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Full Length Research Paper

Kinetics of endoglucanase and cellobiohydrolase production by parent and mutant derivative of moderately thermotolerant *Bacillus subtilis* GQ 301542 on optimized medium

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A moderately thermotolerant bacterial strain was isolated from the hot spring of Tatta Pani (AJ and K) Pakistan and was designated as *Bacillus subtilis* strain GQ 301542 after biochemical, morphological and 16S rDNA sequence analysis. This strain and its catabolite repression resistant mutant CRM197 were utilized for the study of different production kinetic parameters of both endoglucanase and cellobiohydrolase. Time course study on one monomeric (glucose), one dimeric (maltose) and two polymeric substrates (α-cellulose and wheat straw) was carried out at different time intervals (4 - 28 h, after each 4 h) for determining the maximum enzyme productivity on a particular substrate. Maximum rate of endoglucanase production by the mutant (53.1 IU/L/h) was significantly (P = 0.0007) higher than that (23.7 IU/L/h of the parental organism following their growth on glucose in Dubos salts medium while the optimum product yields ($Y_{p/s}$) was calculated as 69.0 IU/g S (parent) and 82.3 IU/g S (mutant) for cellobiohydrolase production. Deoxy-D-glucose resistant mutant was significantly (p = 0.03 to 0.0007) improved over its parental strain with respect to some substrate consumption and all product formation parameters and can easily degrade cellulosic biomass for production of fermentable carbohydrates.

Key words: Cellobiohydrolase, endoglucanase, thermotolerant, *Bacillus subtilis*.

INTRODUCTION

Cellulases are defined by their ability to cut the β-1,4 glycosidic bonds. The complete enzyme hydrolysis of cellulosic materials needs different types of cellulases: endoglucanase (1,4-β-D-glucan-glucanohydrolase; EC 3.2.1.4), exocellobiohydrolase (1,4-β-D-glucan glucohydrolase; EC 3.2.1.91) and β-glucosidase (β-D-glucoside glucohydrolase; EC 3.2.1.21) (Yi et al., 1999). There are three different types of cellulases that have got applications in different industries such as food, brewery, wine, pulp and paper, textile, detergent, feed and agriculture (Bhat, 2000; Li et al., 2009; Sehnem et al., 2006). Cellulases have attracted much interest because of their enormous potential to convert cellulose, a natural abundant and renewable energy resource to valuable

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products such as soluble oligosaccharides, glucose, alcohols, and other industrially important chemicals (Bhat and Bhat, 1997; Bhat, 2000; Howard et al., 2003; Sun and Cheng, 2002). Majority of studies on cellulase production have focused on fungi, with relatively lesser emphasis on bacterial sources (Bhat, 2000; Camassola et al., 2004; Kotchoni et al., 2003).

Bacteria, due to their high diversity, faster growth and capability to produce highly thermostable enzymes, are ideal for use in industries as highly potent and robust sources of industrially important enzymes (Bhat, 2000; Camassola et al., 2004; Haki and Rakshit, 2003; Kotchonios et al., 2003; Vielle and Zeikus, 2001). The enzymes isolated from some extremophiles have proven to be of great use in the biotechnology industry (Liang et al., 2009; Sun and Cheng, 2002; Yang et al., 2010) as they possessed high-level activity under high temperatures and posed lesser problem of contamination and saved cooling energy (Yang et al., 2010). However, commercial cellulases are still too costly to economically degrade cellulose in industrial bioconversion of lignocellulosic substrates (Howard et al., 2003). Faster growing thermophilic bacteria may support the formation of thermostable and inexpensive cellulases for industrial applications. In the present study, we optimized fermentation process variables for hyperproduction of cellobiohydrolase and endoglucanase in shake flasks. Responses of variation of process parameters were measured by observing their influence on different growth kinetic parameters with relation to substrate utilization and product formation by employing a moderately thermophilic *Bacillus* strain on easily available and low cost substrate compared with easily and slowly metabolizable substrates, respectively.

