Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 50 (2008) 114–120
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Optimisation of β-glucuronidase production from a newly isolated *Ganoderma applanatum*

Rym Chouiter, Ipsita Roy, Christopher Bucke *

Fungal Biotechnology Research Group, University of Westminster, 115 New Cavendish Street, London W1W 6UW, UK

Available online 16 September 2007

Abstract

Media optimisation was attempted for β-glucuronidase production from a newly and locally isolated (Oxfordshire, UK) fungal strain of *Ganoderma applanatum*. Both fungal growth and β-glucuronidase activity were found to be greatly affected by varying the carbon or the nitrogen source with gum arabic and yeast extracts being the best carbon and nitrogen sources, respectively. Their concentrations were optimised at 8 g L^{-1} for the former and 2 g L^{-1} for the latter.

Work then proceeded to enhance the yield of β-glucuronidase in a controlled environment. Control, batch and fed-batch cultivations were performed in 2-L bioreactors using the optimised medium supplemented with cellobiuronic acid as inducer. Time profiles of biomass dry weight, carbohydrate consumption and β-glucuronidase production were obtained and the results showed that production of β-glucuronidase was noticeably increased by the addition of cellobiuronic acid in both batch and fed-batch fermentations. Although the addition did not produce a variation in the pattern of growth seen between control, and induced fermenters, higher levels of the enzyme were attained when adopting a fed-batch process with 1.09 U mL^{-1} of culture, corresponding to a 5-fold enhancement in β-glucuronidase production rate compared with batch fermentation.

Keywords: β-Glucuronidase production; Diglycoside inducer; *Ganoderma applanatum*; Media optimisation; Submerged culture

1. Introduction

β-Glucuronidase (GUS, EC 3.2.1.31) is an exoglycosidase that catalyses hydrolysis of β-linked D-glucuronides to yield their various derivatives and free glucuronic acid. It has been isolated from snail, human, dog, cow, rabbit, rat [1] and a variety of bacteria belonging to the family *Enterobacteriaceae* and genera *Streptococcus, Peptostreptococcus, Lactobacillus, Catenabacterium, Bifidobacterium, Corynebacterium, Propionibacterium* and *Clostridium* [2]. β-Glucuronidase activity is however absent in most other organisms, especially plants, fungi and most bacteria. Until now only five fungal species have been found capable of producing the enzyme, *Kobayasia nipponica* [3], *Aspergillus niger* [4], *Penicillium canescens, Scopulariopsis* sp. [5] and recently *Ganoderma applanatum*. β-Glucuronidase has several applications. As a gene, it is used as a positive selection marker for transformed plants [6], for bacteria carrying endogenous glucuronidase gene [7] and for fungi carrying exogenous glucuronidase gene acquired by lateral gene transfer [5]. As an enzyme, it can act on a variety of substrates, such as commercially available *p*-nitrophenyl β-D-glucuronide, 5-bromo-4-chloro-3-indolyl β-D-glucuronide and 4-methylumbelliferyl β-glucuronide. Besides, the enzyme is very versatile as it can be detected by both cytochemical and spectrophotometric assays. Other extensive uses of this enzyme are its exploitation as a tool for the controlled degradation of proteoglycans in structural studies and for research purposes in diagnostic and research laboratories [4]. Production of β-glucuronidase by fungi in submerged fermentation has been carried out using cellobiuronic acid as sole carbon source as its hydrolysis releases two metabolisable sugar residues, a D-glucopyranuronic acid and a D-glucopyranoside [5,8]. However, there have been no previous attempts to optimise media for the production of β-glucuronidase in fungi.

Successful production of a desired enzyme in a submerged fermentation requires the knowledge of the growth conditions as they alter both the biomass and product formation. Carbon, nitrogen and oxygen are the most abundant components of the cell, so their forms, concentrations, and relative availability in the medium are critical in media design [9]. Inducers can also

* Corresponding author. Tel.: +44 2079115000x3966; fax: +44 2079115087.
E-mail address: buckec@wmin.ac.uk (C. Bucke).

1381-1177/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2007.09.015

be added to the growth medium to stimulate and maximise the production of valuable enzymes. Diglycosides derived from polysaccharides are widely used as inducers. They freely enter the cell and trigger the synthesis of the respective enzymes. Xylobiose induces xylanases [10] while cellobiose, trehalose, sophorose and lactose induce cellulase synthesis [11]. Submerged fermentation can be carried out as batch or fed-batch fermentation. In batch fermentation, all materials (media components and inoculum) are added to the system at the start of the process. Fed-batch fermentation, on the other hand, allows addition of media components and inducers to the system during the fermentation process [9].

