EFFECT OF CARBON AND NITROGEN SOURCES ON CELLULASE PRODCUTION BY *STREPTOMYCES* STRAIN 21B

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INTRODUCTION

Cellulosic materials are the most abundant and renewable organic resources which are available to man (Kosaric et. al. 1980). Cellulosic wastes may be agricultural, forestry or urban in origin. If an economical process is developed, cellulose could be converted into cellobiose or glucose which could then form the substrate base for the production of single cell protein or other fermentation products. Enzymic hydrolysis is one way of achieving this conversion.

Though microbial sources of cellulase is varied and numerous, the fungal cellulase system has been widely studied. The *Trichoderma viridae* have been the initial microorganisms of choice due to their high cellulolytic activities toward crystallined cellulose (Mandels *et. all* 1974; Peiterson, 1975; Andren *et. al.* 1976a; Mandels & Sternberg, 1976; Gose Sabai, 1979).

It has also been reported that cellulases from *Thermomonospora fusca* Yx can hydrolyze crystalline cellulose at a faster rate than would any of the known cellulases from mesophilic fungi (Crawford, 1975).

Although a number of bacteria have been shown to be cellulolytic, there has however, been very few studies on the effect of carbon and nitrogen sources on the production of cellulase by bacteria.

The purpose of the present investigation was to study the influence of various carbon sources, nitrogen sources and Tween 80 (a surfactant) on the production of cellulase by a new isolate *Streptomyces* strain 21B. The activities of the new isolate were compared with those of *Thermomospora fusca* Yx, a well known cellulase producer.

MATERIALS AND METHODS

Cultures

Streptomyces strain 21B used was originally isolated from decaying plant materials by Njoku & Antai (1987). ***Thermomonospora fusca*** Yx was obtained from Professor David B. Wilson (Biochemistry, molecular and cell Biology section, Cornell University).

Substrates used.

Lignocellulose substrates used were prepared from sugar cane (***Saccharum officinarum)*** bagasse and elephant grass (***Panicum maximum)*** as described by Crawford (1978).

Lignocellulose Pretreatment.

To delignify the lignocellulose substrates the method of Toyama and Ogawa (1975) was employed. In brief, the extractive — free lignocellulose substraies were autoclaved in 1.0% NaOH solution for 1h at 121°C.. The autoclaved lignocellulose substrates were then washed with several c anges of distilled water until there was no trace of NaOH left as confirmed by absence of of pink colour development when a few drops of 1.0% phenophthalein solution was added to the rinse water. The washed lignocellulose substrates were then dried in a hot air oven at 70°C for 24h and stored until needed. This lignocellulose was then described as pretreated lignocellulosic substrate.

Media.

The basal medium used consisted of the following in grams/litre:— Na_2HPO_4. $7H_2O$, 4.0g; KH_2PO_4. 1.0g; NaCl, 0.2g; $MgSO_4$ $7H_20$, 0.02g; $CaCl_2$. $2H_20$,. 0.05g; Pridham and Cottlieb's truce elments (1ml/L). The following nitrogen sources were used: 0.7% peptone (Oxoid), 0.3% ammonium sulphate, 0.14% Urea, 0.36% sodium nitrate. The carbon sources used were: 1.0% glucose, 1.0% cellobiose, 1.0% lactose, 1.0% carboxymethyl cellulose (CMC) and 1.0% ground cotton wool.

Effect of different carbon sources on cellulose production

Five millilitres of active cells of *streptomyces* strain 21B was inoculated into 100ml of mineral salts broth contained in 250 ml flask which were supplemented differently with 1.0% glucose, 1.0% cellulose, 1.0% lactose, 1.0% carboxymethyl cellulose (CMC) and 1.0% ground cotton wool. The flasks were set up in duplicates. To one set of the flasks was added 1.0% of sterilized delignified sugar cane bagasse lignocellulose while to the other set was added 1.0g of sterilized delignified grass lignocellulose and incubated at 37°C in a rotary shaker Gallenkamp model BKS 350 at 100 rev/min for 14 days. At the end of the incubation period, 1.0 ml samples were removed and assayed for cellulose filter paper activity (FP activity) according to the method of Mandels *et al.* (1976).

