Journal of Microbiology Research 2013, 3(6): 224-227
DOI: 10.5923/j.microbiology.20130306.05

Effect of Different Carbon and Nitrogen Sources on the Growth of Two Thermophilic Actinomycetes and Their Cellulases Production

Md Towhid Hossain*, Md Abul Kashem, Md Abul Manchur, Md Nural Anwar

Department of Microbiology, University of Chittagong, Chittagong-4331, Bangladesh

Abstract Thermophilic cellulases have emerged as promising alternatives in biotechnological applications. At present, most of the enzymes used in industrial processes have been isolated from mesophiles microorganisms due to the difficulties to grow thermopiles in high scale. The present investigation deals with optimization of the production medium with different carbon and nitrogen sources to observe their effect on the growth and production of extracellular proteins, reducing sugar, saccharification percentage, biomass yield and cellulases of the selected isolates at high temperature. In the present study, the isolate *Streptomyces longisporus* (A_1) showed maximum production (activities) of CMCase (265.75 U/ml) and Avicelase (86.61 U/ml) in the presence of lactose and fructose as suitable carbon sources respectively; on the other hand the isolate *Streptomyces bobili* (A_4) exhibited maximum production (activities) of FPase (177.16 U/ml) and β-glucosidase (209.65 U/ml) with galactose. KNO_3 was found suitable nitrogen source for CMCase production (activities) of the both isolates. These findings will be helpful in prospect for increase the production of industrial enzyme.

Keywords Thermophilic, Cellulase, Actinomycetes

1. Introduction

Cellulose is the most abundant organic compound found in nature. However, cellulosic residues from different sources have great potential as renewal energy, food, fertilizer, chemicals and other valuable products[1-3].

Cellulose is the major carbohydrate synthesized by plants. Plant residues in soil consist of a large amount of cellulose. Therefore, the degradation of cellulosic biomass by cellulolytic microorganisms such as fungi[4], actinomycetes [5], gliding bacteria and true bacteria[6] play a vital role in the biosphere.

Nowadays, closer attentions have been made to thermophilic microorganisms that are capable of producing extracellular hydrolases and these enzymes exhibit unusual heat stability, which could be put to practical advantages.

In view of the above, the present work undertaken to study the effect of different carbon and nitrogen sources for the maximum production of cellulases and β-glucosidase by two thermophilic actinomycetes. This investigation adapted the estimation of reducing sugar as the index of cellulose degradation.

* Corresponding author:
towhid.mbio@cu.ac.bd (Md Towhid Hossain)
Published online at http://journal.sapub.org/microbiology

2. Materials and Methods

Two cellulolytic actinomycetes were provisionally identified as *Streptomyces longisporus* (A_1) and *Streptomyces bobili* (A_4) according to Bergey's Manual[7]. They were screened for the production of extracellular protein, reducing sugar and enzyme activity. In present investigation Winstead's medium[8] was used as basal medium with changing carbon (at the rate of 1.2%) and nitrogen (at the rate of 0.2%) sources.

To test the cellulose and β-glucosidase activity in different carbon and nitrogen sources the selected isolates were inoculated in 50 ml Winstead's medium in 100 ml conical flasks. After 12-15 days of incubation the culture filtrate were collected (by centrifuged at 12000 rpm at 4°C for 10 min) and analyzed for proteins, reducing sugars and enzyme activity.

2.1. CMCase Activity

Two milliliter of culture filtrate was added to 2 ml of 1% carboxy methyl cellulose (CMC) prepared in 0.1M citrate, pH 4.6 and 1 ml of 0.1 M citrate buffer in a test tube and incubated at 45°C for 2h.

2.2. FPase Activity

Two milliliter of culture filtrate was added to 1ml of 0.1M citrate buffer at pH along with 50mg Whatman No.1 filter paper strip in a test tube and incubated at 45˚C for 2h.

2.3. Avicelase Activity

Two milliliter of culture filtrate was added to 1ml of 1 % avicel (microcrystalline cellulose) prepared in 0.1M citrate buffer pH 4.6 and 1ml of citrate buffer in a test tube and incubated at 45˚C for 2h.

