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Optimization of Process Parameters for the Production of Inulinase from *Aspergillus niger*

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Fifty cultures of inulinase producing fungi were isolated from 5 different soil samples using a synthetic medium with inulin as the sole carbon source. Among these, an isolate AUP19 exhibited maximum inulinase activity and was identified as *Aspergillus niger*. Studies were carried out on concentration of inulin, different carbon and nitrogen sources, process parameters like optimum temperature, pH, fermentation time, and inoculum level using this strain. The optimized process parameters for inulinase production are incubation temperature, 28°; fermentation time, 72 h; pH, 6.5 and inoculum level, 10% (v/v). Maximum inulinase production (176 u/ml) in indented flasks was achieved under optimized conditions with medium containing inulin, 5%; galactose, 1%; corn steep liquor, 1% and $(NH_4)\ H_2PO_4$, 1%.

Inulinases are β-2,1-fructanohydralases (E.C.3.2.1.7), which convert inulin to fructose in a single step. Inulin, a polyfructan, occurs as a reserve carbohydrate in the roots and tubers of some members of Compositae and Graminae family like Jerusalem artichoke, chicory and dahlia[1,2]. Fructose is intensively emerging as a safe and alternative sweetener to sucrose[3], which causes problems, related to corpulence, carcinogencity, atherosclerosis and diabetes. Ultra high fructose glucose syrup (UHFGS) stands in the first position in the world growing market for which no proper technical know-how is available for higher yields. Inulinases are also used in the inulin clearance test[4]. Though this enzyme was first isolated from plants, it is difficult to extract it in sufficient quantities from plant sources. Also, the acid hydrolysis of inulin to fructose displays several drawbacks. This has diverted interest towards the microbial inulinases. Another timely application of inulinase consists of the direct fermentation of inulin to ethanol with inulinase producing yeasts. Inulinases are produced by yeasts[5,6] filamentous fungi[7-10] and bacteria[11-15]. Gill *et al.*[16]reported a streptomyces species, which produces high levels of extracellular inulinase Optimization of inulinase production was studies by vranesic *et al.*[17] with *K. bulgaricus* by using response surface methodology. The growing potential of inulinase prompted us to screen for newer inulinase producing organisms. In this paper, we report the medium optimization and process parameters for maximal inulinase production.

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MATERIALS AND METHODS

For the isolation of inulinase-producing fungi, soil samples were collected from local gardens, cultivated fields and composts. The isolation medium contained (g/l) $(NH_4)_2SO_4$ 0.5; KH_2PO_4 3; $NaNO_3$, 1.5; $MgSO_4 7\ H_2O$, 0.01 and inulin, 3 with pH 7. Inulin was sterilized separately and added to the medium before inoculation. One gram of each soil sample was added to 50 ml of isolation medium in 250 ml Erlenmeyer flask and incubated at 28° on rotary shaker (220 rpm) for 72 h for enrichment. One millilitre of this growth suspension was added to 50 ml of isolation medium (containing 2% agar) and thoroughly mixed before plating and incubated at 28° for 72 h. Discrete colonies (50 isolates) were picked up, grown in liquid medium and evaluated for inulinase activity. Out of 50 isolates, one was found to be a potent inulinase producer (80 u/ml) and it was designated as AUP19. The isolate was subjected to taxonomic studies[18] and was identified as *Aspergillus niger*.

Optimization studies:

Four different production media were tried for inulinase

production as basal medium and their composition is presented in Table 1. Fifty millilitres of basal medium (which produced highest inulinase from selected four media) in 250 ml flask was inoculated with 5% (v/v) level of 72 h old inoculum and incubated on rotary shaker (220 rpm) at 28° for 96 h. Samples in triplicate were withdrawn from each flask and assayed for extracellular inulinase content. The effects of different carbon sources (sucrose, glucose, fructose, galactose, maltose and dextrose), concentration of inulin (1-6%), different nitrogen sources [$NaNO_3$, KNO_3, NH_4C1, NH_4NO_3, $(NH_4)H_2PO_4$, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, corn steep liquor, soybean meal, tryptone, peptone and yeast extract], initial pH (2.5-8.5), incubation period (12 to 168 h), inoculum level (1.5 to 15%) temperature (20 to 50°), and agitation (plain flasks and indented flasks) were examined on inulinase production.