The aim of the present work is to develop a medium for β-glucuronidase production and to maximise its production from a newly isolated strain of *G. applanatum* CB1 using the diglycoside cellobiuronic acid in both batch and fed-batch fermentations. The strategy is to investigate fungal growth, substrate consumption and β-glucuronidase production in order to analyse the dynamic characteristics of β-glucuronidase production and the preferred culture method to obtain a higher enzyme yield.

2. Materials and methods

2.1. Materials

All analytical-grade chemicals were purchased from Sigma (Dorset, UK) and DIFCO (Oxford, UK). Solvents were obtained from BDH (Poole, UK). Diethyl amino ethyl (DEAE)-Sephacel resin was obtained from Sigma (Dorset, UK).

2.2. Micro-organism

The strain of *G. applanatum* CB1 used in this study was isolated by Prof. C. Bucke from a *Fagus sylvatica*, Christmas Common Oxon, UK and deposited at the University of Westminster (collection number 990). The culture was maintained in potato-dextrose agar, stored at 4 °C and transferred monthly.

2.3. Production of cellobiuronic acid from gellan gum

Cellobiouronic acid was prepared by partial acid hydrolysis of gellan gum followed by purification by ion exchange chromatography. A variation on the method developed by Jefferson [8] was used. Gellan gum (42.3 g) was dissolved in 40 mL of 72% sulphuric acid. After 45 min, the solution was diluted to 500 mL to give an 8% gellan gum solution in 1 M sulphuric acid. The solution was heated with reflux at 100 °C for 4 h. It was then rapidly cooled down to stop the hydrolysis and filtered through two layers of Whatman No. 1 filter paper. The supernatant was neutralised by addition of pellets of barium carbonate, filtered through two layers of Whatman No. 5 filter paper and decolourised by addition of 6 g of activated charcoal. The solution was filtered yet again through two layers of Whatman No. 5 filter paper. About 400 mL of the hydrolysate were applied to a column (5 cm × 30 cm) containing 400 mL of DEAE-Sephacel anion exchange resin pre-equilibrated in 20 mM sodium acetate. The resin was washed with two column volumes of 2 mM sodium acetate, four column volumes of HPLC grade water before the cellobiuronic acid was eluted with 0.15 M acetic acid at a flow rate of 3 mL min^{-1}. The pooled fractions were titrated with a barium hydroxide until the mixture was just acidic to phenolphthalein. An equal volume of ethanol was then added and a precipitate was formed. The solution was allowed to stand for 3 h and the precipitate was recovered by filtration and dried. Cellobiuronic acid in the form of a white amorphous material was obtained.

2.4. 2.4. Inoculum preparation

The inoculum was prepared in five 1 L-flasks containing 200 mL of *Ganoderma* complete (GC) medium [12]. The medium consisted of (g L^{-1}): Glucose 20, Sucrose 20, Peptone 4, Casamino acid 5, Yeast extract 10, $MgSO_4 \cdot 7H_2O$ 0.50, KH_2PO_4 0.46, K_2HPO_4 1; and was inoculated with 10 plugs/flask aseptically removed from the growing edge of culture plates using a sterile cork borer No. 5. Flasks were incubated at 26 °C in an orbital shaker with a 2-cm throw at 200 rpm for 5 days. Fungal pellets obtained were then aseptically filtered and homogenized for 5 s in an autoclaved blender (Osterizer pulse matic 10, Oster, USA) containing 350 mL of distilled water. This mixture was used as inoculum.

2.5. Optimisation of medium for β-glucuronidase production

For the study on the effects of different carbon and nitrogen sources, the mushroom minimal (MM) medium [12] was used as the basal medium. It consisted of (g L^{-1}): carbon source 5, nitrogen source 1, $MgSO_4 \cdot 7H_2O$ 0.50, KH_2PO_4 0.46, K_2HPO_4 1. The liquid cultures were carried out in 500-mL flasks containing 100 mL of medium, inoculated with 10% (v/v) pre-grown inoculum, and incubated at 26 °C in a rotary shaker with a 2-cm throw at 200 rpm for 14 days. After incubation, the whole culture broth was assayed for fungal biomass and β-glucuronidase production.

2.5.1. Effect of carbon sources on the production of β-glucuronidase

To assess the effect of carbon sources on *G. applanatum* CB1 growth and β-glucuronidase production, the MM medium was supplemented with 1 g L^{-1} of bactopeptone extract and 5 g L^{-1} of different carbon sources. Glucose, galactose, mannose, arabinose, cellobiuronic acid, xanthan, gellan, arabic and locust bean gums were used as carbon sources. Medium without any carbon source was used as a control. In a subsequent study, the influence of increasing concentrations of the best carbon source (1–10 g L^{-1}) was assessed.