A similar experiment was set up with ***Thermomonospora fusca*** Yx as active inoculum. The incubation temperature was increased to 55°C, while the incubation period was reduced to 12 days. The incubation period of 12 or 14 days was determined in a preliminary experiment as the period that the organisms produced the highest level of cellulose.

Effect of nitrogen sources on cellulose production.

To determine the effect of nitrogen sources on cellulose production, approximately equal amounts of nitrogen in various nitrogenous compounds were added differently into 100 ml of mineral salts medium contained in 250 ml conical flasks. Delignified sugar cane bagasse or grass lignocellulose was sterilized at 121°C for 2 h and added 1.0g differently to the conical flasks.

Concentrated active cells (5.0 ml) of either *Streptomyces* strain 21B or *T. fusca* Yx was added to each flask.

The flasks inoculated with *streptomyces* strain 21B were incubated at 37°C for 14 days while those inoculated with *T. fusca* Yx were incubated at 55°C for 12 days. At the end of incubation period, triplicate 1.0 ml sample was obtained from each flask and assayed for cellulose FP - activity according to the methods of Mandels. *et.al.* (1976).

Effect of Tween 80 on cellulose production

To determined the effect of Tween 80 on cellulose production, by the organisms, 0.1 ml to 0.7 ml of Tween 80 was added to 100 ml of mineral salts containing 1.0g of either delignified sugar cane bagasse of grass lignocellulose. The concentration of Tween 80 in the broth was therefore 0.1% to 0.7% respectively. Concentrated active cells (5.0 ml) of either *Streptomyces* strain 21B or *T. fusca* Yx was added to the flasks. At the end of 14 days incubation period for *Streptomyces* strain 21B and 12 days for *T. fsca* Yx the activities of the crude cellulose were determined using the filter paper method of Mandels *et.al.* (1976).

Results.

Effects of different carbon sources on cellulose production.

The effect of carbon sources on cellulose production by the two organisms is presented in Tables 1 and 2. No cellulose activity was detectable when glucose and lactose were incorporated as the additional carbon sources to the lignocelluloses.

When 1.0% CMC was added as the additional carbon source, cellulose activity of 1.2 FPU ml^{-1} was obtained for *T. fusca* Yx growing on sugar cane bagasse. On comparing this result with that of the control, it was observed that *T. fusca* Yx cellulose production capacity was enhanced by about 11%. Enhancement of cellulose production by *Streptomyces* strain 21B was observed when 1.0% ground cotton was added as additional carbon source (Table 1). It was also observed that there was repression of cellulose synthesis when the cultures were supplemented with glucose, lactose or cellulose regardless of whether sugar cane bagasse or elephant grass lignocellulose was the substrate (Tables 1 and 2).

There was a general increase in the pH of the culture filtrate, with the decrease in pH being most drastic in culture supplemented with either glucose, lactose or cellulose as additional carbon source (Tables 1 and 2). No cellulose activity was detected in these culture filtrates.

Effect of nitrogen sources on cellulose production

The effect of nitrogen sources on cellulose production is presented in Tables 3 and 4. *T. fusca* Yx produced cellulose with the highest cellulose activity of 1.4 F P U ml^{-1} as it was growing on sugar cane bagasse lignocellulose supplemented with 0.76 peptone as nitrogen source. *Streptomyces* strain 21B produced cellulose with 1.2 F P U ml^{-1} as it was growing on grass lignocellulose supplemented with 0.7% peptone as nitrogen source. From the results obtained 0.7% peptone was observed to enhance cellulose production by the two organisms regardless of the type of lignocellulose which served as the substrate. All the other nitrogen sources tended to supress cellulose production. Sodium nitrate was observed to be the worst nitrogen source for cellulose production by either *T. fusca* Yx or *Streptomyces* strain 21B (Table 3 and 4). Sugar cane bagasse lignocellulose was observed to be the best substrate for cellulase production by *T. fusca* Yx, while grass lignocellulose was observed to be the best substrate for cellulose production by *Streptomyces* strain 21B.