Method for determination of inulinase activity:

The broth was centrifuged at 10 000 g for 20 min at 4° and the clear supernatant was used for the enzyme assay. To 2 ml of 0.2 % inulin solution, 2 ml acetate buffer (pH 4.6) and 0.5 ml test sample were added and incubated at 50°. After 20 min, the tubes were kept in boiling water bath for 10 min to inactivate the enzyme and then cooled to room temperature. The reaction mixture was assayed for fructose content by DNS method[19] and absorbances were measured at 575 nm using an UV/Vis spectrophotometer (Systronics 117). The inulinase activity was calculated from the standard curve of fructose solution. One unit of inulinase activity was defined as the amount of enzyme required to produce 1 μM of fructose under the assay conditions. All the experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Of the four media studied Medium IV containing (g/l) inulin, 3; $(NH_4)H_2PO_4$, 0.5; KH_2PO_4, 3; $NaNO_3$, 1.5; $MgSO_4.7H_2O$, 0.01 at pH 7 gave maximum inulinase production (80 u/ml) and was selected as basal medium for subsequent studies. Among various carbon sources (Table 2), galactose gave maximum inulinase yield (133 u/ml) followed by maltose (86 u/ml) and other sugars. As such galactose (1%) was used in all subsequent experiments. In order to establish the optimum concentration of inulin for maximum inulinase production, various concentrations were incorporated into the medium and the effects were studied. A control was also run without the addition of inulin. The results (Table 2) showed that 5% inulin was optimum concentration for maximum enzyme production (149 u/ml) and same concentration was used in all subsequent experiments.

The effect of various organic nitrogen sources such

TABLE 1: COMPOSITION OF DIFFERENT MEDIA FOR INULINASE PRODUCTION

Ingredients	Quantities (g/l) of different mediums			
	Medium No. I	Medium No II	Medium No III	Medium No IV
Inulin	10	10	5	3
Corn steep liquor	20	-	-	-
Yeast extract	-	10	5	-
Peptone	-	-	10	-
Sodium Chloride	-	5	5	-
$(NH_4)\ H_2\ PO_4$	12	-	-	0.5
KCl	0.7	-	-	-
$MgSO_4.7H_2O$	0.5	-	-	0.01
$FeSo_4.7\ H_2O$	0.01	-	-	-
KH_2PO_4	-	-	-	3
$NaNo_3$	-	-	-	1.5
PH	4.5 to 5.0	5	7.2	7

TABLE 2: EFFECT OF VARIOUS CARBON SOURCES AND CONCENTRATION OF INULIN ON INULINASE PRODUCTION

Carbon sources (1%w/v)	Inulinase activity (u/ml)	Inulin concentration (%)	Inulinase activity (u/ml)
Control	70±1.42	Control (without inulin)	—
Sucrose	65±1.1	1	79±1.23
Glucose	68±1.3	2	86±1.28
Fructose	72±1.12	3	125±1.32
Galactose	133±1.63	4	132±1.41
Maltose	86±0.92	5	149±1.50
Dextrose	71±0.85	6	133±1.48

as corn steep liquor, soya bean meal, tryptone, peptone and yeast extract were studied. Among these peptone and corn steep liquor stimulated the extra cellular inulinase production, where as yeast extract and soya bean meal had no influence. Among various inorganic nitrogen sources, $(NH_4)H_2PO_4$ showed slight increase in inulinase production (152 u/ml) and others did not exhibit appreciable changes (data not shown). Derycke and Vandamme[8] reported increased productivity with $(NH_4)H_2PO_4$ employing *Aspergillus niger*. In all the subsequent experiments both corn steep liquor and $(NH_4)H_2PO_4$ at 1% level were incorporated in the production medium.

For determining the optimum pH, a range of 2.5 to 8.5 was tested (fig. 1). It was found that pH 6.5 was optimal for inulinase production (155 u/ml). Below pH 5.5 inulinase production was reduced drastically. Similar type of results (pH 6.5) reported by Selvakumar and Pandey[20] with *K. marxianus* for inulinase production. In all subsequent experiments initial pH was adjusted to 6.5. The effect of Incubation period (fig. 2) was investigated on inulinase production and maximum inulinase production (162 u/ml) was obtained in 72 h. The effect of incubation temperature was studied on the production of inulinase (fig. 3) and 28° (164 u/ml) was found to be optimum temperature.

Inoculum level was also an important factor for the production of inulinase. Experiments using different inoculum levels (1.25% to 15%) were conducted (fig. 4). Higher enzyme production (165 u/ml) was observed at 10 % (v/v) inoculum level. The effect of aeration on enzyme production was studied by changing the volumes of medium in the EM flasks (volume of the medium to volume for the flask ratio (v/v) 1:30, 1:20, 1:10, 1:5 and 1:2.5). The maximum inulinase production (168 u/ml) was observed at medium volume to flask volume ratio of 1:20 (v/v). A maximum inulinase productivity of 176 u/ml was achieved with indented flasks while an activity of 168 u/ml was

