Biosci. Biotech. Res. Asia Vol. **3,** No.(**1**) 85-88 (2005)

INVESTIGATION IN TO THE CELL WALL BOUND BETA-GLUCOSIDASE OF *Penicillium purpurogenum*

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(Received April 17, 2005; Accepted June 01, 2005)

ABSTRACT

Penicillium purpurogenum was found to produce cell wall bound β-glucosidase reaching to its peak after 4 days of incubation, which parallels with the growth of fungus. The cell wall bound enzyme exhibited optimum activity at pH 5.5 & 50°C. The ionic detergent SDS at 0.1% concentration shows maximum solubilization of cell wall bound enzyme in to the extraction buffer. The enzyme activity was mildly enhanced by metal ions like K^+, Na^{2+} and strongly inhibited by Hg^{2+}. EDTA shows little inhibition of the β-glucosidase activity.

Keywords: β-glucosidase, SDS and *Penicillium purpurogenum*.

INTRUDOCTION

β-glucosidase (EC. 3.2.1.21) β-glucoside glucohydrolase catalyzes the hydrolysis of β-glucosidic linkages of aryl and alkyl β-glucosides. β linked oligosaccharides, and several other oligosaccharides with the release of glucose[1,2]. The enzyme was important in industrial saccharification to remove the cellobiose, an inhibitor of other cellulolytic enzymes[3]. β-glucosidase is also the key enzyme in flavor industry releasing aromatic compounds from glucosidic precursors present in fruits and fermentation products[4, 5]. In the present paper the various conditions for the enhanced production of cell wall bound β-glucosidase have been optimized. The effect of pH, temperature, agitation and additives was also monitored.

MATERIAL AND METHODS

Microorganism

P. purpurogenum isolated in our laboratory and identified by IMT, MTCC, Chandigarh, India was used. The microorganism was maintained on potato dextrose agar (PDA) slants with periodic subculturing at every 2 months. The cultures were stored at 4°C. All other chemicals were of analytical grade. Sucrose; p-nitrophenyl-β-D-glucopyranoside (β-PNPG) were the products of SRL, Mumbai. *P. purpurogenum* was cultivated in Czapek Dox (CD) medium used by Patil and Shastri (1981)[6]. Sucrose, the preferred carbon source was separately autoclaved and mixed with sterilized CD medium to get 1% concentration. Erlenmeyer flasks of 250 ml capacity each containing 50 ml medium with initial pH of 5.5 before autoclaving at 15 lbs steam pressure for 15 min were inoculated with 0.1 ml spore suspension of organism (10^4 spores/ml) and incubated under static condition at 30°C. Cell wall bound β-glucosidase activity was monitored for 20 days.

Effect of environmental factors on β-glucosidase production

The effect of various environmental factors like incubation period, initial pH, agitation, various carbon sources, different nitrogen sources, different NaCl concentration was studied on β-glucosidase production.

Characterization of β-glucosidase

β-glucosidase activity and stability was monitored at different temperatures (30 to 70^{0}C). The enzyme activity was monitored from pH 2 to 7 by using the buffers of various pH ranges. The buffers used were HCL-KCL (0.1M, pH 2), Sodium citrate (0.1M, pH 3), Sodium acetate (0.1M, pH 4 to 6) and Citrate-Phosphate buffer (0.1M, pH 7). The effect of EDTA and metal ions was also monitored on cell wall bound β-glucosidase activity by incorporating at 0.1mM concentration in reaction mixture.

Solubilization of cell wall bound β-glucosidase

Detergents like Triton X-100, Tween 80,

Tween 20, and sodium dodesyl sulphate (SDS) were used to solubilize the cell wall bound β-glucosidase at 0.01, 0.05, 0.1, 1 and 5%. These detergents were added in reaction mixture to solubilize the cell wall bound β-glucosidase as suggested by Gupta *et al.* (2004).

β-glucosidase assay

Cell-wall-bound enzyme activity was determined as described by Saloheimo *et al.* (2002). β-glucosidase assay was done by the method of Riou *et al*. (1998) using β-PNPG as a substrate[9]. One unit of β-glucosidase activity was considered as that amount of enzyme, which liberated 1 mmol of *p*- nitrophenol min^{-1} under experimental conditions.

RESULTS AND DISCUSSION

Very few reports are available on the studies of cell wall bound enzymes produced by microorganism. Cell wall associated β-glucosidases are reported in yeast like *Candida curvata*[10], fungi like *Chaetomium thermophile var. coprophile*[11], *Sporotrichum pulverulentum*[12], *Trichoderma reesi*[13], and *T. koningii*[14].