MATERIALS AND METHODS

Isolation of thermotolerant bacterial strain and fermentation medium

A thermotolerant cellulose degrading strain was isolated from leaves and mud samples from hot spring of Tatta Pani, Azad Jammu and Kashmir (AJ and K), Pakistan by enrichment technique using Dubos salts medium supplemented with α-cellulose and after incubation on a vibratory shaker at 50℃ for 72 h. Enriched cultures were plated on carboxymethyl cellulose (1%, w/v) solid medium and colonies which produced yellow haloes on Congo red staining were sub-cultured several times to get a gram +ve bacterial organism which was named as TP-strain 1. The bacterial isolate was tentatively identified by the microscopic examination, physical and biochemical reactions using qualitative test screening (QTS, DESTRO Laboratories, Karachi, PAKISTAN) kit. The organism was tentatively identified as *Bacillus* strain.

The strain was further confirmed by 16S rRNA analysis by using universal primers P1 and P6 (Chun and Bae, 2000) which amplified the desired *gene* when DNA sample of the TP- strain was used as a template in a PCR reaction, for which following parameters were adopted: an initial denaturation at 95℃ (5 min), followed by 35 cycles of 95℃ for 1 min, 50℃ (1 min), 72℃ (1 min), with a final extension at 72 ℃ for 10 min. The polymerase chain reaction (PCR) product of 16S rRNA was visualized on 1% agarose gel by UV illumination (on gel doc) after staining with 0.3 µl ethidium broimide. The band of 16 S rRNA was cut and purified using Gel extraction kit (Qiagen, Germany). The purified PCR product was sent for sequencing to MacroGen International (Germany) and the results were compared using homologous analysis online with NCBI blast.

The above purified strain of *Bacillus subtilis* GQ 301542 was subcultured four times on carboxymethyl cellulose (CMC) plates and used for conducting these research experiments. The fermentation medium for seed culture was prepared in Dubose salts medium containing (g/L): $K_2HPO_4.7H_2O$ 1.0, $NaNO_3$ 0.5, Mg $SO_4.7H_2O$ 0.5, KCl 0.5, $FeSO_4.7H_2O$ 0.01, yeast extract 2.0 and glucose 2.0. Enzyme production was carried out initially in basal salt medium (pH 7.0) at 45℃ in an orbital shaking incubator (200 rpm).

Random mutagenesis of strain for developing deoxy-D-glucose-resistant mutant (CRM197)

For enhancing enzyme activity, the culture was mutated by using γ-radiations as described previously (Rajoka, 2005). The pure colony of *Bacillus*-TP strain was grown on Dubos salt cellobiose medium and suspended equally in 30 mL McCartney vials and exposed to 0.2 - 1.2 k Gray gamma rays in gamma cell radiation chamber (Mark-V). The survivors were permitted to grow on Dubos salt-cellobiose (1%) –DG medium at 50˚C for 48 h. Enriched cells were plated on CMC-DG-Dubos medium. Among 2000 variants, faster growing population was screened by Congo red screening. Selection plates had 30 - 50 colonies per plate and 9 colonies were selected randomly from the selection plates and tested for enoglucanase activity and also for β-glucosidase activity according to methods mentioned previously (Rajoka, 2005). From these 9 colonies, only one colony designated *B. subtilis* CRM197 was selected for further enzyme production and maintained on nutrient agar plates and slants.