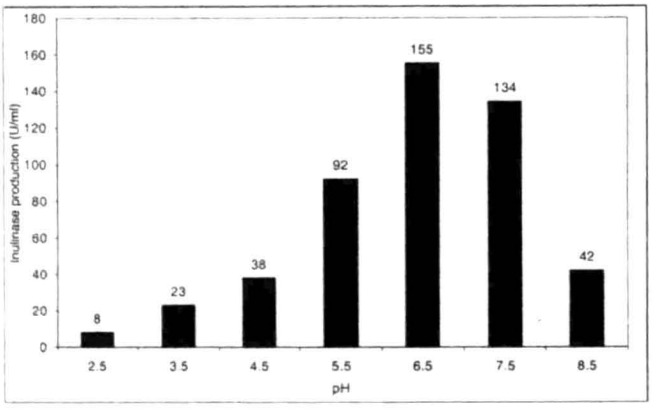

Fig. 1: pH vs inulinase production

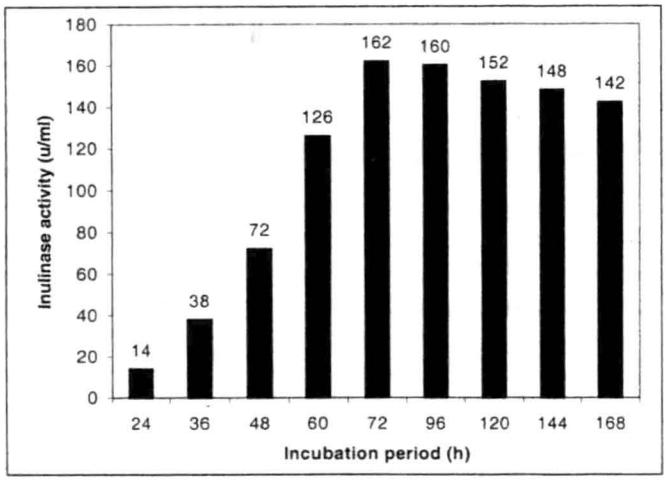

Fig. 2: Effect of incubation period on inulinase production

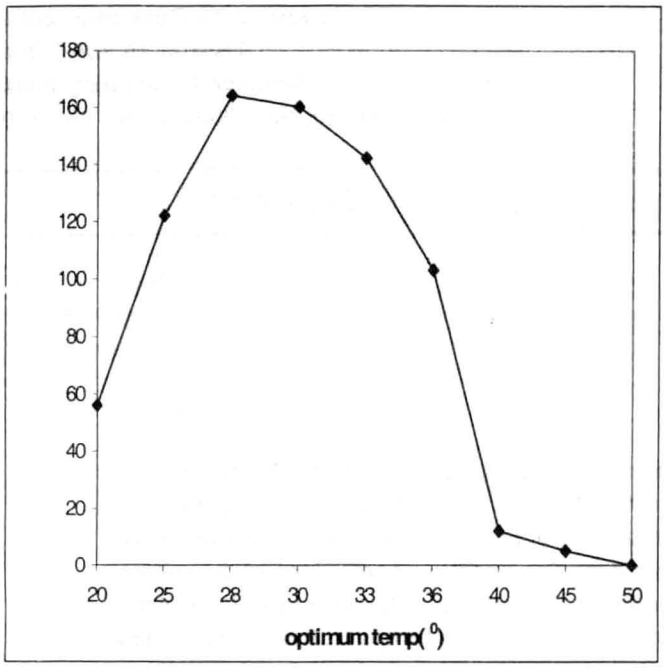

Fig. 3: Effect of optimum temperature on inulinase production

obtained using plain flasks under optimized conditions with *A. niger* AUP19.

From the results, it can be concluded that maximum production of inulinase by *Asprigillus niger* AUP19 achieved with galactose (1%) as carbon source, 5% inulin, Corn steep liquor(1%) and $(NH_4)H_2PO_4$(1%) as nitrogen sources, initial pH of 6.5, incubation temperature of 28° and 10% inoculum level. It is also observed medium volume to flask volume ratio of 1:20 (v/v) was most suitable. While better enzyme yields were obtained with indented flasks.

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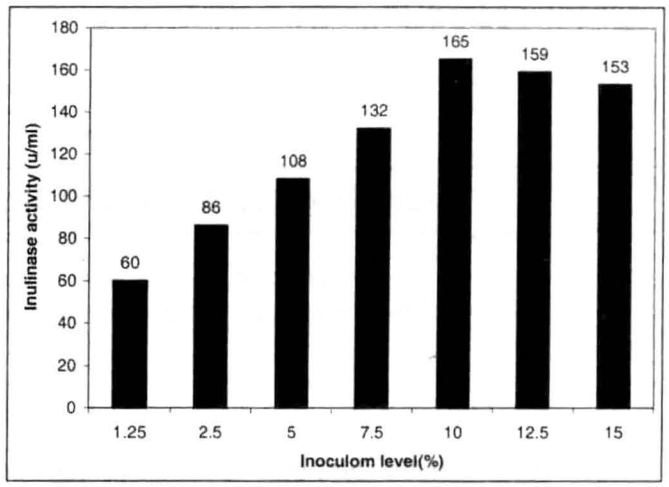

Fig. 4: Effect of inoculum level on inulinase production

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