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FULL FACTORIAL DESIGN TO STUDY FERMENTATIVE PRODUCTION OF INULINASE USING INULIN FROM KUTH (*SAUSSUREA LAPPA*) ROOT POWDER BY *ASPERGILLUS NIGER* VAN TEIGHEM UV11 MUTANT

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Abstract

*Kuth (*Saussurea lappa*) as a source of inulin for fermentative production of inulinase has been tested for the first time. Preliminary shake-flask experiments in the fermentative production of inulinase using kuth as the carbon source, in combination with different organic and inorganic nitrogen sources, gave the highest yield of inulinase activity (302 Uml^{-1}) with corn steep liquor as the nitrogen source. The full factorial design using $3^3=27$ experiments with varying combinations of kuth and CSL concentrations and agitation rates was tried. The results obtained were analysed using SPSS/PC+ (Version 4·0). This is the first use of a full factorial method for optimising fermentative production of inulinase.*

Key words: Aspergillus niger, full factorial design, inulinase, *Saussurea lappa.*

INTRODUCTION

Inulin is a polymer of plant origin that serves as a reserve carbohydrate in Compositae and Graminae. Jerusalem artichoke and chicory plant roots containing inulin have been used for fermentative production of inulinase by microorganisms (Manzoni & Cavazzoni, 1992; Ferreira *et al.*, 1991; Gupta *et al.*, 1988; Byun & Nahm, 1978). *Saussurea lappa* (kuth or costus — trade name) belongs to the Compositae family and contains inulin in the roots. Kuth root as a rich source of inulin has been utilized for high-fructose syrup production by chemical hydrolysis (Kulkarni *et al.*, 1969). There is no report on fermentative production of inulinase using kuth as the source of inulin. The present work was planned to utilise *Saussurea lappa*, a source of inulin, for fermentative production of inulinase by *Aspergillus niger* van Teighem UV11.

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METHODS

Carbon and nitrogen analysis of kuth root powder and corn steep liquor (CSL)

Total carbon and nitrogen contents of the kuth root powder from the local market, Bombay (powdered in a flour mill to 60 mesh size), and CSL (Anil Starch Industries, Ahmedabad) were determined by Total Carbon Analyzer (Beckmann, USA) and Kjeldhal methods, respectively.

Determination of inulin content of kuth root powder

The inulin content of kuth root powder was determined using the Streptkov phosphomolybdic–permanganate method (Winton & Winton, 1960).

Fermentation of kuth root powder in shake-flask cultures for inulinase production

Preparation of inoculum

Aspergillus niger van Teighem UV11, a UV mutant of a soil isolate (Poorna & Kulkarni, 1994), was subcultured on synthetic-medium agar (Allais *et al.*, 1987) and incubated at 30 ± 2°C for 72 h. The spores were harvested and suspended in 0·85% saline containing 0·01% Tween 80 to give approximately $2{\cdot}5\times10^6$ spores per ml (by haemocytometer count). A 5% inoculum of this suspension was used for all the experiments.

Medium

The fermentation medium (B) in shake-flask cultures consisted of the following composition (per litre): kuth root powder, 10·0 g; KH_2PO_4, 5·0 g; CSL, 10·0 g; trace element solution (Allais *et al.*, 1987), 1 ml; pH 5·4; sterilised by autoclaving at 121·6°C for 15 min.

Portions (50ml) of medium in 250 ml flasks were inoculated with 5% inoculum prepared as described above and incubated at 30 ± 2°C on a rotary shaker (150 rpm). Samples were withdrawn at specific intervals to determine inulinase activity. These conditions