Chapter 5

Induction and repression of β-glucosidase
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5.1. Introduction

Enzymes may be classified as constitutive or inducible based on the mode of their expression. An inducible enzyme or adaptive enzyme is expressed only under some physiological state in presence of some triggering agents which are called inducers. These inducers are generally chemical substances that are assimilated by the cell or produced by it that stimulates the production of the adaptive enzyme. In contrast, constitutive enzymes are produced continuously without any influence from external inducing factors. Many enzymes are prone to repression whereby the production of enzyme is reduced or temporarily shut down as per the requirement of the cell. The factors, mostly chemical substances that take part in repression are called repressors. Induction and repression mechanisms are generally part of the cellular economy where certain enzymes are produced only when they are required which in turn are determined by certain external/internal conditions. For example, lactose assimilation enzymes are required by a microbe only when lactose is present in the medium and induction and repression mechanisms ensure that the enzymes are synthesized only when medium contains lactose and otherwise the expression of these enzymes is shut down.

Generally cellulases are inducible enzymes produced only when cellulose is present in the medium. Bacterial cellulases as in Clostridium thermocellum, Cellulomonas flavigena and Pseudomonas fluorescens var. cellulosa are constitutive in nature while fungal cellulases are generally inducible (Suto and Tomita, 2001). However, a low level of cellulase expression in the absence of cellulose has been reported in fungi by many investigators. Cellulose is a polymer which the cells cannot assimilate. It is believed that a minor amount of constitutive cellulase expression cause initial degradation of cellulose, resulting in the production of mono or oligosaccharides that act as signal molecules for full scale induction of the cellulosolytic enzymes. Beta-glucosidases play a key role in this induction by regulating the production and uptake of
cellobiose (Berry and Dekker, 1984). Beta-glucosidase with its transglycosylation activity has the ability to convert cellobiose into disaccharide sugars like sophorose which is demonstrated to have very high potential for cellulase induction (Loewenberg and Chapman, 1977; Saloheimo et al., 2002; Suto and Tomita, 2001; Umile and Kubicek, 1986).

5.1.1. Model of cellulase induction in *Trichoderma reesei* (Suto and Tomita, 2001)

*Trichoderma reesei* spores have cellobiohydrolase on the surface, which degrades cellulose to cellobiose. These oligosaccharides are then hydrolyzed into glucose and transglycosylated to sophorose by a constitutive β-glucosidase on the spore surface. The spore germinates to form mycelium and glucose (carbon source), sophorose (inducer) and cellobiose. These oligosaccharides enter the mycelium. The mycelium bound β-glucosidase convert cellobiose to glucose and sophorose. The sophorose induces cellulase expression and the resultant proteins are secreted outside. The secreted cellulase degrades cellulose into glucose and cellobiose. While the glucose is assimilated, some cellobiose is converted to inducer by the constitutive β-glucosidase continuing the cycle (Fig 5.1).

Figure 5.1: Schematic diagram showing cellulase induction in *Trichoderma reesei*  
(Suto and Tomita, 2001)
5.1.2. Model of cellulase induction in Penicillium purpurogenum

(Suto and Tomita, 2001)

In *Penicillium purpurogenum*, constitutive cellulase degrades cellulose into cellobiose saccharides and glucose which enters the mycelium and glucose is assimilated as carbon source. The extracellular β-glucosidase of the fungus lacks transglycosylation property (Kurasawa et al., 1992) and it is believed that the intracellular β-glucosidase convert cellobiose saccharides to gentiobiose which later induces cellulase. The cellulase so produced is secreted outside which degrades cellulose, producing glucose and cellobiose saccharides. These are absorbed by the mycelia and the cycle continues (Fig 5.2).

