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COMPARISON OF OPTIMIZATION CONDITIONS FOR ELEVATED BACTERIAL AND FUNGAL INULINASE

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Abstract

Tithonia roduntifolia is a noxious weed which threatens the environment and biodiversity due to its rapid invasion. This inulin rich weed was investigated for its potential in fructose production by subjecting it to bacterial and fungal inulinase. Inulinases catalyze the hydrolysis of inulin to D-fructose (fructose syrup), which has gained an important place in human diets today. In addition, inulinases are finding other newer applications.

The aim of this work was to compare and optimize the cultural and production parameters for the synthesis of Inulinase by novel bacterial and fungal isolates. The process parameters influencing: fructose formation, cell density and the enzyme production were studied. We emphasized on parameter like substrate concentration, incubation period, pH, temperature and salt concentrations. The optimum temperatures of bacterial and fungal inulinases were 30 and 40 ºC respectively. Fungal Inulinase was more thermostable and remained stable in acidic pH. Among the various substrate studied Tithonia spp. showed maximum inulinase yield. The incubation time for maximum fructose formation was estimated via TLC indicating the fast product formation with incomplete substrate degradation from bacterial inulinase, whereas fungal inulinase produced fructose after 96 hours but there was complete utilization of substrate. This study reveals that, sugar yield was significantly increased under optimized condition which was higher than earlier reports and promises the use of *Tithonia roduntifolia* as a feedstock for fructose production.

Keywords: Inulin, Inulinase,*Tithonia roduntifolia*, Fructose

Introduction

Inulin is linear β (2, 1) linked fructose polymer [3] that occurs as a reserved carbohydrate in many plants belonging to Composite or Asteraceae family. Various plant materials, such as dahlias, chicory, and Jerusalem artichokes, have already been reported [1] as effective source of inulin, yet there has been no previous report on the utilization of *Tithonia roduntifolia* for high-fructose syrup preparation. *Tithonia roduntifolia* is considered as weed in western Maharashtra and has drawn our attention as a potential crop for inulinase production because of its high inulin content, economical and ready availability. It acts as an inexpensive & abundant substrate for the production of high fructose syrup [8] which has beneficial effects in diabetic patients, stimulates calcium absorbtion in postmenopausal women and stimulates growth of *Bifidobacter* in intestine. Fructose syrup is also useful in pastry and confectionary production, as it prevents desiccation and sugar crystallization. There was a need to isolate high yielding inulinase producing micro-organisms by using *Tithonia roduntifolia* as natural source of inulin. The hydrolysis of inulin is catalysed by inulinase where splitting of terminal fructose unit successively occurs from the non- reducing end of the inulin. Thus wide and newer applications [2] of inulinase in food, pharmaceutical, cosmetic industries have forced to screen the high yielding inulinase producing organism. Accordingly, the present study investigates the comparative study of optimization conditions on natural inulin hydrolysis by bacterial and fungal inulinase.

Materials and methods

Preparation of *Tithonia roduntifolia* stems extract

Tithonia roduntifolia stem was collected, washed and blended by a mixer.

Isolation of inulinase producing Microorganisms

Rhizospheric soil of *Tithonia roduntifolia* plant was inoculated into medium containing synthetic inulin as the only 'C' source (inulin 20g/l, $NaNO_3$ 20g/l, K_2HPO_4 1g/l, $MgSO_4$ 0.1g/l). The inoculated flasks were incubated at 28^{0}C and inulinase activity was checked for 3 successive days (after 24, 48, 72 hrs) by performing inulinase assay.

Extracellular Inulinase assay

Inulinase activity was determined by the 3, 5-dinitrosalicylic acid method [6]. One unit of inulinase activity was defined as the amount of enzyme which produced 1µ mole of fructose under the assay conditions [4].

Effect of different concentration of *Tithonia roduntifolia* stem extract Mixtures of basal medium ($NaNO_3$ 20g/l, K_2HPO_4 1g/l, $MgSO_4$ 0.1g/l) with Tithonia stem extract of different concentrations (2, 4, 6, 8, 10%) were prepared its effect on inulinase yield was estimated.

Time course of inulin hydrolysis by inulinase

It was done by TLC analysis with 1-butanol: ethanol: water (5:5:5) as solvent system and the spots were detected with p- anisidine hydrochloride [5] on a hot plate.

Process parameter optimization

The effect of range of pH (3, 4, 5, 6, 7, 8 9 & 10), incubation temperature (20°C, 30°C, 40°C, 50°C and 60°C) and NaCl concentration (0, 2, 4, 6, 8, 10, 12 and 14%) on fructose production[7], cell density & inulinase activity of the *Arthrobacter* sp & *Fusarium* spp was carried out by employing the submerged fermentation method.