2.5.2. Effect of nitrogen sources on the production of β-glucuronidase

For selection of the best nitrogen source, the MM medium was supplemented with 5 g L^{-1} gellan gum and 1 g L^{-1} of different nitrogen sources, i.e. ammonium sulphate, ammonium

nitrate, urea, casein, casamino acid, glutamic acid, soybean, yeast and bactopeptone extracts. Medium without any nitrogen source was also used. The influence of increasing concentrations of the best nitrogen source (0.5–5 g L^{-1}) was also examined.

2.6. Induction of β-glucuronidase production

2.6.1. Production of β-glucuronidase in stirred tank reactors

Production of β-glucuronidase by *G. applanatum* CB1 in stirred tank reactors (STR) was carried out in the optimised medium, with gum arabic and yeast extract used as carbon and nitrogen sources, respectively. The production medium contained (g L^{-1}): gum arabic 8, yeast extract 2, $MgSO_4 \cdot 7H_2O$ 0.50, KH_2PO_4 0.46, K_2HPO_4 1. Two-litre controlled fermenters (LH Fermentations Ltd., Bucks, UK) of similar design and aspect ratio were used with a total medium volume of 1.35 L and inoculated with 0.15 L of inoculum (10% v/v). The dissolved oxygen tension (DOT) was set at 100% air saturation at the beginning of the run and kept above 30% air saturation with stirrer speed ranging from 250 to 500 rpm. The airflow rate and temperature were kept at l.0 vvm and 26 °C, respectively. pH, DOT, fungal biomass, total carbohydrates and β-glucuronidase activity were monitored throughout the 14-day run. The fermenter vessels had a diameter of 12 cm and a stirrer shaft length of 17.5 cm with two 5-tip impellers. The top and bottom impellers were 5 and 3 cm in diameter, respectively. The distances between the top of the shaft and the top impeller and between the top and bottom impellers were 11 and 4 cm, respectively. The bottom impeller was placed 2 cm above the sparger.

2.6.2. Addition of cellobiuronic acid

Cellobiuronic acid was used as diglycoside inducer for the production of β-glucuronidase. Cellobiuronic acid was added at a concentration of 200 mg L^{-1} after 2 days of incubation in the batch fermenter and at the same concentration after 2 and 8 days of culture in the fed-batch fermenter. No diglycoside inducer was added to the control fermenter.

2.7. Assays

5-ml samples from the fermenters were withdrawn every two days and centrifuged at 10,000 g at 4 °C for 30 min. Supernatants were then pipetted out, supplemented with 20 mM benzaminidium chloride, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride and kept on ice. They were assayed for β-glucuronidase activity.

Precipitated cell biomasses were washed with 100 mM phosphate buffer pH 6.5 and biomass production was measured as cell dry weight (CDW) per litre of culture broth. Samples were left to dry at 60 °C to constant weight.

Total carbohydrate concentrations were determined using the phenol-sulfuric acid method [13]. 0.2 mL of a 5% (w/w) solution of phenol in HPLC grade water was added to 0.2 mL of sample, standard or blank, and mixed by gentle shaking. 1.0 mL of concentrated sulphuric acid was rapidly added. Tubes were vigorously mixed and left to cool to room temperature for 30 min. The absorbance of the reaction mixture was read at 490 nm in a spectrophotometer. A standard calibration curve was prepared using varying concentrations of glucose within the detection range.

β-Glucuronidase activity was quantified with the substrate *p*-nitrophenyl β-D-glucuronide. The reaction mixture consisted of 0.9-mL portion of the substrate solution at 5 mM concentration in 50 mM sodium acetate buffer pH 4.8 mixed with 0.1 mL of sample. The mixture was incubated at 45 °C for 10 min in the dark. Hydrolysis was terminated by the addition of 2 mL of ice cold 500 mM sodium carbonate. The absorbance of the liberated *p*-nitrophenol was read at 400 nm. A calibration was constructed using varying concentrations of *p*-nitrophenol.

3. Results and discussion

3.1. Optimisation of media for β-glucuronidase production

A variety of different carbon and nitrogen sources were screened for their ability to support the growth of *G. applanatum* CB1 cultures and β-glucuronidase production in shaken flasks. Different concentrations of the most appropriate sources were then tested to examine their effect on both biomass growth and enzyme production. Carbon sources were chosen for their high content in glucuronic acid residues. Such polysaccharides were gellan gum, gum arabic and xanthan gum with glucuronic acid contents of 25, 20 and 20%, respectively. Other saccharides were either the disaccharide cellobiuronic acid or simple sugars (glucose, galactose, mannose and arabinose). The choice of these simple sugars was based on the monomeric composition of the polysaccharidic gums. Nitrogen sources, on the other hand, were selected randomly. They included organic and inorganic compounds. Low concentrations of carbon and nitrogen sources were used in order to lower the viscosity of the media, when using gums, which could have had repressive effects on the synthesis of the enzyme. Increased viscosity leads to poor mixing and oxygen transfer and hence to lower enzyme levels. Control media with no carbon or nitrogen sources were also used.