![Cellulase induction diagram](image)

Figure 5.2: Schematic diagram showing cellulase induction in *Penicillium purpurogenum* (Suto and Tomita, 2001)

5.1.3. Beta-glucosidase – a constitutive or inducible enzyme

Reports indicate that β-glucosidase may occur as either an inducible or a constitutive enzyme. The inducible enzymes were also subject to repression by specific sugar molecules. In *Monilia* sp., one intracellular β-glucosidase (IG 1) was constitutive
while the other intracellular isoform (IG 2) and extracellular β-glucosidase (EG 1) were inducible. Cellobiose and D-glucose was found to repress β-glucosidase (IG 2 and EG 1) formation at higher substrate levels. This repression by cellobiose was suggested to be due to accumulation of glucose within the cell due to the action of intracellular β-glucosidase especially IG 1 (Berry and Dekker, 1984). In *Neurospora*, cellobiose was found to be the inducer for β-glucosidase production. Both aryl β-glucosidase and cellobiase were induced with cellobiose, though higher concentration of cellobiose (10 mM) was found to cause significant inhibition to cellobiase production (Eberhart and Beck, 1973). In *Streptomyces*, carboxy methyl cellulose and cellobiose was found to induce two intracellular β-glucosidases. Glucose added along with cellobiose caused repression of enzyme synthesis, possibly due to inhibition of inducer transport in to the cell (Pérez-Pons et al., 1995). In *Shewanella*, three isoforms of β-glucosidase were reported when grown in cellobiose and glucose as carbon source. However, production of the three isoforms were higher in cellobiose as carbon source (Cristóbal et al., 2009).

Beta-glucosidase production in fungal strains varies in response to the chemical nature of sugar molecule. Also, chemicals reported to be an inducer in one strain may have no effect or may even act as repressor in the other strains. Sophorose is a well known inducer of cellulase. However in *Trichoderma reesei*, sophorose caused repression of β-glucosidase. The low levels of β-glucosidase in *Trichoderma reesei* may be associated with cellulase inducer sophorose which represses β-glucosidase synthesis to prevent itself from getting hydrolyzed. Thus keeping low concentrations of β-glucosidase maximizes sophorose concentration in mycelium causing induction of cellulase (Sternbergt and Mandels, 1980).

*Byssochlamys fulva* NII 0930 was found to produce several isoforms of the BGL and it was observed that the levels of expression of these proteins were differing when grown in different carbon source leading to the inference that at least some of the BGL isoforms might be inducible. Experiments were conducted to study the effect of carbon sources on induction/repression of BGLs in the fungus.
5.2. Materials and Methods

5.2.1. Preparation of mycelia

Mandel & Weber media was prepared as outlined in section 2.2.2. Four liters of media was added in to a 5 L fermenter and autoclaved. Filter sterilized glucose was added aseptically in to the media to a final concentration of 1 % w/v. Spores from 8 day old culture, suspended in 0.01 % tween 80 were used as inoculum. An agitation of 150 rpm was provided and the system was run at 30 °C for 48 h. Dissolved oxygen was maintained at 50 % level. The mycelia were harvested, washed thrice with sterile buffer and then suspended in sterile citrate buffer (0.05 M, pH 4.8).

5.2.2. Carbohydrates used for induction/repression studies

Monosaccharides, oligosaccharides, polysaccharides and derivatives of carbohydrates were tested as inducers or repressors. The carbohydrates used were glucose, thioglucose, cellobiose, cellobiose octaacetate, lactose, galactose, sucrose, maltose, fructose, salicin, rhamnose, adonitol, arabinose, inositol, mannitol, mannose, melibiose, sorbitol, rutin, quercetin, ribose, xylose, glycerol, trehalose, ascorbic acid, para-nitrophenyl β-D glucopyranoside (pNPG), methyl umbelliferyl β-D glucopyranoside (MUG), sophorose, cellulose, avicel, carboxy methyl cellulose (CMC), microcrystalline cellulose, pectin, xylan, starch, inulin, thiocellobiose and xylitol. To study the combined effect of two sugars, a combination of sophorose (0.5 mg/50 ml) and thioglucose (1.0 mM), thiocellobiose (0.5 mg/50 ml) and thioglucose (1.0 mM), salicin (1.0 mM) and thiocellobiose (0.5 mg/50 ml) were used.
5.2.3. Induction/repression of $\beta$-glucosidase by carbohydrates