2.4. B-glucosidase Activity

Two milliliter of culture filtrate was added to 2ml of 1% salicin prepared in 0.1M citrate buffer pH 4.6 and 1ml of citrate buffer in a test tube and incubated at 45˚C for 2h.

2.5. Reducing Sugar

The amount of reducing sugar was determined by Nelson's Modification of Somogyi Method[9] measuring absorbance at 500nm. Enzyme activity was expressed Uml {The amount of reducing sugars (μg) released/ml filtrate/hour}.

2.6. Protein Estimation

Protein was determined by Lowry method[10] measuring the absorbance at 600 nm and compared with standard curve prepared by Bovine serum albumin. Biomass was measured by dry weight method. After collection of the supernatant, the biomass residue was dried at 80°C and the yield was expressed as mg gm^{-1} of substrate.

2.7. Saccharification

Saccharification percentage was calculated by applying the following equation:

$$\text{Saccharification}\ \% = \frac{\text{mg of reducing sugar / ml}}{\text{mg of substrate / ml}} \times 100$$

2.8. Statistical Analysis

Possible treatment differences among the factors (such as carbon, nitrogen sources, enzymes activity), were investigated by Complete Randomized Design (CRD) and Duncan's Multiple Range Test (DMRT) by running MSTATC software.

3. Results and Discussion

In the present investigation, two thermophilic actinomycetes *Streptomyces longisporus* (A_1) and *Streptomyces bobili* (A_4) were screened using Winstead's medium to observe the effect of different carbon and nitrogen sources on the growth and production of enzyme profiles of the isolates. After incubation, the effect of different carbon and nitrogen sources were recorded (Table 1). The liquefaction of the medium gradually increases with the increase of incubation time and found to liquefy the medium completely at 12-14 days by both the isolates.

The effect of different carbon and nitrogen sources on the production of extracellular proteins, reducing sugar and saccharification percentage and biomass yield of the selected isolates (A_1 and A_4) were recorded (Table 2). The isolate(A_1) showed maximum biomass yield (713 mg/gm cellulose) and extracellular protein (2325 μg/ml) in the presence of glucose, although highest reducing sugar (892 μg/ml) and saccharification percent (7.43) were recorded with lactose. On the other hand, the isolate (A_4) exhibited maximum extracellular proteins (2227μg/ml), reducing sugars (962 μg/ml), saccharification percent (8.02) while fructose used as carbon source, but the maximum biomass yield (711mg/gm cellulose) was recorded with glucose. On the other hand, both of the isolate showed maximum production of extracellular proteins, reducing sugars, biomass and saccharification percentage while KNO_3 was used as nitrogen source. This result suggested that glucose as carbon source and KNO_3 as nitrogen source is suitable for maximum biomass yield.

Table 1. Effect of carbon and nitrogen sources on the liquefaction of medium (cellulase activity) by the selected isolates

Isolates	Incubation period (day)	Carbon sources				Nitrogen sources		
		Glucose	Fructose	Lactose	Galactose	NH_4Cl	KNO_3	$(NH_4)_2SO_4$
A_1	4	+	+	+	+	+	+	++
	6	+	+	+	+	+	++	++
	8	++	++	++	++	+	++	++
	10	++	+++	+++	+++	++	+++	+++
	12	+++	+++	+++	+++	+++	+++	+++
	14	++++	++++	++++	++++	++++	++++	++++
A_4	4	+	+	+	-	+	+	+
	6	+	++	++	+	+	+	+
	8	++	++	++	++	++	++	++
	10	+++	+++	+++	+++	+++	+++	+++
	12	+++	++++	+++	+++	+++	++++	+++
	14	++++	-	++++	++++	++++	-	++++

Incubation temperature 42 ±2˚C, Initial pH 7.5, ++++ High, +++ moderate, ++ Low, + Scanty liquefaction of medium

Table 2. Effect of carbon and nitrogen sources on the production of extracellular protein, reducing sugar, saccharification and biomass by the selected isolates