3.1.1. Effect of nature and concentration of the carbon sources

The effect of nine carbon sources on the growth of *G. applanatum* CB1 and on β-glucuronidase activity was investigated in shaken flask cultures after 14 days of incubation. In this set of experiments, various carbon sources were used at a concentration of 5 g L^{-1} in the basal MM medium supplemented with 1 g L^{-1} of bactopeptone. Cell dry weight and β-glucuronidase activity detected in the culture broth of all the carbon sources are given in Fig. 1A. β-Glucuronidase yields, expressed in U g^{-1} of biomass, are given in Table 1.

The results obtained showed that *G. applanatum* was capable of growing on all tested carbon sources with significant differences in β-glucuronidase activities (Fig. 1A). The highest enzyme titre and yield (0.36 U ml^{-1} and 187.24 U g^{-1}, respectively) were produced on gum arabic followed by gellan gum and cellobiuronic acid, i.e. when the organism was grown in presence of substrates with high glucuronic acid content. Although cel-

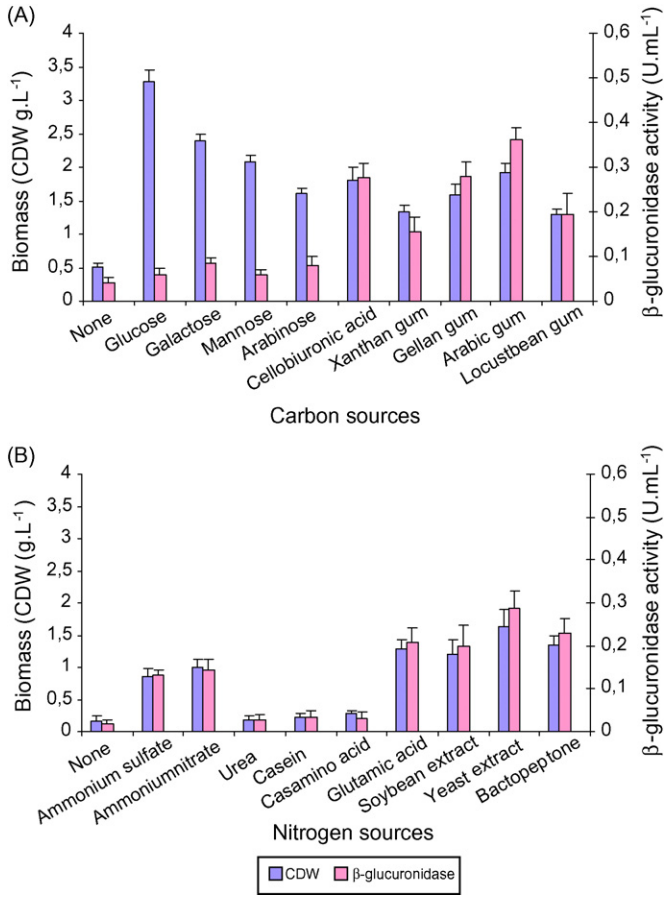

Fig. 1. Effect of carbon (A) and nitrogen (B) sources on fungal growth and β-glucuronidase production after 14 day of growth on MM medium.

lobiuronic acid had the highest content (50%), β-glucuronidase production was lower than in gum arabic grown culture. Cellobiuronic acid, being a disaccharide, enters the cell rapidly and triggers the biosynthesis of the β-glucuronidase. Mass secretion of the enzyme leads to large amounts of cellobiuronic acid being rapidly degraded to monomers in the cell. This can cause glucose repression of the enzyme synthesis. Glucose repression or glucose effect is the energy saving response that occurs in fungi when enzymes used to metabolise other carbon sources are dispensable in the presence of glucose [14]. Readily metabolised sugars such as glucose yielded high biomass content (3.27 g L^{-1}) but low β-glucuronidase activities after 14 days of growth. The enzyme levels were even lower than when no inducer was present (control). There was a 77% decrease (Table 1) in β-glucuronidase production when glucose was used compared to the control. This further indicated that simple sugars acted as repressors of the enzyme production which could be credited to the enzyme regulation by glucose repression. Growth occurred in the medium without carbon sources (starvation conditions) which could be due to the carbon content of the bactopeptone (12%). β-Glucuronidase was also synthesised in small amounts in this medium. It can, therefore, be concluded that a low level of β-glucuronidase is constitutively formed by *G. applanatum* CB1. This basal level of the enzyme might be produced by the fungus to hydrolyse complex polysaccharides to produce soluble molecules which enter the cell and trigger induction. A similar phenomenon of induction was thoroughly studied for both xylan and cellulose degrading enzymes [15,16].