Fresh mycelia harvested from fermenter were added to 50 ml sterile citrate buffer (0.05 M, pH 4.8) in 250 ml conical flasks so as to achieve a final biomass of 0.5 mg/ml. Polysaccharides (cellulose, avicel, carboxy methyl cellulose, microcrystalline cellulose, pectin, xylan, starch or inulin) were autoclaved separately and added to the induction medium to a final concentration of 0.01 % w/v. Monosaccharides, oligosaccharides and their derivatives were filter sterilized using 0.22 µ syringe filter. Except thiocellobiose and sophorose, all other filter sterilized sugars were added to a final concentration of 1.0 mM. Thiocellobiose and sophorose were added to a final concentration of 1.0 mg/ml. To study the combined effect of known inducers and repressors, combination of two sugars were also tested. These included sophorose (0.5 mg/50 ml) and thioglucose (1.0 mM), thiocellobiose (0.5 mg/50 ml) and thioglucose (1.0 mM), salicin (1.0 mM) and thiocellobiose (0.5 mg/50 ml). The flasks were incubated at 30 °C in an incubator shaker at 200 rpm. Samples (5 ml) were taken at 8 h, 16 h, 24 h and 32 h of incubation and centrifuged at 12000 rpm, 4 °C for 15 min. The supernatants were assayed for $\beta$-glucosidase activity and glucose tolerance as per section 2.5.1.

5.2.4. Native PAGE, zymogram analysis and silver staining

Ten milliliters of the culture supernatant from 32 h incubated flasks of the induction/repression study were precipitated using 4 volumes of acetone as outlined in section 2.4. The precipitate was air dried, dissolved in 100 µl citrate buffer and native PAGE (8 % gel) and zymogram analysis using MUG as substrate was carried out as explained in section 2.6. The gel was visualized under a UV illuminator and photographed using an imaging system (Syngene-GBox, UK). The gel was washed twice with distilled water and silver staining of protein bands were performed according to a modified protocol of Merril et al., 1981. The silver stained gel was photographed under white light using the imaging system (Syngene-GBox, UK).
5.3. Results and Discussion

5.3.1. Enzyme production after 8 h of incubation

Beta-glucosidase production was noticed in all the experimental flasks. In the control, even in the absence carbohydrates, BGL production was noticed (Fig 5.3). This indicates the constitutive nature of BGL secretion by Byssochlamys fulva NII 0930. In Neurospora crassa, a 6 h conidial induction with monosaccharides and disaccharides on cellobiase and aryl β-glucosidase was reported. As in the present study, control flasks with no added sugar showed β-glucosidase production (Eberhart and Beck, 1973).

Figure 5.3: BGL and GBGL production after 8 h of incubation

Within the first 8 h there was no significant induction or repression of BGL. However, a significant reduction in GBGL production was noticed with pNPG. BGL production was comparatively high with xylose, inulin and MUG. Compared to other
sugars, GBGL production was highest with inulin followed by cellulose, galactose and MUG.