For enzyme production, 0.8 g α-cellulose or alkali-pretreated wheat straw (milled and separated by 0.45 mm sieve, 40 mesh) were added to 500 mL flasks containing 90 mL Dubose-salt medium (pH 7.0) and whole contents were sterilized for 30 min at 121℃. Non-induced (glucose-grown) and saline washed cell suspensions were used to inoculate the enzyme production medium (1:10) containing the carbon sources (glucose and maltose were added after filter-sterilization). The inoculated flasks were incubated at 45 ± 2.0℃ in a refrigerated vibratory shaking incubator (250 rpm) where temperature could be controlled automatically. Control temperature was 45℃. After each 4 h, the enzymes were recovered from each flask by centrifugation (12,000 x g for 10 min, 10℃) and preserved for enzyme assays. Fermented insoluble substrates (α-cellulose and wheat straw) were separated by centrifugation (8,000 x g for d 10 min, 10℃) and substrate free enzyme extract was used to follow enzyme activity. Substrates were made cell free by suspending substrate in water containing Tween 80 (1%) and shaking at 250 rpm for 30 min. These preparations were re-centrifuged at low speed (8,000 x g) to separate substrate from cell mass. The cells were recovered by re-centrifuging them again as above (12,000 x g for 10 min, 10℃) and their cell absorbance was measured to determine cell mass using a standard curve. Further growth studies were carried out considering different process variables such as selection of a proper nitrogen additives (ammonium sulphate, potassium nitrate, corn steep liquor, urea, and diammonium hydrogen phosphate used at 0.07 g nitrogen/L), initial pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5) of the medium and temperature of fermentation (20, 25, 30, 35, 40, 45, 50, 55, 60, and 65℃) by adopting search technique varying process variables one at a time as described previously (Iftikhar et al., 2008).

Determination of time course for enzyme production

B. subtilis strain was inoculated into Dubose salts medium containing glucose, maltose, α-cellulose, and wheat straw as substrates in separate flasks in triplicate and incubated at 50°C (found optimum) for a period of 28 h. The cellulase activity, substrate and cell mass were measured at regular intervals of 4 h, as mentioned earlier and maximum enzyme production time was determined (Iftikhar et al., 2008).

Endoglucanase assay

Endoglucanase activity in 50 mM sodium acetate buffer (pH 5.0) at 50°C was determined using carboxymethyl cellulose (CMC) as a substrate. The reaction mixture contained 1 mL of enzyme with 1 mL of 1% (w/v) CMC solution and 1 mL of 50 mM Na-acetate buffer (pH 5.0). The flask was kept in shaking water bath (100 rpm) at 50°C for 30 min. After completing incubation period, 3 mL of DNS (3,5-dinitrosalicylic acid) reagent (Miller, 1959) was added and the reaction was quenched by placing the tubes in boiling water bath for 10 min, and then immediately cooled in ice. The reducing sugars were estimated colourimetrically at 590 nm (Rajoka, 2005).

Exoglucanase assay

Exoglucanase activity in 50 mM sodium acetate buffer (pH 5.0) at 50°C was determined using 0.5 gm cotton as a substrate. The enzyme preparation (1 mL) was reacted with the substrate (cotton, 0.02 g) in 2 mL of 50 mM sodium acetate buffer to which 100 µL Tween 80 was added and kept in shaking water bath (100 rpm) at 50°C for 30 min. After completing incubation period 3 mL of DNS (3,5-dinitrosalicylic acid) reagent was added and the reaction was quenched by placing the tubes in boiling water bath for 10 min, and then immediately cooled in ice. The reducing sugars were estimated colourimetrically at 540 nm (Rajoka, 2005).

Determination of growth kinetic parameters

Growth kinetic parameters were determined as described by Pirt (1975). The value of µ, that is, specific growth rate was calculated from plot of ln (X) versus time. Various kinetics constants were determined by using the following formulae:

Product yield coefficient with respect to cell mass ($Y_{p/x}$) = dP/dx
Product yield coefficient with respect to substrate ($Y_{p/s}$) = dP/ds
Specific rate of product formation ($q_{p)}$ = μ / $Y_{p/x}$
Substrate utilization (q_s) = μ /Yx/s
Cell mass productivity (Qx), and volumetric rate of S consumption were determined from a plot of g dry cells/L, and S (g/L) present in the fermentation flasks.

Statistical analysis

The results were analyzed statistically using analysis of variance with the application of the Tukey-Kramer multiple comparison test for a $p < 0.05$ using the Graphpad Instat software 3.0. For analysis of interaction of substrates and organisms, completely randomized block design (CRBD) with two-factor factorial was applied using MStat C software (Michigan State University, MI, USA).