Results and Discussion

An attempt was made to study the efficient bioconversion of inulin rich plant waste into an industrially valuable product. The *Arthrobacter* spp and *Fusarium* spp were selected for the present study after screening 30 isolates for their capacity to synthesis extracellular inulinases (981 and 1278 U/ml respectively) after being grown on basal growth medium containing Tithonia stem extract 8% (w/v) as a sole carbon source (Table 1). Different concentrations of composite plants extracts: 3% for Jerusalem artichoke and 5% for sunflower were reported by other authors as carbon sources for inulinases production.

The exo- or endo-nature of the crude inulinase was determined using TLC. As the incubation time prolonged, the amount of released fructose from inulin increased while the initial amount of inulin in the reaction mixture decreased gradually. (Fig. 1 & 2) The optimum time of inulin hydrolysis by inulinase from *Arthrobacter spp* was observed to be 36 hrs at pH 7 & 30 °C and was of endo nature. In case of fungal inulinase, though late (after 96 h at 30 °C) but inulin in the reaction mixture was completely hydrolyzed into fructose as the major hydrolytic product indicating that the enzyme produced by *Fusarium* spp showed high exo-inulinase activity. The comparative study of process parameters (pH, temperature and salt conc.) influencing fructose formation, cell density and the enzyme production by *Arthrobacter* spp and *Fusarium* spp were as follows.

Maximum fructose was formed at 30 °C under pH 7 and 2% salt conc. in fungal and bacterial fermented broth (Fig 3). But *Fusarium* spp. Produced fructose even at pH 4, up to 60°C & up to 10% NaCl. Fig 4 represents the effect of pH, temperature and salt % on bacterial and fungal biomass production in presence of 8% *Tithonia* extract as inulin source. Elevated enzyme activity by *Arthobacter* spp was detected at 30 °C (Fig 5). Inulinase by *Fusarium* spp was found to be thermostable (up to 50°C), acid tolerant (up to pH 4) and osmotolerant (6% NaCl). The optimum pH for activity was found to be 7 and 6 for bacterial and fungal inulinase respectively. The pH stability exhibited by the fungal inulinase was between 4- 8, but at 6 pH for bacterial inulinase.

Table 1: Effect of different concentration of *Tithonia* stem extract

Tithonia stem extract %	Inulinase activity EU/ml	
	Arthrobacter spp	*Fusarium* spp
2	243	201
4	322	541
6	776	843
8	**981**	**1278**
10	440	649

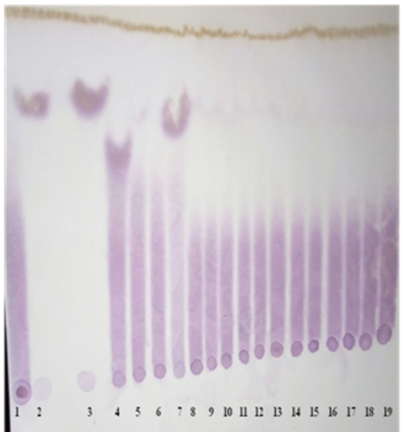

Figure 1: TLC of sample withdrawn after every 12 hrs interval [spots 4-19] of fermentation of inulin rich media with *Arthrobacter* spp; spot 1, 2 & 3 as std Inulin & fructose respectively.

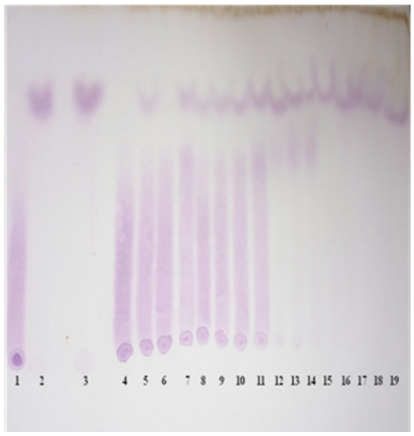

Figure 2: Thin layer chromatography of products of inulin hydrolysis by *Fusarium* spp. 1= inulin; 2, 3 = fructose; 4 to 19= products from reaction times taken at 0 to every 12 hrs interval, respectively.

