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Effect of Carbon and Nitrogen Sources on the Production of Reducing Sugars, Extra-cellular Protein and Cellulolytic Enzymes by Two Cellulolytic Bacterial Isolates

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Abstract: Two thermophilic cellulolytic bacterial isolates were tested to determine the effect of carbon and nitrogen sources on the production of extra-cellular proteins, reducing sugars and cellulolytic enzymes. Lactose was found to be the most potential carbon source for Avicelase (342.52 U mL^{-1}) and ß-glucosidase (256.89 U mL^{-1}) activity where as NH_4Cl was found to be the potential nitrogen source for CMCase (144.68 U mL^{-1}) activity.

Key words: Thermophilic bacteria, cellulases, ß-glucosidase, carbon and nitrogen effect

INTRODUCTION

Cellulolytic microorganisms play an important role in the biosphere by reducing cellulose[1] and they also convert cellulose into various economically important products like monomeric sugars[2], single cell proteins or microbial biomass proteins[3], compost[4] and antibiotics[5] etc. to everyday use for man.

The cellulases produced by thermophilic microorganisms exhibit unusual heat stability which could be put practical advantages[6,7]. Bacterial cellulase has received good attraction because of fast growth of organisms and rate of enzyme production[8]. Cellulose degradation mainly depends on nitrogen level, temperature, aeration, moisture, pH and presence of other carbohydrates and the proportion of lignin in the residue[9]. Carbon is needed for the synthesis of cell structure and energy for microorganisms where as nitrogen is key nutrient substance for microbial growth and hence organic matter breakdown.

The present study has been undertaken to determine the suitable carbon and nitrogen sources for the maximum production of bacterial cellulases and ß-glucosidase. This investigation adapted the estimation of reducing sugar as the index of cellulose degradation.

MATERIALS AND METHODS

Two thermophilic bacterial isolates and were provisionally identified as *Cellulomonas fimi* (B_2) and *Cellulomonas cellusea* (B_4) according to Bergey's Manual[10]. They were maintained in Nutrient Agar medium and tested for the production of extra-cellular proteins, reducing sugars and enzyme activity. For this purpose, Winstead's medium[11] 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 active cellulase and ß-glucosidase activity in different carbon and nitrogen sources the bacterial isolates were inoculated in 50 mL Winstead's medium in 100 mL conical flasks. After 3-5 days of incubation the culture filtrate (prepared by centrifugation at 12000 rpm, 10 min and 4°C) were analyzed for proteins, reducing sugars and enzyme activity.

CMCase activity: Two milliliter of culture filtrate was added to 2 mL of 1% CMC prepared in 0.1 M citrate buffer, pH 4.6 and 1 mL of 0.1 M citrate buffer in a test tube and incubated at 45°C for 2 h.

FPase activity: Two milliliter of culture filtrate was added to 1 mL of 0.1 M citrate buffer at pH along with 50 mg Whatman No. 1 filter paper strip (1×6 cm) in a test tube and incubated at 45°C for 2 h.

Avicelase activity: Two milliliter of culture filtrate was added to 2 mL of 1% Avicel (microcrystalline cellulose) prepared in 0.1 M citrate buffer pH 4.6 and 1 mL of 0.1M citrate buffer in a test tube and incubated at 45°C for 2h.

ß-Glucosidase activity: Two milliliter of culture filtrate was added to 2 mL of 1 % Salicin prepared in 0.1M citrate buffer pH 4.6 and 1 mL of 0.1M citrate buffer in a test tube and incubated at 45°C for 2 h.

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The amount of reducing sugar was determined by Nelson's modification of Somogyi method[12] measuring absorbance at 500 nm. Enzyme activity was expressed by U mL {the amount of reducing sugars (μg) released/mL filtrate/hour}[13].

Protein was determined by Lowry method[14] measuring the absorbance at 600 nm and compared with standard curve prepared by using Bovine Serum Albumin. Bacterial biomass was determined by optical density method, measuring the absorbance at 600 nm[15].

Saccharification percentage was calculated by applying the following equation:

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

RESULTS AND DISCUSSION

In the present study two thermophilic cellulolytic bacteria *Cellulomonas fimi* (B_2) and *Cellulomonas cellusea* (B_4) were tested to see the effect of four carbon sources (glucose, fructose, lactose and galactose) and three nitrogen sources [NH_4Cl, KNO_3 and $(NH_4)_2SO_4$] on the growth. These carbon and nitrogen sources were added to the basal medium (Winstead's medium) and the effect of different carbon and nitrogen sources were recorded (Table 1).

The effect of carbon and nitrogen sources on the production of reducing sugars, extra-cellular proteins, saccharification percentage and biomass yield of the isolates B_2 and B_4 were recorded (Table 2).

The maximum protein production and reducing sugars level of culture filtrate were recorded with fructose and lactose, respectively by the isolate B_2. The maximum biomass yield and saccharification percentage were recorded with glucose and lactose, respectively by B_2. The isolate B_2 showed maximum production of protein, reducing sugar level, biomass yield and saccharification while KNO_3 used as nitrogen source (Table 2).