Table 1
β-Glucuronidase yields after 14 day of growth on MM medium supplemented with 1 g L^{-1} bactopeptone and 5 g L^{-1} carbon source

Substrates	β-Glucuronidase yield (U g^{-1})	(%) Compared to control
None	79.05	100.00
Glucose	18.32	23.17
Galactose	35.52	44.93
Mannose	28.00	35.42
Arabinose	50.00	63.25
Cellobiuronic acid	153.33	193.97
Xanthan gum	116.90	147.88
Gellan gum	174.40	220.62
Gum Arabic	187.24	236.86
Locust bean gum	149.23	188.78

Varying concentrations of gellan gum were tested to determine whether the production of β-glucuronidase could be improved in the growth medium (Fig. 2A). Increasing concentration of gum arabic up to 8 g L^{-1} went together with an increase in β-glucuronidase production (0.62 U mL^{-1}). Further increase in

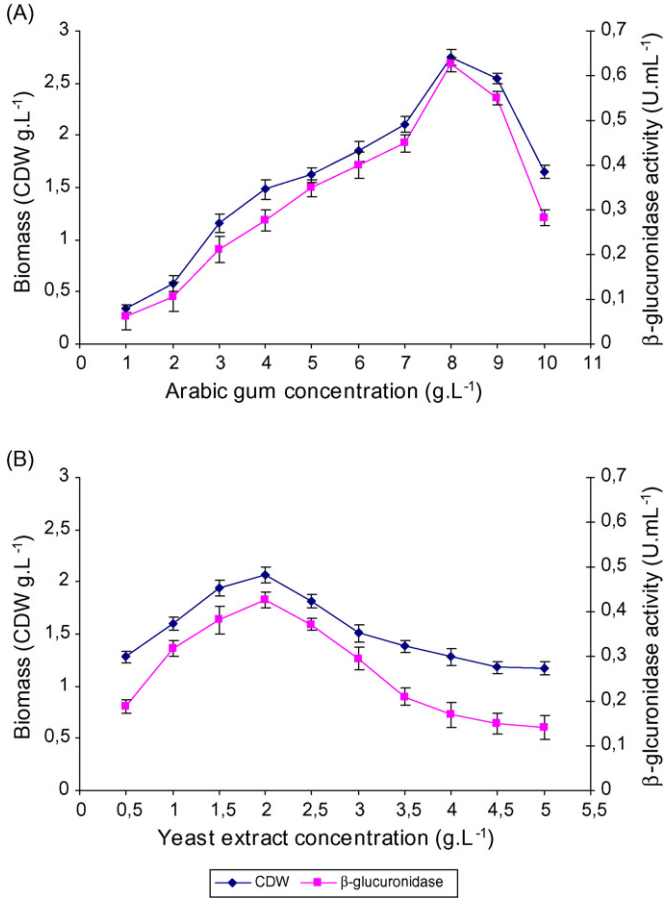

Fig. 2. Effect of different gum arabic (A) and yeast extract (B) concentrations on cell growth and β-glucuronidase production after 14 days of growth on MM medium.

Table 2
β-Glucuronidase yields after 14 day of growth on MM medium supplemented with 5 g L^{-1} gellan gum and 1 g L^{-1} nitrogen source

Substrates	β-Glucuronidase yields (U g^{-1})	(%) Compared to control
None	102.40	100.00
Ammonium sulphate	151.97	148.40
Ammonium nitrate	144.42	141.03
Urea	154.25	150.63
Casein	142.85	139.50
Casamino acid	103.44	101.01
Glutamic acid	161.74	157.95
Soybean extract	167.36	163.43
Yeast extract	176.32	172.19
Bactopeptone extract	170.49	166.49

arabic gum concentration lead to a decrease in both cell biomass and enzyme production.

3.1.2. *Effect of the nature and concentration of nitrogen sources*

In this parallel study, the MM medium was supplemented with 5 g L^{-1} of gellan gum and 1 g L^{-1} of various inorganic and organic nitrogenous sources. After 14 days incubation, CDW and β-glucuronidase activity were determined in culture filtrates. Fig. 1B shows the effect of the nitrogen sources on biomass weight and β-glucuronidase production. β-Glucuronidase yields (U g^{-1} of biomass) are given in Table 2 for each nitrogen source.

Organic and inorganic nitrogenous sources were good sources for fungal growth and production of β-glucuronidase (Fig. 1B). Amongst them, yeast extract was the best nitrogen source for the growth of *G. applanatum* CB1 (1.63 g L^{-1}) and the production and yield of β-glucuronidase (0.28 U mL^{-1} and 176.32 U g^{-1}, respectively). This could be attributed to yeast extract being an abundant source of nitrogen compounds, growth factors (purines, pyrimidines, vitamins), as well as minerals [17].

