al., 1990), *Kluyveromyces* (Parekh and Margaritis, 1986; Rouwenhorst et al., 1988) have been investigated for their inulinase activity. In the present study enzyme production characteristics and properties of a thermostable inulinase was obtained from a strain of *A. niger* isolated from dried fig samples were investigated.

MATERIALS AND METHODS

**Microorganism** *A. niger* strain designated as *A. niger* A42 was from our laboratory culture collection. The organism was maintained on 2% Jerusalem artichoke extract and 1.5% agar slants.

**Preparation of Jerusalem artichoke** Jerusalem artichokes were washed with cold water, sliced and then dried in a Lab. Type Demaco Tray Drier at 80°C to a constant weight. The dried slices were then milled to a fine powder with a hammer mill.

**Medium and Cultivation** The medium for enzyme production contained 1.0% Jerusalem artichoke 0.23% NH₄NO₃, 0.37% (NH₄)₂HPO₄, 0.1% KH₂PO₄, 0.05% MgSO₄, 0.15% Yeast Extract. The initial pH was 5.0. Medium was autoclaved at 115°C for 30 min. Spores were suspended with 0.001% Tween 80 and 1 ml of this was used to inoculate 250 ml flasks. Flasks containing 50 ml medium were incubated at 28°C on an orbital shaker at 200 rpm.

**Crude enzyme** The mycelia were removed by centrifugation at 1500 g for 10 min and the supernatant was used as the crude enzyme solution throughout the experiments for enzyme assays. Biomass was determined as dry cell weight.

**Inulinase activity** The reaction mixture contained 1 ml of 5% inulin in 0.1M sodium acetate buffer (pH 4.5) and 1 ml of diluted crude enzyme. The mixture was held at 50°C for 30 min.

**Invertase-type activity** The reaction mixture contained 1 ml of 0.1 M glucose in 0.1 M sodium acetate buffer (pH 5.0) and 1 ml diluted crude enzyme. The mixture was held at 50°C for 5 min. In both of the enzyme assays, the amount of reducing sugar was measured by the

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3,5-dinitrosalicylic acid method (Miller, 1959). One unit (U) of enzyme activity was defined as 1 micromole of reducing sugar produced per minute.

Cation determination: Cation contents of the crude enzyme solution was determined by using Pye Unicam SP 9 Atomic Absorption Spectrophotometer for each cations in standard assay conditions. All the chemicals used throughout the experiments were analytical grade reagents.

RESULTS AND DISCUSSION

Firstly, the effect of substrate concentration on the production of inulinase from A. niger A42 was tested (Figure 1). A. niger A42 was inoculated to various concentrations of substrate (Jerusalem artichoke) and incubated for 120 hours.

![Figure 1. Effect of substrate concentration on the production of inulinase from A. niger A42, at 28°C, for 120 hours.](image)

1% substrate concentration, gave the highest inulinase activity (4800 U g substrate⁻¹). The activity against both sucrose and inulin decreased at substrate concentrations above 1%.