Effect of different concentration of Tween 80 on cellulose production

Tween 80 was observed to enhance cellulose production by the two organisms regardless of which lignocellulose type was the substrate for the growth of the organisms (Tables 5 and 6).

There were increasing levels of cellulase production as the concentration of Tween 80 was increased from 0.1% to 0.5% with 0.5% supporting the highest level of cellulase production (Tables 5 and 6). Concentration of Tween 80 above 0.5% tended to suppress cellulase production.

Discussion.

It has previously been established that a variety of parameter such as concentration and nature of the lignocellulose substrate, concentration of other nutrients and pH had a marked influence on cellulase production by a particular organism.

In this study five different carbon sources were used as cellulase inducers, the highest cellase activity was obtained by growing *T. fusca* Yx on sugar cane bagasse supplemented with 1.0% CMC, a pure cellulose and *Streptomyces*, strain 21B on grass lignocellulose supplemented with 1.0% ground cotton a complex cellulose. The three sugars (glucose, lactose and cellobiose) were observed to depress cellulase production by the two organisms.

The explanation for this is that the three sugars are easily metabolisable sugars which are readily broken down into organic acids resulting in the lowering of the pH. This decrease in pH was observed to cause a concomitant decrease in cellulase activity. Similar observation has previously been reported by Mandels and Sternberg (1976).

It has been reported that at pH values below pH 3.0 considerable cellulase activity was lost, but if pH was controlled at around 5.0 during the consumption of the sugar, the enzyme activities remained stable (Mandels et al., 1975). In the present study, it was observed that at pH 3.3 to 4.0, no enzyme activity was detected in the fermentation medium (Tables 1 and 3). Optimum cellulase activity was obtained when the pH of the lignocellulose culture filtrate was 6.0 thus further confirming the fact that a low pH of the culture can adversely affect cellulose sources.

The results obtained from the study on the effect of nitrogen sources on cellulase production here revealed that the organisms under study preferred organic nitrogen sources to inorganic sources for optimum cellulase production. Sternberg (1976) obtained similar results when he added peptone to lignocellulose culture media for production of cellulose from *T. reesei*.

The results however differ from that of Stewart and Parry (1981) who found that $(NH_4)_2 SO_4$ added as nitrogen source supported a much higher level of cellulase production by *Aspergillus famigatus* than peptone. Their results actually showed that peptone was one of the worst nitrogen source for cellulose production by this organism.

From the results obtained from this study and those reviewed in this paper, it appears that different organisms prefer different nitrogen sources for optimal cellulose production.

Results obtained have further revealed that increasing the concentration of surfactant in the culture broth resulted in increases in cellulase production by the two organisms.

Tangu *et. al.* (1981) had previously reported slight increases in cellulase production by some *Trichoderma reesei* strains when 0.2% Tween was added to the culture broth.

SUMMARY

Utilization of different carbon sources for production of cellulases by *T. fusca* yx and a newly isolated *Streptomyces* strain 21B is reported. During growth in liquid culture containing delignified lignocellulose supplemented with additional carbon source, the new isolate released cellulases into the medium: The amount released was comparable to that of a well known cellulase producer *T. fusca* Yx and depended on the nitrogen source, the type of additional carbon source and Tween 80 concentration added to the medium. Addition of glucose, lactose and cellobiose was observed to repress cellulase synthesis, while carboxymethl cellulose enhanced cellulase synthesis.

Peptone (0.7%) added as nitrogen source enhanced cellulase synthesis, while $NaNO_3$, $(NH_4)_2 SO_4$ and urea had a depressing effect on cellulase synthesis. Different concentration of Tween 80 (0.1% to 0.5%) was observed to enhance cellulase synthesis. Results obtained have shown that the new isolate is a good producer of cellulase.

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Table I

Cellulase production resulting from the growth by *Streptomyces* strain 21B and *T. fusca* Yx on sugar cane bagasse lignocellulose supplemented with different carbon sources.