Influence of varying concentrations of yeast extract on the growth of *G. applanatum* CB1 and the production of β-glucuronidase is shown in Fig. 2B. 2 g L^{-1} of yeast extract provided the maximum CDW and β-glucuronidase production, i.e. 2.06 g L^{-1} and 0.43 U mL^{-1}, respectively. Further increase in the concentration led to a decrease in the enzyme production.

Results of this set of experiments indicate that gum arabic and yeast extract were the most suitable carbon and nitrogen source for the production of β-glucuronidase at the concentrations of 8 and 2 g L^{-1}, respectively.

3.2. *Fermentation of G. applanatum CB1 for β-glucuronidase production in stirred tank reactors*

Production of biomass and β-glucuronidase, and consumption of carbohydrates are compared between the control fermentation with no cellobiuronic acid added, batch induced fermentation to which 200 mg L^{-1} of cellobiuronic acid were added on day 2, and fed-batch induced fermentation to which addition of diglycoside inducer at 200 mg L^{-1} was repeated twice at days 2 and 8.

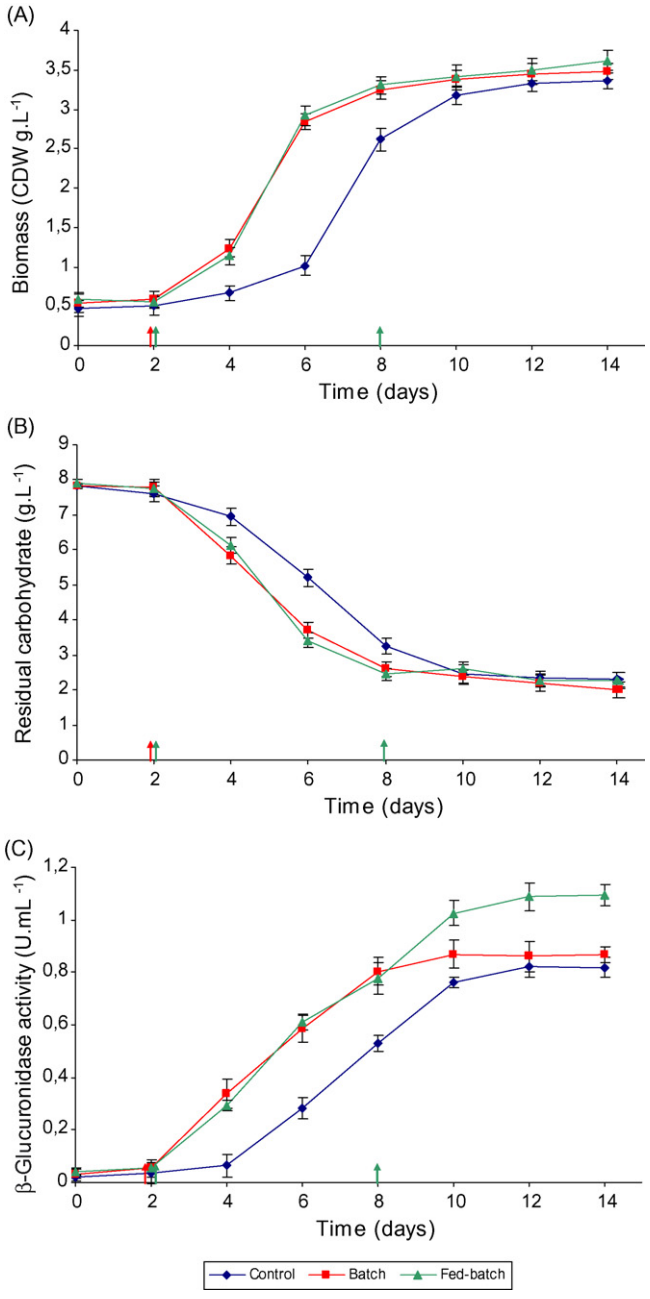

Fig. 3. Time courses of dry biomass yield (A), carbohydrate consumption (B) and activities of β-glucuronidase (C) of *G. applanatum* CB1 in control, batch induced and fed-batch induced fermentations. Colour coded arrows indicate the time of addition of cellobiuronic acid (red arrow for batch and green arrows for fed-batch fermentations).

The phenomenon of induction was confirmed through comparison between control and induced fermentations; while comparison between batch and fed-batch fermentations provided an insight into the mechanism of induction.

