Production of inulooligosaccharides from inulin by a dual endoinulinase system

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ABSTRACT

The production of inulooligosaccharides from inulin by a dual endoinulinase system of *Pseudomonas* sp and *Xanthomonas* sp. was investigated the optimum conditions for a dual endoinulinase reaction were as follows : pH,5.8; temperature, 50℃; substrate concentration, 50 g/l; enzyme ratio, 3:1 as *Xanthomonas* endoinulinase to *Pseudomonas* endoinulinase. Under optimum conditions, the maximum yield of oligosaccharides was 90.5% in total sugar basis by dual endoinulinase system

INTRODUCTION

Recent interest in process development for the production of oligosaccharides has concentrated on high-content commercial products[1-2]. Inulin consists of linear chains of β-2,1-linked D-fructofuranose terminated by a glucose residue through a sucrose type linkage at the reducing end[3]. It has been widely investigated as a source for the production of ultra-high-fructose syrup through enzymatically with endoinulinase[4].

MATERIALS AND METHODS

Materials

Pure, non-hydrolyzed inulin from dehlia tubers (sigma) was used

Enzyme preparation

Xanthomonas sp. was cultivated aerobically at 37℃ for 24 h in a 250 ml flasks containing 50ml medium composed of (as g/l) inulin 20, yeast extract 20, $(NH_4)_2HPO_4$ 5, $NH_4H_2PO_4$ 2, MnCl2· 4H2O 0.5, KCl 0.5, MgSO4· 7H2O 0.5, FeSO4· 7H2O 0.01 and *Pseudomonas* sp.was cultivated at 45℃ for 60 h in a 250 ml flasks containing 50ml medium composed of inulin 10, $(NH_4)_2HPO_4$ 8, corn steep liquor 15, KCl 0.5, MgSO4· 7H2O 0.5, and FeSO4· 7H2O 0.03. The broths were removed by centrifugation (10,000×g, 20 min) the resulting culture filtrate, after filtration through membrane filter (Milipore, 0.45μm) was directly used as enzyme without futher purification

Enzyme assay

Endoinulinase of *Xanthomonas* sp. and *Psudomonas* sp. activities were assayed with 50 g/l

inulin at 45℃ and 55℃ for 60 min, respectivly. One enzyme unit was defined as the amount of hydrolyzed inulin (μmole) per min, under the above conditions

Enzyme reaction

Dual endoinulinases reaction were carried out 416.67 U/gram inulin at 50℃ in a water bath

Analytical methods

The products of enzymatic reaction were analyzed by HPLC using a cation ion exchange column (Aminex HPX-42C,Biorad) and a refracive index detector. the column was at 85℃ and water was used as the mobile phase at a flow rate of 0.6 ml/min. the total inulooligosaccharides were estimated as the sum of all oligofructosides which have a degree of polymerization (DP) ranging from DP2 to DP6

RESULTS

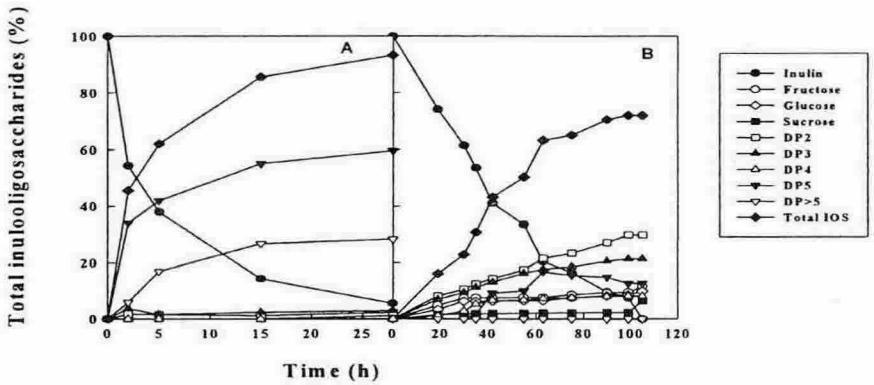

Fig.1. Typical time courses of inulooligosaccharide production from inulin by endoinulinase from *Xanthomonas* (A) and *Pseudomonas* (B) sp.

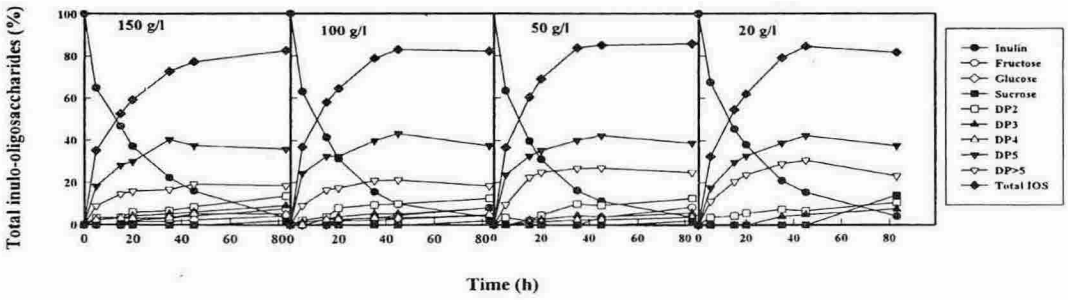

Fig 2. Effect of substrate concentration on the inulo-oligosaccharide production by a dual enzyme system.

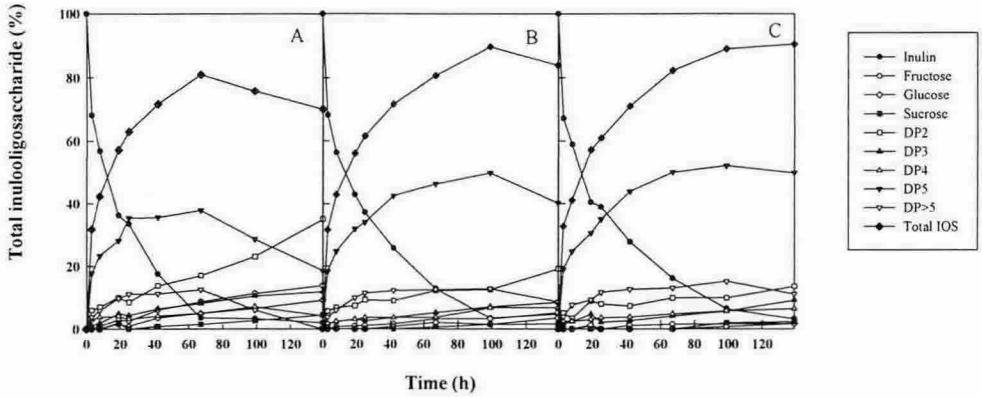

Fig.3. Effect of enzyme ratio on the inulooligoaccharides production by dual enzyme system (A) 1:1 (*Xanthomonas* : *Pseudomonas*) enzyme ratio, (B) 2:1, (C) 3:1

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