RESULTS AND DISCUSSION

The themostable TP-strain was a gram positive, rod-shaped, aerobic and motile~~-~~. Further identification was conducted by QTS-24 kit out as suggested by the manufacturers. The result of different reactions was confirmed from the standard table values given by the manufacturers. The kit results showed that the organism had the ability to utilize 15 carbon sources, including glucose, cellobiose, glycerol, maltose, sucrose, lactose, mannitol, arabinose, xylose, sorbitol, mannitol, and raffinose. The bacterial sequence libraries revealed that the TP strain is in closest data base match with *B. subtilis* with maximum identity of 98% but the query coverage was 100%. The nucleotide sequence of the 16S rRNA was submitted in the Genbank data base of Japan with the accession No. GQ301542.

Generally, the synthesis of cellulases in different organisms is induced by cellulose and repressed by glucose or other readily metabolizable sugars in the growth medium. The main inducers of these enzymes are cellulose, cellobiose, sephorose, lactose and other sugars. Potential bioconversion processes of lignocellulosic materials depend mainly on the good sources of cellulolytic and hemicellulolytic enzymes, the nature of lignocellulosics and the optimal conditions for production and catalytic activity of the cellulases. Studies have shown that carbon and nitrogen sources, optimum pH, and temperature, are important limiting factors for the maximum production of cellulases and were optimized.

Effect of substrates on kinetic parameters of endoglucanase production

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Endoglucanase production was induced when *B. subtilis* GQ301542 was grown in the presence of 0.8% glucose, maltose, α-cellulose or wheat straw in basal Dubos medium in shaken cultures but maximum activity occurred on glucose at an initial pH of 7.0, temperature 50°C for 24 h. Time course study showed that the maximum enzyme production was observed after 24 h of incubation at 50°C (Figure 1) in time course. Short incubation period for enzyme production offers the potential for inexpensive production of enzymes as previously reported by Ogel et al. (2001). Incubation time necessary for optimum production varied with different substrates by the same organism. In the present study, the enzyme production was increasing steadily right from the beginning and reached the maximum at 24 h of incubation. Incubation for 28 h caused denaturation of enzymes and lowers the activity of endoglucanase that was observed (Figure 1).

The maximum value of Q_p of endo-glucanase was ~~13.99~~32.0 U/L/h with maltose for parental strain of *B. subtilis* and that of mutant the value was 34.~~61~~3~~0~~ U/L/h with glucose (Table 1) whereas the minimum Q_p value of parent and mutant strains were 17.4 and ~~8.03~~21.8 U/L/h on wheat straw, respectively. These activities of mutant derivative were significantly higher than those reported by

a potent strain of *Pencillium echinulatum* (Camassola et al., 2004;