The mycelium of *P. purpurogenum* has been found to contain quite a good amount of β-glucosidase. To investigate the properties and in order to enhance the production experiments were designed and conducted in triplicates. The mean of all the results were analyzed by applying single linear regression. The optimum incubation time for cell wall bound enzyme production was found to be after 96 hrs. (Fig-1). The optimum incubation time for cell wall bound lipase from a bacterial isolate SJ-15 was after 48 hrs as reported by Gupta *et al.* (2004), while Sandhu *et al.* (1985) observed that for cell wall bound â-glucosidase by *C.curvata* the optimum incubation period was 2 days[10].

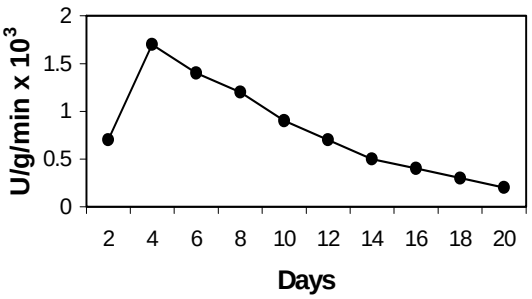

Fig. - 1: Effect of incubation time on cell wall bound β-glucosidase production by *P. purpurogenum* upto 20 days

The initial pH of the cultivation medium is one of the important variables for maximum β-glucosidase production. The favorable pH gives the organism better condition to grow and produce more enzymes. The initial pH of 5.5 is found to be good for cell wall bound β-glucosidase production by *P. purpurogenum*. Sandhu *et al.* (1985) also reported pH 5 as preferred initial pH by *Candida curvata* for cell wall bound β-glucosidase[10]. Cell wall bound lipase from a bacterial isolate SJ-15 was maximum at pH 6.5 Gupta *et al.* (2004). Ota *et al.* (1982) have also reported pH of 6.5 to be optimum initial pH for maximum cell wall bound lipase production by *Sacchromyces lipolytica*[15].

Agitation completely inhibits the growth of the organism, thus no β-glucosidase production was observed by *P. purpurogenum*.

Effect of various carbon sources was checked on cell wall associated β-glucosidase including sucrose. The sucrose was selected as the best carbon source (Table-1). Sucrose at 1% concentration was observed to be best for maximum enzyme production. Sandhu *et al*. (1985) had observed cellobiose at 0.5% concentration as the preferred carbon source for maximum β-glucosidase production by *C. curvata*[10]. Whereas soybean oil at 1% concentration was the preferred carbon source over other carbon sources for optimum cell wall bound lipase from a bacterial isolate SJ-15 as observed by Gupta *et al.* (2004).

$NaNO_3$ at 2.5 % was found to be the best nitrogen source for maximum β-glucosidase production by *P. purpurogenum* (Table-2). Gupta *et al.* (2004) have studied effect of different nitrogen sources on lipase production by a bacterial isolate SJ-15 and obtained maximum cell wall bound enzyme production with urea and $NaNO_3$ at 0.3% concentration[7].

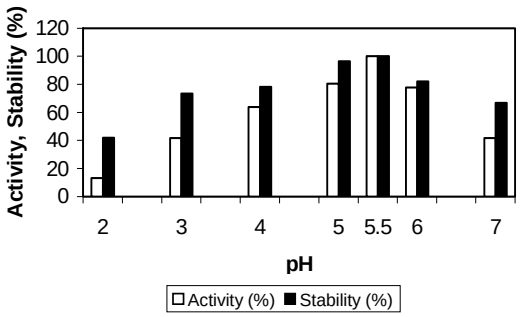

Fig. - 2: Effect of pH on activity and stability of cell wall bound β-glucosidase by *P. purpurogenum*

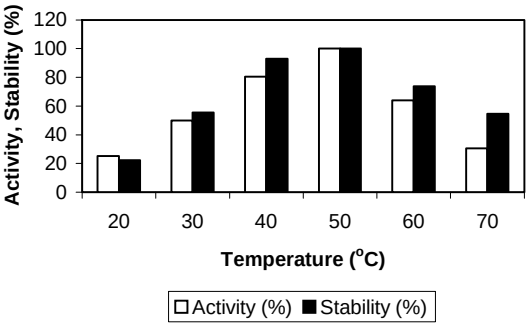

Fig. - 3: Effect of temperature on activity and stability of cell wall bound β-glucosidase by *P. purpurogenum*

Table - 1: Effect of different carbon sources on cell wall bound β-glucosidase production by P. purpurogenum after 4 days

Different Carbon Sources (1%)	Enzyme production (U/g/min)
Cellobiose	1631.42
Glucose	1007.76
Isomaltose	1343.58
Maltose	1439.53
Rutin	1295.61
Sucrose	1775.34
Xylose	1055.73

Table - 2: Effect of Different Nitrogen Sources on cell wall bound β-glucosidase production by *P. purpurogenum* after 4 day

Different Nitrogen Sources (2.5%)	Enzyme production (U/g/min)
$NaNo_3$	1679.39
KNo_3	1439.53
$(NH_4)_2No_3$	1247.63

Maximum β-glucosidase production was obtained at 0.6% NaCl in the cultivation medium. The presence of sodium ions in the surrounding environment has proved to be essential for effective transport through membranes. The use of NaCl by Damaso *et al*. (2002) has also indicated the significant impact of NaCl on production of xylanase by *Thermomyces lanuginosus*[16].