On the other hand, the isolate B_4 produced maximum extra-cellular protein, reducing sugar level, biomass yield and saccharification percentage while fructose, lactose, fructose and lactose used as carbon sources, respectively. Besides, KNO_3 was found to induce

Table 1: Effect of carbon and nitrogen sources on the growth

		Carbon sources				Nitrogen sources		
Isolates	Incubation period (Day)	Glucose	Fructose	Lactose	Galactose	NH_4Cl	KNO_3	$(NH_4)_2SO_4$
B_2	3	++	++	+++	+	+	++	++
	4	+++	+++	+++	++	++	+++	++
	5	+++	+++	+++	+++	+++	+++	+++
B_4	3	++	++	+	+++	++	++	+++
	4	+++	+++	++	+++	+++	+++	+++
	5	+++	+++	+++	+++	+++	+++	+++

Incubation temperature 42±2°C, Initial pH 7.5, +++ = High liquefaction, ++ = Moderate liquefaction, + = Low liquefaction

Table 2: Effect of carbon and nitrogen sources on extra-cellular protein production, reducing sugar level, saccharification and biomass production by B_2 and B_4

Carbon and nitrogen sources	Extra-cellular Protein (μg mL^{-1})	Reducing sugar (μg mL^{-1})	Biomass yield (Absorption at 600 nm)	Saccharification (%)
B_2				
Glucose	1200.0	746.45	0.829	6.21
Fructose	1950.0	648.81	0.715	5.41
Lactose	1807.0	907.87	0.622	7.56
Galactose	864.0	802.36	0.595	6.68
NH_4Cl	450.0	251.97	0.429	2.10
KNO_3	750.0	481.89	0.529	4.02
$(NH_4)_2SO_4$	487.5	308.66	0.405	2.56
B_4				
Glucose	1020.0	699.21	0.592	5.82
Fructose	1491.0	631.49	0.618	6.26
Lactose	513.0	856.69	0.495	7.14
Galactose	663.0	798.42	0.488	6.65
NH_4Cl	442.5	49.61	0.462	0.41
KNO_3	453.0	171.65	0.412	1.42
$(NH_4)_2SO_4$	390.0	151.97	0.402	1.27

Table 3: Effect of carbon and nitrogen sources on the production of cellulases and β-Glucosidase by B_2 and B_4

	Enzyme activity (μg mL^{-1})			
Carbon and nitrogen sources	CMCase	Avicelase	FPase	ß-Glucosidase
B_2				
Glucose	93.53	18.70	2.95	85.63
Fructose	15.75	41.34	56.10	11.81
Lactose	7.87	165.35	36.61	12.79
Galactose	27.56	17.72	14.17	36.42
NH_4Cl	144.68	41.39	40.16	20.67
KNO_3	4.92	21.65	12.40	3.94
$(NH_4)_2SO_4$	20.67	122.04	64.96	15.75
B_4				
Glucose	13.78	13.78	60.24	126.97
Fructose	20.87	1.97	5.91	22.64
Lactose	23.62	342.52	104.53	256.89
Galactose	82.68	19.68	63.78	33.46
NH_4Cl	7.87	9.84	1.77	4.92
KNO_3	1.97	49.21	1.18	19.69
$(NH_4)_2SO_4$	35.43	25.59	10.63	4.92

maximum production of extra-cellular protein, reducing sugar and saccharification percentage. But the biomass yield was found to induce by NH_4Cl (Table 2).

The crude enzymes of the culture filtrates were allowed to react with 1.2% CMC/Filter Paper/Salicin substrate during enzyme-substrate reaction. The CMCase, FPase, Avicelase and ß-Glucosidase activity of crude enzymes of isolate B_2 and B_4 were shown in the Table 3.

Table 3 shows the induction of CMCase, Avicelase, FPase and ß-Glucosidase production (activities) by glucose, lactose, fructose and glucose, respectively. NH_4Cl was found better for the production of CMCase and ß-Glucosidase and $(NH_4)_2SO_4$ was found better nitrogen source for the production of Avicelase and FPase by the isolate B_2.

From the Table 3 it is evident that the isolate B_4 produced (activity) maximum CMCase in presence of galactose and maximum Avicelase, FPase and ß-Glucosidase in presence of lactose as carbon source in the medium. The isolate B_2 showed maximum CMCase and FPase production (activity) while $(NH_4)_2SO_4$ used as nitrogen source and maximum Avicelase and ß-Glucosidase production while KNO_3 used as nitrogen source.

The induction or repression of microbial cellulolytic enzyme production due to addition of different nitrogen sources in the medium was reported by many workers[16-19]. The present observations showed similarities with these reports. The enhance production or repression of microbial cellulases by carbon sources in the medium was also reported by many workers[17-26]. Present results on the induction or repression of cellulases production with carbon sources are in concurrence with many of the above reports.

The findings of present study suggested that the lactose is suitable carbon source for the maximum production of extra-cellular enzyme with a special reference to Avicellase, FPase and β-Glucosidase. Similar results were also reported by other workers[27].

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