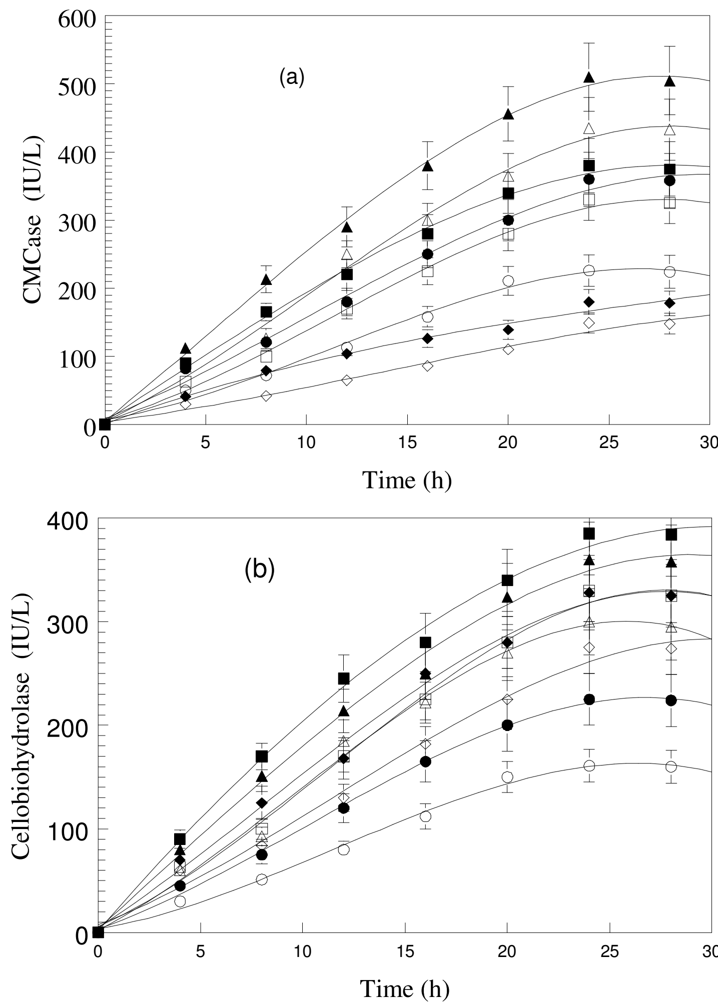

Figure 1. Time course of endo-glucanase (a) and cellobiohydrolase (b) production by parental and DG-resistant mutant of *B. subtilis* following growth on glucose (∆,▲), maltose (○,●), α-cellulose (□, ■) and wheat straw (◊,♦) in Dubos salt basal medium (pH = 7.0) at 45°C in shaking incubator as described in materials and methods. Data presented are mean values ± SD of *n* = 3 experiments.

Sehnem et al., 2006). The maximum $Y_{p/s}$ values for both parent and mutant strains of *B. subtilis* were 53.0 U/g with glucose as substrate and 57.50 U/g/S, respectively. These values were again higher than values reported by Zang et al. (2008) and other authors (Camassola et al., 2004; Sehnem et al., 2006). The statistical analysis (Table 1) of Q_pQp of endoglucanase enzyme showed that the effect of strain was more significant as compared to substrates (F = 694.80; p <= 0.050001), and the interactive effect of both strains and substrates (Table 2) was also found highly significant (F = 201.32; $p < 0.05$). All carbon sources exerted highly significant influence on

production of both endo-glucanase and that mutant was significantly improved through mutagenesis.

Table 1. Effect of carbon sources on kinetic parameters of endoglucanase and exoglucanase production by parent (P) and mutant strains of *Bacillus subtilis* in basal Dubos salt medium (pH 7.0) at 45°C.

Exoglucanse				Endoglucanse			
Substrate	**Strain**	**Qp (IU/L/h)**	**Yp/s IU/g substrate**	**Carbon sources**	**Strain**	**Qp (IU/L/h)**	**Yp/s IU/g substrate**
Glucose	P	14.0[b]	41.0[a]	Glucose	P	45.4[a]	53.0[a]
	M	34.6[a]	45.8[a]		M	51.5[a]	57.5[a]
Maltose	P	11.0[b]	34.5[b]	Maltose	P	32.0[b]	50.5[a]
	M	15.6[b]	39.0[a]		M	36.3[b]	56.3[a]
α-Cellulose	P	9.2[b]	31.9[c]	α-Cellulose	P	15.5[c]	34.0[c]
	M	14.8[b]	40.3[a]		M	20.6[c]	40.6[c]
Wheat Straw	P	2.4[c]	34.8[b]	Wheat Straw	P	17.1[c]	40.6[c]
	M	8.0[b]	40.6[a]		M	21.8[c]	46.9[b]
p value		0.0001	0.0001			0.0003	0.0046

Each value is a mean of *n*=3 experiments. Values followed by different letters in each column differ significantly using Tukey-Kramer test of multiple combinations using PadGrapg Instat 3.0 version.