Isolates			Extracellular protein (μg/ml)	Reducing sugar (μg/ml)	Biomass yield (mg/gm cellulose)	Saccharification (%)
A_1	Carbon sources	Glucose	2325 A	694 E	713 E	5.78 J
		Fructose	1464 B	667 F	466 H	5.56 J
		Lactose	699 E	892 D	633 G	7.43 J
		Galactose	1173 C	881 D	383 I	7.34 J
	Nitrogen sources	NH_4Cl	600 B	161 E	50 G	1.34 H
		KNO_3	667 A	262 C	133 F	2.18 H
		$(NH_4)_2SO_4$	600 B	204 D	50 G	1.71 H
A_4	Carbon sources	Glucose	1395 B	746 G	711H	6.21 L
		Fructose	2227 A	962 C	369 K	8.02 L
		Lactose	840 E	781 F	516 I	6.51 L
		Galactose	849 DE	853 D	385 J	7.11 L
	Nitrogen sources	NH_4Cl	435 C	51 H	216 E	0.42 I
		KNO_3	534 A	129 G	250 D	1.08 I
		$(NH_4)_2SO_4$	480 B	127 G	133 F	1.06 I

Note: The same letter(s) in the row indicates no significant difference at $p<0.05$ (ANOVA and DMRT)

Table 3. Effect of carbon and nitrogen sources on the production of cellulose and β-glucosidase by the selected isolates

Isolates			Enzyme Activity (U/ml)			
			CMC-ase	Avicelase	FPase	β-glucosidase
A_1	Carbon sources	Glucose	13.78 N	5.91 P	88.58 E	49.21 F
		Fructose	11.81 O	118.11C	18.89 K	46.26 H
		Lactose	265.75 A	14.76 M	47.24 G	17.85 L
		Galactose	135.83 B	43.31 I	30.71 J	91.70 D
	Nitrogen sources	NH_4Cl	7.87 E	11.81 C	14.76 A	12.80 B
		KNO_3	11.81C	9.84 D	10.04 D	1.97 H
		$(NH_4)_2SO_4$	9.84 D	4.92 F	4.08 G	3.94 G
A_4	Carbon sources	Glucose	19.68 J	4.92 L	35.43 H	98.45 C
		Fructose	32.48 I	3.94 M	81.50 E	4.92 K
		Lactose	39.37 G	19.68 J	33.07 I	7.78 K
		Galactose	51.18 F	86.61 D	177.2 B	209.4 A
	Nitrogen sources	NH_4Cl	1.97 J	3.94 H	2.95 I	1.97 J
		KNO_3	33.46 A	6.89 E	1.77 J	14.76 C
		$(NH_4)_2SO_4$	13.78 D	15.75 B	5.91 F	4.92 G

Note: The same letter(s) in the row indicates no significant difference at $p<0.05$ (ANOVA and DMRT)

The crude enzymes of the culture filtrates were allowed to react with different substrate (1.2 % CMC/filter paper/salicin) during enzyme substrate reaction. The CMCase, Avicelase, FPase, and β-glucosidase activity of crude enzymes of the isolates were shown in Table 3. The CMCase (265.75 U/ml), Avicelase (118.11 U/ml), FPase (88.58U/ml), and β-glucosidase (91.54 U/ml) production (activities) by the isolate A_1 were induced in the presence of lactose, fructose, glucose and galactose as carbon source respectively. On contrary, KNO_3 was found suitable for production of CMCase (11.81U/ml) and NH_4Cl was better nitrogen source for the production of Avicelase (11.81U/ml), FPase (14.76U/ml), and β-glucosidase (12.80U/ml) by the isolate A_1.

In table 3, the isolate A_4 showed maximum production (activities) of CMCase (51.18U/ml), Avicelase (86.61U/ml), FPase (177.16U/ml) and β-glucosidase (209.65U/ml) while galactose was used as carbon source in the medium. The isolate A_4 showed maximum CMCase (33.46U/ml) and β-glucosidase (14.76U/ml) production (activities) in the presence of KNO_3, and maximum Avicelase (15.75U/ml), FPase (5.91U/ml) production (activities) while $(NH_4)_2SO_4$ was used as nitrogen source in the medium.

The induction or repression of microbial cellulolytic enzymes due to the presence of different nitrogen sources [11-14] and carbon sources[13-18] in the medium were reported.