In *Neurospora crassa* among the sugars tested, for strain ‘74-ORS 1a’ maximum induction was noted with cellobiose. An induction of 25 % as compared to cellobiose was noted with maltose, followed by D-galactose, D-glucose and mannose. A low level of induction was noted with D-fructose, trehalose and D-xylose while enzyme activity in D-arabinose, melibiose and lactose was comparable to control. Flasks with D-fucose, L-fucose and melibiose showed absence of cellobiase. A similar pattern was noticed with ‘33(2-6)A’ strain but lactose was found to repress enzyme production (Eberhart and Beck, 1973). In the present study, cellobiose had no significant effect in BGL induction while mannose, D-glucose, melibiose and galactose showed slight inductive effect. Lactose had a repressive effect on BGL production. In *Neurospora crassa*, for aryl β-glucosidase, cellobiose was the promising inducer, followed by galactose. Maltose, xylose, D-arabinose, mannose and D-glucose showed moderate induction. In strain ‘74-ORS 1a’ repression was noted with D-fucose, L-fucose, melibiose, melezitose, trehalose and lactose. In ‘33(2-6)A’ strain, D-fucose, D-fructose, D-glucose, D-mannose, melibiose was found to repress enzyme activity (Eberhart and Beck, 1973).

Among heteroglucosides and sugar derivatives, arbutin showed induction of both enzymes in the two strains of *Neurospora crassa*. In both strains, salicin and pNPG was found to be the inducer and quercetin to be the repressor of aryl β-glucosidase (Eberhart and Beck, 1973). In the present study, pNPG had significant effect on GBGL production and showed considerable levels of repression. The control flask recorded a GBGL activity of 4.37 U/ml while flask with pNPG showed only 0.68 U/ml GBGL activity.

5.3.2. Enzyme production after 16 h of incubation

With 16 h of incubation, induction and repression of BGL and GBGL synthesis were evident, even though the basal level expression continued. Xylose was the best
inducer for BGL, followed by inulin and MUG (Fig 5.4). Among the cellulosic substrates, cellulose and microcrystalline cellulose behaved as inducers for BGL while avicel and CMC had no significant effect on induction. Production of BGL in cellobiose flask was comparable to that of control. Repression of BGL synthesis was evident with thioglucose and sorbitol causing marked decrease in BGL production. Interestingly, glucose was found to exhibit a minor inductive effect on BGL production. For GBGL production, xylose and inulin was found to be the best inducers followed by cellulose and MUG. Repression of GBGL was evident with thioglucose and cellobiose octaacetate. Minor levels of repression to GBGL production was noted with ribose, sorbitol, salicin, rhamnose, thiocellobiose, inositol, avicel, galactose, lactose, quercetin, starch and xylose. In Scytalidium thermophilum, addition of glucose, fructose, cellobiose and ethanol caused induction of BGL and among the monosaccharides, cellobiose was found to be the best inducer. Sucrose, mannitol, glycerol and sorbitol behaved as repressors to BGL production (Kaur et al., 2006)

Figure 5.4: BGL and GBGL production after 16 h of incubation
5.3.3. Enzyme production after 24 h of incubation

With 24 h of incubation inulin showed high levels of BGL induction (Fig 5.5) followed by xylose, mannitol and cellulose (≥ 44 U/ml) as compared to control (31.8 U/ml). Inulin is a polymer of fructose and glucose based oligosaccharides. In rats, administration of inulin resulted in increased β-glucosidase activity in gut. This result indicates the stimulatory effect of inulin in β-glucosidase secretion by gut micro flora (Rowland et al., 1998). There was marked repression of BGL production with thioglucose (25.74 U/ml) followed by sorbitol (28.2 U/ml). As for GBGL production, among the tested chemicals xylose was the best inducer followed by cellulose, MUG, inulin, mannitol, melibiose and fructose. Cellobiose octaacetate and thioglucose showed marked repression of GBGL production.

Figure 5.5: BGL and GBGL production after 24 h of incubation
5.3.4. Enzyme production after 32 h of incubation

With 32 h of incubation, xylose showed high levels of BGL induction followed by inulin, mannitol, cellulose and glycerol (≥ 50 U/ml) as compared to control (38.1 U/ml). There was marked repression of BGL production with thioglucose (25.4 U/ml) followed by sorbitol (30.4 U/ml). As for GBGL production, among the tested chemicals xylose was the best inducer followed by mannose and mannitol. Thioglucose and cellobiose octaacetate showed repression of GBGL (Fig 5.6).