Table 2..The *F* values for effect of carbon sources on kinetic parameters of CMCase, and CBH production by parent and its mutant *B. subtilis* CRM197 on basal Dubos salt medium (pH = 7.0) at 45 °C using CRB with two-factor factorial design.

SOV	df	$Y_{p/s}$ (CMCase)	Q_p (CMCase)	$Y_{p/s}$ (CBH)	Q_p (CBH)
Organisms(A)	1	24.7550 **	87.5823**	56.7983**	694.8063**
Carbon sources(B)	3	839.9356**	128.8544**	1313.8870**	469.7127**
AxB	3	0.9010	13.3685**	7.6172**	201.3222**
Error	16				
Total	23				

Effect of substrates on kinetic parameters of exoglucanase production

The maximum value of kinetic parameters like Q_p on exoglucanase activity of both parent and mutant strains was 14.6 and 34.6 U/L/h with glucose. The maximum $Y_{p/s}$ values of parent and mutant strains were 41.0 and 45.8 IU/g S, respectively. This productivity was significantly higher than reported by Hanif et al. (2004) on *Aspergillus niger* and Rajoka (2004) on *Cellulomonas flavigena.* The statistical analysis (Table 1) of Q_p and $Y_{p/s}$ of exoglucanase enzyme showed that all substrates differed significantly ($p = 0.0003$ and 0.0046, respectively), with respect to supporting the synthesis of exoglucanse. CRBD with two-factor factorial analysis indicated that the effect of substrates ($F = 447.70$; $p < 0.05$) was highly significant than that of strains.

In the study, the maximum exo-glucanase production was observed after 24 h of incubation. There was low enzyme activity detected after 8 h of fermentation and this is because the action of cellulases during early stages of growth resulted in cellobiose production that can induce the biosynthesis of enzymes at later stages. Further, the incubation time required for the formation of such enzymes also varied according to the substrate and microorganism, which confirmed the finding of Devanthan et al. (2007) who also observed similar trend using *A. niger*. Earlier Camassola et al. (2004) also reported lower production of cellulases by *Penicillium echinulatum* during early stages of growth. Low production of cellulases on complex substrate is slow in almost all organisms and is a versatile phenomenon in nature. It was found that initial pH of the fermentation medium (pH 6.0), ammonium sulphate as nitrogen source, temperature 45°C and glucose (0.8%) supported maximum activities of both endo-glucanase and exo-glucanase (Rafique, 2008) and were used in further studies.

Growth kinetic parameters on optimized medium for substrate utilization under shake flask fermentation

Time course studies were performed using parental and mutant (CRM197) strains of *B. subtilis* in above optimized medium and results are depicted in Figure 2 and substrate consumption parameters were determined as described in materials and methods. The specific growth rate (μ) was 0.23 h^{-1} (Table 3) by mutant derivative of *B.*

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subtilis with glucose as a substrate and ammonium sulphate as a nitrogen source. The maximum substrate