Activity of β-glucosidase was determined from pH 2 to 7, where maximum activity was obtained at pH 5.5 (Fig-2). The β-glucosidase activity was meseared from 20 to 70°C and the optimum enzyme activity was obtained at 50°C (Fig-3). The pH 7 and 45°C was reported by Saloheimo *et al*. (2002) as optimum pH and temperature for cell wall bound β-glucosidase from

Table - 3: Effect of addition of surfactant on solubilization of cell wall bound β-glucosidase activity by *P. purpurogenum*

Different Surfactants	Mean activity (U/g/min)
Control (No surfactant)	1727.37
Triton X-100 (5%)	2638.87
Triton X-100 (1%)	2063.18
Triton X-100 (0.1%)	1775.34
Triton X-100 (0.05%)	1535.47
Triton X-100 (0.01%)	1295.61
Tween 20 (5%)	2255.07
Tween 20 (1%)	2159.13
Tween 20 (0.1%)	1919.26
Tween 20 (0.05%)	1775.34
Tween 20 (0.01%)	1535.47
Tween 80 (5%)	1775.34
Tween 80 (1%)	1967.23
Tween 80 (0.1%)	1727.37
Tween 80 (0.05%)	1679.39
Tween 80 (0.01%)	1583.45
SDS (5%)	336.13
SDS (1%)	480.05
SDS (0.1%)	2782.79
SDS (0.05%)	2159.13
SDS (0.01%)	1775.34

Table - 4: Effect of addition of metal ion and EDTA on cell wall bound β-glucosidase activity by *P. purpurogenum*

Different Additives (0.1 mM)	Mean Activity (U/g/min)
Control (No additives)	1775.34
$CaCl_2.2H_2O$	1439.53
$CoCl_2.6H_2O$	1631.42
$CuSO_4.5H_2O$	1343.58
$FeCl_3$	1439.53
$FeSO_4.7H_2O$	1727.37
$HgCl_2$	192.21
KCl	2159.13
$MgSO_4.7H_2O$	1295.61
$MnCl_2.4H_2O$	1487.50
NaCl	1871.29
$PbSO_4.H_2O$	480.05
$ZnSO_4.7H_2O$	815.86
EDTA	1295.61

Trichoderma reesei[8]. Riou *et al*. (1998) reported optimum activity at pH 5 for β-glucosidase from *Aspergillus oryzae*[9]. The pH 5 and temperature 37°C was the optimum pH and temperature for getting maximum cell wall bound β-glucosidase activity for *C. curvata* as observed by Sandhu *et al.* (1985). The cell wall bound lipases from a bacterial isolate SJ-15 as studied by Gupta *et al.* (2004) also shows optimum activity at pH 8.5 and temperature 50°C[7].

The cell wall bound enzyme activity was determined as described above in presence of various detergents at different concentration. (Table - 3). It was observed that sodium dodesyl sulphate (SDS) at 0.1% solubilized the enzyme from intact biomass to maximum extent. Effect of detergents was also studied on cell wall bound enzyme by Gupta *et al*. (2004) for lipase solubilization from *Bacillus* species where SDS was found to be significant[7]. In case of *Sclerotium rolfsii* the cell associated β 1-3 glucosidase was solubilized maximally by Triton X-100[15].

The effect of metal ions was studied on the activity of cell wall bound β-glucosidase. The experiment shows that the presence of K^+ and Na^{2+} ions induces the β-glucosidase to some extent while other ions are ineffective. The presence of Hg^{2+} in reaction mixture indicates strong inhibitory response on β-glucosidase activity. The addition of EDTA has a mild inhibitory response on cell wall bound β-glucosidase activity (Table - 4). Chen *et al.* 1994 shows induction in β-glucosidase activity by Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, while Ag^+, Fe^{2+}, Cu^{2+} and Hg^{2+} shows inhibition of â-glucosidase activity by fungus *Orpinomyces* sps[18]. The effect of metal ions on microbial lipases was also studied by Nishio *et al.* (1987) and Yamaguchi and Mase, (1991). Thus, the mycelium of *P.purpurogenum* also harbors cell wall bound β-glucosidase. Taking into consideration the industrial application of β-glucosidase, it is worth to purify and characterize the cell wall bound β-glucosidase of *P. purpurogenum*. Work on this line is in progress.

ACKNOWLEDGEMENT

Authors thank The Head, University Department of Biochemistry, Nagpur University, Nagpur, India, for laboratory facility and encouragement.

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