3.2.1. *Production of biomass*

Differences in biomass concentration (CDW) between control, batch and fed-batch induced fermentations over the course

of the growth are shown in Fig. 3A. Growth rate of biomass between days 2 (after the first addition of cellobiuronic acid) and 6 was calculated as the slope in a plot of natural logarithm of growth against time (Fig. 4A).

G. applanatum CB1 grew slowly in the early days of growth as it adapted to the new environment. The addition of cellobiuronic acid to both induced fermentations did not produce a variation in the pattern of growth seen between controls, batch induced and fed-batch induced fermentations (Fig. 3A). However, it shortened the lag phase after inoculation in both induced fermentations. Further supply of cellobiuronic acid to the fed-batch fermenter did not lead to an increase in biomass. This result indicates that the addition of the diglycoside inducer has triggered the production of β-glucuronidase which upon hydrolysis of the gum arabic has led to an increase in the growth of the fungus (growth rate of induced and control fermenters were ~0.39 and 0.17 day^{-1}, respectively between day 2 and 6).

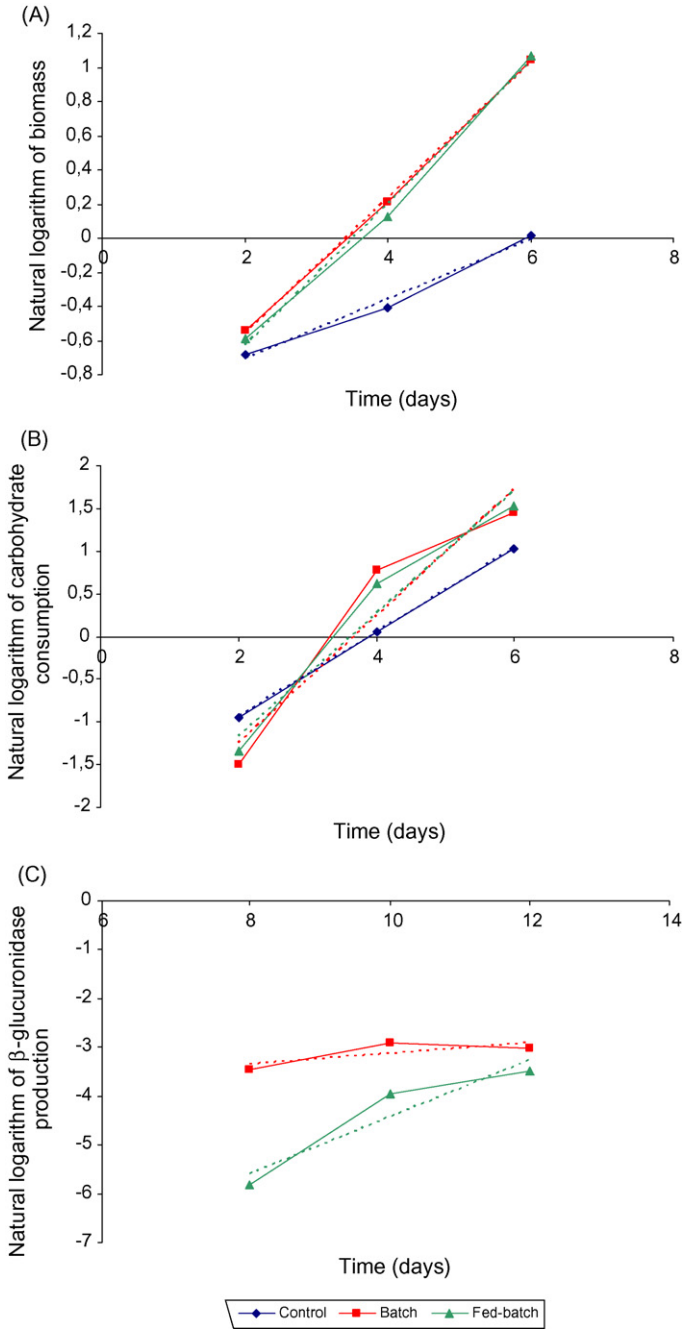

Fig. 4. Growth rate of biomass (A), consumption rate of carbohydrates (B) and production rate of β-glucuronidase (C) in control and induced fermentations during the growth phase. Dotted lines represent typical trend lines.

3.2.2. Consumption of carbohydrates

It is clear that the increase in substrate consumption followed the increase in biomass formation. In the control fermenter, when the residual concentration of carbohydrate was under 2.5 g L^{-1} at day 10, *G. applanatum* CB1 stopped growing (Fig. 3B). This was also observed in the induced fermentations but at an earlier stage of growth (day 8).

Addition of the diglycoside inducer has triggered a decrease in the concentration of carbohydrate in the medium (carbohydrate consumption rate of induced and control fermenters were ~0.72 and 0.49 day^{-1}, respectively between day 2 and (6) (Fig. 4B). In the control fermenter, the lag phase lasted 4 days. During this time, only the constitutive levels of enzyme activity were expressed, which led to a slow hydrolysis of gum arabic.