Our results suggested that, galactose is suitable carbon source for the maximum production of CMCase, Avicelase, FPase, and β-glucosidase by the isolate A_4, on the other hand

KNO_3 is suitable nitrogen source for the maximum production of CMCase by both the isolate. Present results on different carbon and nitrogen sources are in concurrence with many of the above reports[11-18].

4. Conclusions

In this investigation, two thermophilic actinomycetes were screened, and both of the isolates were identified as suitable species for the production of thermostable cellulase. Different carbon and nitrogen sources of the production medium were also fixed for better production of thermostable enzymes. The screening of microorganism for the production of thermostable cellulase will open a new and simple route for synthetic processes and consequently the present work will be a very good baseline for the large scale production.

REFERENCES

[1] Mee-Young, M., Chahal, D.S. and Vlach D., 1978, Single cell protein from various chemically presents wood substrate using *chaetemium cellulolyticum*, Biotechnol. Bioeng., 20:107-118.

[2] Linko, M., 1977, An evaluation of enzymatic hydrolysis of cellulosic materials. Adv. In Biochem. Eng., 5:25-48.

[3] Bisaria, V. S. and Ghose T. K., 1981, Biodegradation of cellulosic materials: substrates, microorganisms, enzymes and products. Enzyme Microbiol. Technol. 3:90-104.

[4] Herr, D. G . Luck and Delleg H., 1978, Formation of cellulose and degradation of cellulose by several fungi. J Ferment. Technol. 26:273-278.

[5] Stutzenberger, F.J., 1971, Cellulase production by *Thermomonospora curvata* isolated from municipal solid waste compost. J. Appl. Microbiol. 22:147-152.

[6] Rajoka M. I. and Malik K. A., 1984, Cellulase, hemicellulase production oc *Cellulomonas flavigena* NIAB 441. Biotechnol. Letters 6:557-600.

[7] Buchanan, R.E. and Gibbons N.E., 1974, Bergey's Manual of Determinative Bacteriology, 8^{th} edn. The Willium and Wilkins Co. Baltimore.

[8] Winstead, N.N., 1963, Production of peptinolytic and cellulolytic enzymes by cucurbit anthacnose fungi. Phytopathology, 53:961-964.

[9] Nelson, N., 1944, A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 153: 375-380.

[10] Lowry, O.H., Rosebrough N.J., Farr A.L. and Randall R.J., 1951, Protein measurement with the *Folin phenol* reagent. J. Biol. Chem., 193: 265-275.

[11] Sasaki, H., Kamagata Y., Takao S., Matangkasombut P. and Bhumiratana A., 1983, Selection and classifiction of active cellulose decomposing fungi. Microb. Utilization Renewable Resour., 3: 65-76.

[12] Hossain, F.M.M., Rahman M.M., Chowdhury N. and Malek M.A., 1999, Production of extra-cellular carboxymehtyl cellulase and cellobiase by a thermophilic *Bacillus* sp. Bangladesh Journal of Microbiology, 16: 115-125.

[13] Huq, M., Rahman M.M., Khan A.R. and Malek M.A., 2002, Production of extra-cellular carboxymehtyl cellulase and Avicelase by a sawdust degrading *Streptomyces* sp. Bangladesh Journal of Microbiology, 19: 1-6.

[14] Alam, M.Z., 2004, Study of five cellulolytic microorganisms and their cellulases. M.Sc. Thesis, Chittagong University, Bangladesh.

[15] Mendels, M. and Reese E.T., 1960, Induction of cellulases in fungi by cellobiose. Journal of Bacteriology, 79: 816-826.

[16] Martin, G.M. and Eberhart B., 1966, Regulation of cellulase and cellobiase in *Neurospora crassa*. Biochem. Biophys. Res. Commun., 24: 782-785.

[17] Donald, J.G., Hoq M.M. and Steiner W., 1995, Induction of cellulose degrading enzymes in *Thermoascus aurantiacus*. Bangladesh Journal of Microbiology, 12: 23-29.

[18] Jaksevse, J.R., Majdance L. and Dordevia J., 1984, Cellulolytic enzyme biosynthesis. Microbiol. J. (Belgr.), 21: 151-164.