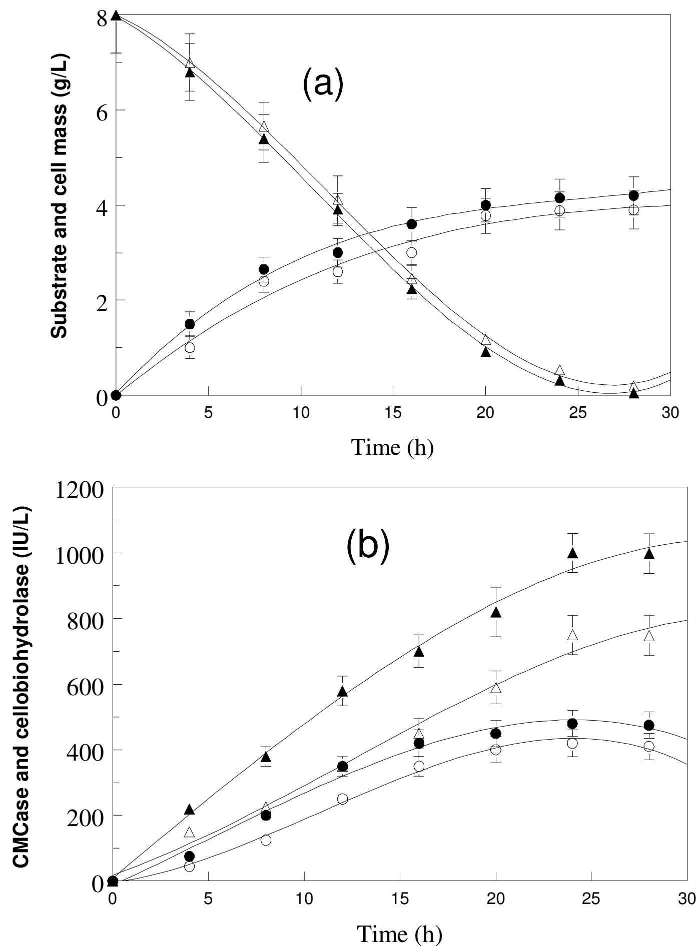

Figure 2. Time course of glucose (Δ,▲) and cell mass (○,●) (a) and (endo-glucanase (Δ,▲) and cellobiohydrolase (○,●) (b) present in culture broth of parental and DG-resistant mutant of *B. subtilis* following growth in optimized Dubos salt medium containing ammonium sulphate as nitrogen source (pH = 6.0) at 50 °C in shaking incubator as described in materials and methods. Data presented are mean values ± SD of *n* = 3 experiments.

consumption rate (Q_s) and cell mass formation (Q_x) were recorded in the case of mutant *B. subtilis* CRM197 and were significantly (p = 0.008 and 0.033 respectively), than those of parental organism. Whereas, the specific substrate consumption rate (q_s), that is, 0.39 g/g/h and $Y_{x/s}$ of 0.49 g/g of the mutant (Table 3) was non-significantly different from those of the parental organism. The substrate consumption kinetic parameters were studied previously by Rajoka (2005) but these values are significantly higher than those reported on wild organism of *Cellulomonas biazotea* and some mutant derivatives reported by him. However, double mutant reported by Rajoka (2005) exhibited similar values as those of *B. subtilis* CRM197 mutant. The variation in results was because of difference in species of microorganisms and production medium conditions. Comparison of results with

other authors is not possible because several authors have not reported these kinetic parameters on *B. subtilis*.

Table 3. Kinetic parameters for substrate consumption and endoglucanse and cellobiohydrolase formation by *B. subtilis* (Parent) and its mutant derivative in shake flasks using glucose and ammonium sulphate as carbon and nitrogen sources, respectively, in Dubos salt medium pH (6.0) at 50℃.

Parameter	Parent	Mutant	P
Substrate consumption parameters			
μ_m* (h^{-1})	0.22	0.23	0.57
Q_S* (g/L.h)	0.21	0.29	0.008
Q_X* (g/L.h)	0.60	0.74	0.030
q_S (g/g h)	0.38	0.39	0.70
$Y_{X/S}$ (g/g)	0.45	0.49	0.18
Endo-glucanse formation parameters			
Q_P* (g/L.h)	48.0	65.5	0.0137
q_P* (g/g h)	39.6	51.8	0.0223
$Y_{P/S}$ (g/g)	91.6	123.3	0.0093
$Y_{P/X}$ (g/g)	180.2	228.6	0.008
Exo-glucanse formation parameters			
Q_P* (g/L.h)	18.5	41.5	0.006
q_P* (g/g h)	29.0	39.1	0.0146
$Y_{P/S}$ (g/g)	69.0	92.3	0.0157
$Y_{P/X}$ (g/g)	132.0	170.0	0.0042

*All kinetic parameters were determined after Pirt (1975). The p values are two-tailed p values determined using GraphPad Instat 3.0 software. Each value is a mean of n=3 experiments. Standard deviation among replicates was very low and has not been presented.