3.2.3. Production of β-glucuronidase

Time courses of β-glucuronidase production at different stages of growth in the three culture conditions are presented in Fig. 3C. Production rate of β-glucuronidase between days 8 (after the second addition of cellobiuronic acid) and 12 was calculated as the slope in a plot of natural logarithm of β-glucuronidase against time (Fig. 4C).

After a long (4 day) lag phase, enzyme production increased steadily in the control fermenter, reaching titres of 0.82 U mL^{-1} after 12 days and plateaued at this level until the end of the experiment. The same pattern was observed in the induced batch fermenter where the maximum production titre was 0.86 U mL^{-1} at day 10. The longer availability of the inducer in the fed-batch fermenter provided a higher β-glucuronidase production with a maximum of 1.09 U mL^{-1} at the end of the fermentation. When compared with the changes in residual carbohydrate and biomass concentrations, it appears that in the initial stage of fermentation, the increase in enzyme activities is growth associated and eventually led to further hydrolysis and utilisation of the carbon source as well as increase in the cell biomass (Fig. 3A and B). However, a repeated addition of the inducer in the fed-batch fermenter at day 8 has not led to an increase in the biomass even though the β-glucuronidase activity increased 5-fold (Figs. 3C and 4C). This could be explained by the fact that at this stage of growth the polysaccharides were hydrolysed into oligosaccharides and utilised. An additional supply of cellobiuronic acid for the biosynthesis of β-glucuronidase did not make any difference in biomass growth.

4. Conclusion

In conclusion, this study shows that the use of different carbon and nitrogen sources results in differences in biomass production

and enzyme activities. Arabic gum and yeast extract were the best carbon and nitrogen sources, respectively, for the production of β-glucuronidase enzyme in *G. applanatum* CB1.

This study also indicates that cellobiuronic acid was a good diglycoside inducer for β-glucuronidase biosynthesis. Batch induced culture was a good system to accelerate the production of β-glucuronidase, however, fed-batch induced culture was an even more effective way to improve enzyme yields as supplementation of diglycoside inducer improved not only the rate of the enzyme production but also its activity. Biomass remained the same making the enzyme yield better.

References

[1] S. Aich, L.T.J. Delbaere, R. Chen, Prot. Express. Purif. 22 (2001) 75–78.
[2] D. Gadelle, P. Raibaud, E. Sacquet, Appl. Environ. Microbiol. 49 (1985) 682–685.
[3] H. Tsuchihashi, T. Yadomae, T. Miyazaki, J. Biochem. 96 (1984) 1789–1797.
[4] H. Kuroyama, N. Tsutsui, Y. Hashimoto, Y. Tsumuraya, Carbohydr. Res. 333 (2001) 27–39.
[5] P. Wenzl, L. Wong, K. Kwang-won, R.A. Jefferson, Mol. Biol. Evol. 22 (2005) 308–316.
[6] C. Basu, A.P. Kausch, J.M. Chandlee, Biochem. Biophys Res. Commun. 320 (2004) 7–10.
[7] S. Bonardi, E. Maggi, A. Bottarelli, M.L. Pacciarini, A. Ansuini, G. Vellini, S. Morabito, A. Caprioli, Vet. Microbiol. 67 (1999) 203–211.
[8] R.A. Jefferson. US patent 6268493 (2001).
[9] P.F. Stanbury, A. Whitaker, S.J. Hall, The Principles of Fermentation Technology, second ed., Butterworth-Heinemann, Oxford, 1995.
[10] J.E. Busch, F.J. Stutzenberger, Bioresource Technol. 60 (1997) 81–85.
[11] S. Thirumale, D. Swaroopa Rani, K. Nand, Proc. Biochem. 37 (2001) 241–245.
[12] B.K. Kim, J.H. Kang, M. Jin, H.W. Kim, M.J. Shim, E.C. Choi, Life Sci. 66 (2000) 1359–1367.
[13] M. Dubois, G.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smoth, Anal. Chem. 28 (1956) 350–356.
[14] H. Ronne, Trends Genet. 11 (1995) 12–17.
[15] W. Liu, Y. Lu, G. Ma, Proc. Biochem. 34 (1999) 67–72.
[16] A. Hanif, A. Yasmeen, M.I. Rajoka, Bioresource Technol. 94 (2004) 311–319.
[17] M.S. Umikalsom, A.B. Ariff, Z.H. Shamsuddin, C.C. Tong, M.A. Hassan, M.T.A. Karim, Appl. Microbiol. Biotechnol. 47 (1997) 590–595.