Figure 5.6: BGL and GBGL production after 32 h of incubation

No β-glucosidase and cellulase production was seen when glucose was used as the carbon source for growth of Trichoderma harzianum. However, mycelial biomass were found to be the highest compared to fungi grown in CMC, sigma cell, lactose, wheat bran or corncob as carbon source indicating effect of glucose in cellulase repression (Aslam et al., 2010). In Trichoderma harzianum, CMC as carbon source was
found to be a cellulase inducer while glucose showed strong repression (Ahmed et al., 2009). *Scytalidium thermophilum* MTCC 4520, when grown in avicel and Solka-Floc produced high titers of BGL than in glucose, fructose, carboxy methyl cellulose, rice straw or wheat bran (Kaur et al., 2006). *Monascus purpureus* produced a constitutive level of β-glucosidase in presence of glucose and higher enzyme production occurred only after glucose depletion. This indicates the inducible nature of β-glucosidase in *M. purpureus* and the catabolic repression controlled by glucose (Daroit et al., 2007). Among rutin, quercetin, cellulobiose and glucose tested for induction, quercetin was found to be the best inducer for glucose tolerant β-glucosidase production in *Aspergillus oryzae* and *Aspergillus niger* (Günata and Vallier, 1999). In *Acremonium persicinum*, extracellular β-glucosidase was found to be an inducible enzyme, prone to repression by simple sugars. Growth of fungi in sophorose containing medium resulted in a 10 fold increase in enzyme production. Gentiobiose, laminaribiose, laminaritriose, laminartetraose, also showed inductive effect while easily metabolized sugars like glucose, sucrose, maltose, fructose, galactose, lactose showed repression (Piston et al., 1999). In *Myceliophthora thermophila*, pNPG was the best inducer for β-glucosidase production and glucose showed repression to enzyme production (Roy et al., 1988).

5.3.5. *Effect of sophorose, thiocellobiose and salicin in thioglucose induced repression of BGL and GBGL production*

In the present study, thioglucose showed repression of both BGL and GBGL production. Addition of thiocellobiose, salicin or sophorose did not reverse the repressive effect of thioglucose on BGL production (Fig 5.7).
The same pattern was noticed for GBGL production but after 16 h of incubation, the repressive effect of thioglucose was partially reduced by salicin (Fig 5.8).
In *Melanocarpus* sp. MTCC 3922, BGL production was high with avicel as carbon source. Compared to glucose, fructose showed a higher repression to BGL synthesis. Addition of glucose, fructose, cellobiose, sucrose, glycerol, sorbitol, mannitol and ethanol to fungi growing in 1 % CMC had repressive effect; maximum repression was noted with glucose, followed by ethanol, mannitol, and sorbitol (Kaur et al., 2006). In *Humicola grisea* var. *thermoidea*, avicel was found to be the best carbon source for β-glucosidase production. However, addition of 1 % glucose in to culture media containing avicel, CMC, sugar cane bagasse, wheat bran or cellobiose caused severe repression of BGL production (Nascimento et al., 2010).

In the production medium containing sucrose as the carbon source, addition of glucose caused repression of β-glucosidase production in *Penicillium purpurogenum*. Addition of glucose did not stop growth of the organism but with increasing concentration of glucose, there was increase in the inhibition of enzyme production (Dhake and Patil, 2005). Growth of *Acremonium persicinum* in *Eisenia bicyclis* laminarin resulted in β-glucosidase production. When glucose was added to the laminarin, only negligible quantities of β-glucosidase were produced. Enzyme was detected only after complete exhaustion of glucose, supplementation of media with additional glucose further delayed the appearance of β-glucosidase. This corresponds to the strong repression β-glucosidase synthesis by glucose (Piston et al., 1999). In the present study, thioglucose was found to show more repressive effect than glucose possibly due to its ‘thio’ group that makes it difficult to get metabolized.