Kinetics of CMCase and CBH production for product formation on optimized medium under shake flask fermentation

In the optimized medium (Dubos salts with ammonium sulphate as nitrogen source, 0.8% glucose as carbon source, initial pH 6.0, temperature 45℃) the maximum CMCase activity was 0.8 and 1.2 IU/mL by both parent and its DG-resistant mutant strains, respectively. Similarly, the maximum activity of cellobiohydrolase was 0.6 and 0.8 IU/mL (Figure 2) for both strains. Recently, Liang et al. (2009) isolated a thermophilic strain of *Brevibacillus* sp. strain JXL which also produced maximum activity on glucose, compared with that on cellobiose. These activities were also higher than those reported earlier on high cellulase activity producing strain of *B. subtilis* (Li et al., 2008). Devanathan et al. (2007) reported lactose as inducer for cellulose production by *A. niger*.

The comparison of production formation rate for both endoglucanase and exoglucanase indicated that endoglucanase (CMCase) and exoglucanase (CBH) formation rates (Q_p) (65.5 and 41.5 IU/L/h, respectively), were maximal for mutant *B. subtilis* (Table 3) and are higher than those reported by Rajoka (2005) by wild and its mutants which showed 23 and 25 IU/L/h, respectively, for exo-glucanase following growth on 1% sugars in kallar grass. Their CBH activity was repressible by the presence of glucose in Dubos salt medium. The specific rate of enzyme production for both enzymes (q_p) was also higher in case of mutant strain that is, 51.8 and 39.1 IU/g/h, respectively. The yield of CMCase and cellobiohydrolase ($Y_{p/s}$) was observed significantly (P = 0.0093 and 0.0157, respectively), higher by mutant strain of *B. subtilis*. The specific yield of enzyme production was maximal for cellobiohydrolase by mutant derivative of *B. subtilis*. Comparison of kinetic data of both endoglucanase and exo-glucanse formation were significantly (p = 0.0223 - 0.0006) higher than those of parental organism. The production kinetic parameters were also determined by other scientists, but their values were low and in some cases comparable with those reported here. The variation in results noted was observed due to different organisms and fermentation media used for specific enzyme production (Rajoka and Yasmeen, 2005).

Conclusion

γ-Ray-induced mutation brought about positive effect on the yield and productivity of both endoglucanase and exoglucanase. Under optimized condition, the mutant strain supported 1.34-fold higher yield of both enzymes and were significantly higher than those reported by other authors. Glucose emerged as the best carbon source in these studies. Glucose accumulating during cellulose

hydrolysis can be a cheaper source for cellulase production using currently developed mutant strain. In better

studied organisms, e.g., *Trichoderma reesei* and *A. niger* and some *B. subtilis* cultures (Howard et al., 2003; Kotchoni et al., 2003), cellulases are repressible but both parental and mutant strains hyperproduced cellulases from glucose. Glucose present in molasses in their country can be used as a cheap and easily available substrate for the production of these industrially important enzymes. They intend to use these enzymes for saccharification of cellulosic substrates for production of value-added products.

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Abbreviations: μ, Specific growth rate (h^{-1}); $\mathbf{Q_s}$, rate of substrate (S) consumption (g S/L/h); $\mathbf{Q_x}$, rate of cell mass formation (g cells/L/h); $\mathbf{q_s}$, specific rate of substrate consumption (g S/g cells/h); $\mathbf{Y_{x/s}}$, g (cells)/ g (S) consumed; $\mathbf{Q_p}$, rate of product formation (IU/L/h); $\mathbf{q_p}$, specific rate of enzyme production (IU/g cells/h); $\mathbf{Y_{p/s}}$, product yield (IU/g S utilized); $\mathbf{Y_{p/x}}$, specific yield of enzyme production (IU/g cells); **CBH,** cellobiohydrolase; **CMC,** carboxymethyl cellulose; **CMCase,** carboxymethyl cellulose.

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