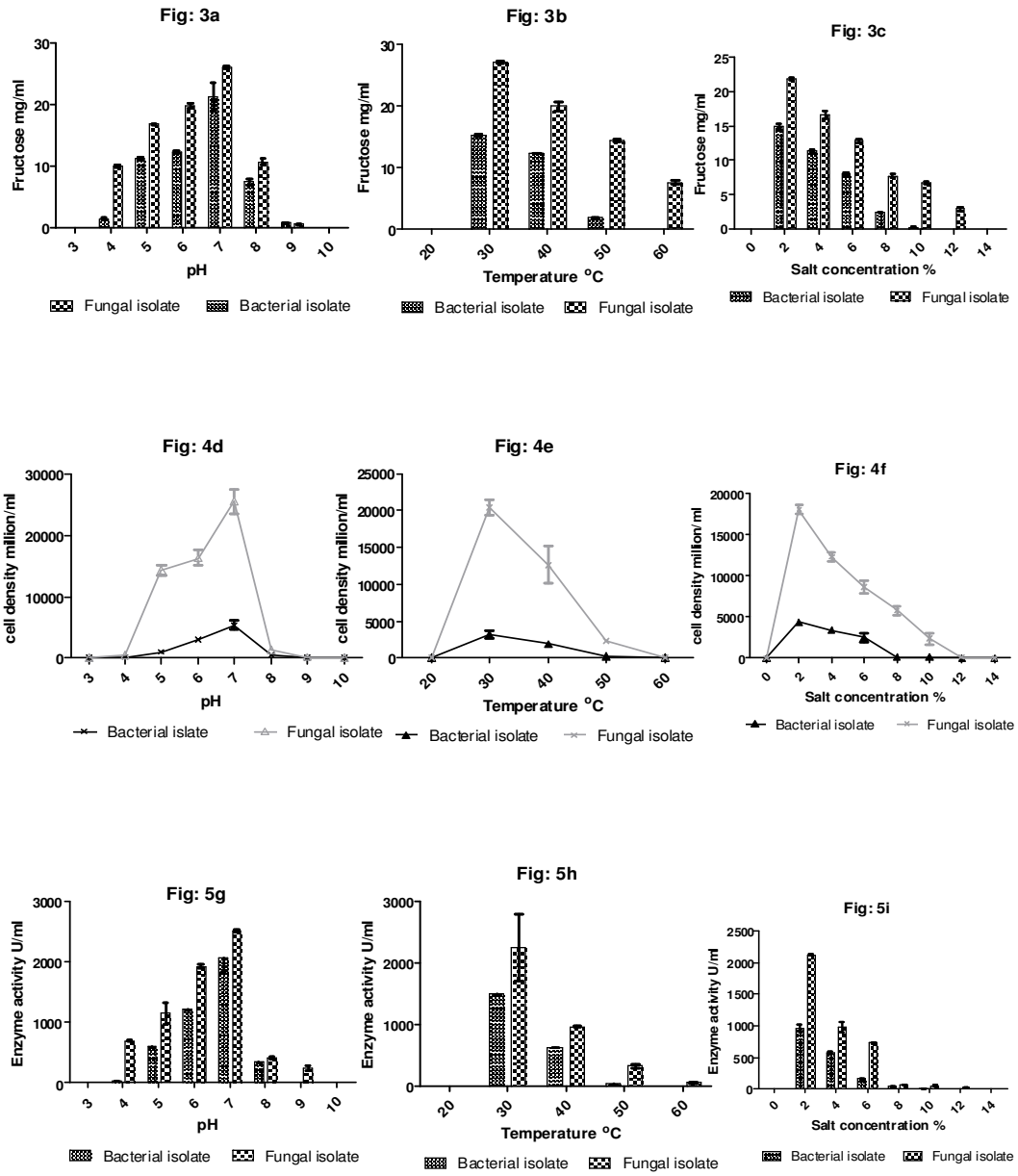

Figure 3 Correlation between fructose production by Nocardiopsis sp. DN-K15 & fungal inulinase producers at various pH (a), temperature (b) and NaCl concentration (c) Each value represents the mean ± SD of two independent experiments

Figure 4 Effect of pH (d), temperature (e) and NaCl concentration (f) on growth of Nocardiopsis sp. DN-K15 & fungal inulinase producing strains. Each value represents the mean ± SD of two independent experiments

Figure 5 Correlation between enzyme activity of Nocardiopsis sp. DN-K15 & fungal inulinase producers at various pH (g), temperature (h) and NaCl concentration (i). Each value represents the mean ± SD of two independent experiments

Conclusion

Inulinases are promising candidates for use as complements in food ingredients and in the production of fermenting sugars. The results showed the use of cheap wild weed: *Tithonia roduntifolia* as substrate for inulinase and fructose synthesis, thus contributing to the reduction in the cost of production medium. The abundance of inulinases production by *Arthrobacter spp* and *Fusarium spp* and its

remarkable higher stability at various process parameters under wide range can have a positive effect at commercial level on food and pharmaceutical industry.

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