### 5.3.6. Native PAGE and zymogram analysis of β-glucosidase produced after 32 h of incubation

Only two bands of activity were noted in the zymogram analyses. The isoforms produced in the experiment corresponds to BGL 2 and BGL 3. Similar bands of activity were noted in all flasks including control (Fig 5.9: A, B, C, D).
In *Debaryomyces pseudopolyomorphus*, cellobiose as sole carbon source in production media caused inductive effect but glucose was found to show repression towards β-glucosidase synthesis. One of the isoforms of β-glucosidase from this yeast was found to be constitutive and was not repressed by glucose (Arévalo Villena et al., 2006). *Monilia* sp. produced two intracellular (IG 1, IG 2) and one extracellular (EG 1)
β-glucosidase. IG 1 was a constitutive enzyme; cellobiose, cellulose and glucose seemed not to affect its production and only a basal level of production occurred compared to IG 2 and EG 1. IG 2 and EG 1 were inducible with highest level of activity when grown in cellulose. Cellobiose and glucose repressed the synthesis of IG 2 and EG1 and enzyme synthesis got de-repressed only when the carbohydrate level decreased in the medium. The repression of synthesis of IG 2 and EG 1 by cellobiose may be due to the accumulation of glucose in cells as a result of hydrolytic activity of IG 1 (Berry and Dekker, 1984).

In *Neurospora crassa*, aryl β-glucosidase and cellobiase were found to be inducible enzymes. Aryl β-glucosidase was found to be induced first, and after a one hour lag, cellobiase was induced. After 7 h of incubation, no further induction was noticed probably due to limitation of cellobiose which was supplied to a final concentration of 1 mM. Prior to conidia formation, the aryl β-glucosidase appeared to be semi constitutive (Eberhart and Beck, 1973). Beta-glucosidase from *Aspergillus nidulans* was found to be constitutive. In this case, the washed mycelia after starvation in saline were transferred to induction medium with various carbon sources at a final concentration of 0.05 % w/v. Beta-glucosidase production in presence of cellobiose, gentiobiose, lactose, sophorose, melibiose, trehalose, amygdalin, esculin, salicin and methyl β-glucoside was comparable to control that lacked any sugar. However, enzyme production showed catabolite repression. Glucose and 2-deoxy glucose (0.05 % w/v) fully inhibited enzyme production while glycerol (0.4 % v/v) reduced the enzyme production (Lee et al., 1996).

BGL production under optimized SmF conditions as outlined in section 4.3.3.2 resulted in the production of 7 isoforms (chapter 6, figure 6.4.). In the induction/repression studies, native PAGE and zymogram indicated only two bands of activity with comparable intensities in all flasks including control. This probably indicates the constitutive nature of the β-glucosidase isoforms BGL 2 and BGL 3 in *Byssoschlamys fulva* while the other isoforms –BGL 1, BGL 4, BGL 5, BGL 6 and
BGL 7 could be inducible. It is likely that these isoforms are produced in low quantities and is hence difficult to detect under the conditions tried for induction studies.

Cellobiose is considered as a potential inducer of cellulase. However in the present study, cellobiose was not found to have any effect on β-glucosidase induction. A similar experiment in *Penicillium purpurogenum* also showed cellobiose to be ineffective in cellulase induction. This was explained to be due to the presence of constitutive extracellular β-glucosidase that cleaved cellobiose into glucose before cellobiose could enter into the cell. Crude intracellular enzyme when incubated with cellobiose showed transglycosylation activity, producing gentiobiose and laminaribose, while crude extracellular enzyme did not show transglycosylation property (Kurasawa et al., 1992). Thiogentiobiose is found to be a non-metabolizable inducer (Kurasawa et al., 1992). Similarly, thiocellobiose can be considered as a non-metabolizable sugar. Thiocellobiose is an inhibitor to β-glucosidase as evident from NMR studies using β-glucosidase from *Streptomyces* (Montero et al., 1998). In the present study, like cellobiose, thiocellobiose also had no inductive effect on β-glucosidase production. However in *Schizophyllum commune*, thiocellobiose was reported as an inducer (Rho et al., 1982).