Carbon source (1.0%)	*Streptomyces* strain 21B[a]		*T. fusca* Yx[b]	
	cellulase activity (FPU/ml)	pH[c]	cellulase activity (FPU/ml)	pH
Glucose	—	3.6	—	3.5
Cellobiose	0.4	5.0	0.5	5.0
Lactose	—	3.5	—	3.6
CMC	0.7	5.9	1.3	6.0
Cotton wool (ground)	0.9	5.9	0.9	6.0
Control[d]	0.8	5.8	1.1	5.9

Table 2.

Cellulose production resulting from the growth of *Streptomyces* strain 21B and *T. fusca* Yx on elephant grass lignocellulose supplemented with different carbon sources.

Carbon source (1.0%)	*Streptomyces* strain 21B[a]		*T. fusca* Yx[b]	
	cellulase activity (FPU/ml)	pH[c]	cellulase activity (FPU/ml)	pH
Glucose	—	3.3	—	3.5
Cellobiose	0.3	5.1	1.4	5.2
Lactose	—	4.0	—	3.5
CMC	0.8	5.9	1.0	6.0
Cotton wool (ground)	1.0	5.7	0.8	5.9
Control[d]	1.0	5.9	1.0	6.0

a. Streptomyces strain 21B was incubated in a shaker incubator set at 37°C.
b. *T. fusca* Yx was incubated in a shaker incubator set at 55°C.
c. The pH values of the crude cellulase (culture filtrate).
d. There was no additional carbon source to lignocellulose.

Table 3

Cellulase production resulting from the growth of *Streptomyces* strain 21B and *T. fusca* Yx on sugar cane begasse lignocellulose supplemented with different nitrogen sources.

Nitrogen source[a]	Cellulase activity (FPU ml^{-1}) *Streptomyces* strain 21B	*T. fusca* Yx
$(NH_4)_2SO_4$ (0.3%)	0.3	0.6
$NaNO_3$ (0.36%)	0.1	0.4
Urea (0.14%)	0.4	0.7
Peptone (0.7%)	0.5	1.4
Control[b]	0.8	1.1

a. The amounts of each nitrogen compound used was calculated based on 3g $(NH_4)_2SO_4$.
b. There was no nitrogen supplmentation.

Table 4

Cellulase production resulting from the growth of *Streptomyces* strain 21B and *T. fusca* Yx on elephant grass lignocellulose supplemented with different nitrogen sources.

Nitrogen source[a]	Cellulase activity (FPU ml^{-1}) *Streptomyces* strain 21B	*T. fusca* Yx
$(NH_4)_2SO_4$ (0.3%)	0.5	0.2
$NaNO_3$ (0.36%)	0.3	0.1
Urea (0.11%)	0.9	0.2
Peptone (0.7%)	1.2	0.3
Control[b]	1.0	0.9

a. The amounts of each nitrogen compound used was calculated based on 3g $(NH_4)_2SO_4$.
b. There was no nitrogen supplmentation.

Table 5.
Effect of different concentrations of Tween 80 on cellulase production by *Streptomyces* strain 21B and *T. fusca* Yx growing on elephant grass lignocellulose.

Tween 80 Concentration (%)	*Cellulase activity (FPU ml^{-1})* *Streptomyces* strain 21B	*T. fusca* Yx
Control[a]	1.0	0.9
0.1	1.04	0.98
0.2	1.06	1.05
0.3	1.10	1.08
0.4	1.23	1.11
0.5	1.27	1.13
0.6	1.18	1.03
0.7	0.95	0.85

a. No Tween 80 was added.

Table 6.
Effect of different concentrations of Tween 80 on cellulase production by *Streptomyces* strain 21B and *T. fusca* Yx growing on sugar cane begasse lignocellulose.

Tween 80 Concentration (%)	*Cellulase activity (FPU ml^{-1})* *Streptomyces* strain 21B	*T. fusca* Yx
Control[a]	0.80	1.10
0.1	0.90	1.16
0.2	0.99	1.20
0.3	1.04	1.25
0.4	1.09	1.20
0.5	1.11	1.31
0.6	1.03	1.22
0.7	0.91	1.10

a. No Tween 80 was added.