The mechanism of induction is not fully understood. Once the inducer enters the cell, it activates certain proteins. These protein activators and activating elements (e.g. CAE in *Hypocrea jecorina*) triggers transcription of cellulase gene (Suto and Tomita, 2001). Some chemicals may have an inductive effect, but due to variation in the localization of the BGL produced, the inductive effect may not be evident. In *Trichoderma longibrachiatum*, cellobiose induced β-glucosidase was mostly localized in cytosol and cell debris, while use of cellulosic substrate resulted in a greater concentration of enzymes in culture filtrate (Sandhu and Sidhu, 1985). Most of the BGL in *Trichoderma pseudokoningii* was found to be cell wall associated when grown in lactose. However, when grown in lactose-sorbitose medium, there occurred rapid release of enzyme into the culture fluid. This effect occurred only when sorbose was added in the beginning of cultivation and no late addition could initiate this phenomenon. The
mechanism is thought to be due to decreased availability of cell wall 1,3 glucan to which β-glucosidase binds. L-sorbose inhibit β-1,3-glucan synthase and hence the newly synthesized BGL doesn’t have enough β-1,3–glucan to get attached and hence got released in to the culture fluid (Kubicek, 1983). Also appearance of increased levels of extracellular β-glucosidase in stationary phase may be due to autolysis of mycelium and not due to increased enzyme synthesis caused by the exhaustion of repressor chemicals (Piston et al., 1999).

Carbon catabolite repressor protein CRE1 could be produced constitutively in the fungal mycelium while the sub-cellular localization of it depends up on the extracellular carbon source. In Sclerotinia sclerotiorum, CRE1 was present in the nuclear fraction of mycelia grown in glucose containing media. When the mycelium was transferred to medium containing other carbon sources, CRE1 was detected in cytosol fractions. Thus the presence of glucose results in localization of CRE1 protein in the nucleus, which binds to the repressor binding region located upstream of cellulase genes and thus regulates transcription (Suto and Tomita, 2001).

Studies in human cell lines indicate differential mode of regulation of gene expression in β-glucosidase production. In epithelial, lymphoblast, histiocyte, glioblastoma and astrocytoma cell lines, a direct relationship was seen between mRNA levels and enzyme activity indicating transcriptional control of BGL expression. Fibroblast, promyelocyte and neuroglioma cell lines also showed a direct relation between mRNA and enzyme activity but compared to first group, enzyme activity was 6 fold than expected. In single monocyte cell line, high levels of mRNA were noted but only moderate level of enzyme activity could be detected. Also mRNA from normal cells was comparable to mRNA from patients with Gaucher’s disease. This indicates that regulation of β-glucosidase activity is complex and may occur at multiple levels (Doll and Smith, 1993). In the present study, isoforms BGL 2 and BGL 3 are constitutive while BGL 1, BGL 4, BGL 5, BGL 6 and BGL 7 may be inducible but the process of induction or secretion seems to be much complicated than the presence or absence of carbohydrates.
5.4. Conclusion

Beta-glucosidase production in Byssochlamys fulva NII 0930 was found to be regulated by complex factors than presence or absence of carbohydrates. BGL 2 and BGL 3 isoforms were found to be constitutively expressed and was secreted even in the absence of carbohydrates. However enhancement of production was noticed with xylose, inulin, mannitol, cellulose and glycerol. Thioglucose was found to repress BGL production. BGL 1, BGL 4, BGL 5, BGL 6 and BGL 7 may be inducible enzymes but more complex factors may be involved in induction and secretion.
Chapter 6

Purification and characterization of the $\beta$-glucosidase isoforms from *Byssochlamys fulva* NII 0930