CONVERSION OF LIGNOCELLULOSESIC SUBSTRATES TO GLUCOSE WITH CELLULOSE OF A NEW ISOLATE STREPTOMYCES STRAIN 21B.

by

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Introduction

There has been considerable research effort on the enzymatic hydrolysis of cellulose materials, and the fungal cellulose systems have been widely studied (Kosaric *et al.* 1980· Bailey and Ratto, 1983).

In addition, a thermophilic actinomycete, *Thermonunospora fusca* YX has been found to optimally decompose lignocellulosic materials (Crawford *et. al.* 1973). Although, other actinomycetes especiallv the *Streptomyces* strains have been shown to degrade both the lignin and cellulose components of lignocellulose (Crawford, 1978; Antai and Crawford, 1981; Antai, 1985; Njoku and Antai, 1987), the ability of these bacteria to produce cellulose capable of hydrolvzing lignocellulosic materials with the resultant release of glucose and other reducing sugars has not been determined

In our investigation, we tested the abilitv of a new isolate, *Streptomyces* strain 21B to produce celluloses when growing on different lignocellulosic substrates. The saccharification and conversion of these lignocelluloses to reducing sugars by cellulases produced by these organisms was also studied and compared to that of two well known cellulase producers *T. fusca* YX and *S. setonii* 75 Vi2.

Materials and Methods

Microorganisms

The *Streptomyces* strains· *Streptomyces* strain 21B was isolated from soil by Njoku & Antai (1988). While *S. setonii* 75 Vi2 was originally isolated from soi sample by Sindon, D.L. (M.S. Thesis. Department of Bacteriology and Bioche·

mistry, University of Idaho, Moscow, U.S.A.). *Thermomonospora fusca* YX was obtained from Professor David B. Wilson (Biochemistry, Molecular and cell Biology section, Cornell University, Ithaca, New York, NY. 14853).

Sunstrates used.

The lignocellulosic substrates were (i) grass lignocellulose: Elephant grass *(Panicum maximum)* were cut a little above the ground from the field. They were dried in a Gallenkamp hot air oven at 70°C for 24h. These were then cut into smaller pieces; (ii) Sugar cane bagasse lignocellulose: Sugar cane *(Saccharum officinarum)* were purchased from the local market, cut into small pieces and dried in a Gallenkamp hot air even at 70°C for 24h. (iii) Sawdust lignocellulose: The sawdust were obtained from the carpenter's shed at the center for special project (CSP) of the Rivers State University of Science and Technology, Port Harcourt, Nigeria.
Wilmington Delaware (USA) was obtained from Berger paints (Nig.) Ltd., Port Harcourt, Nigeria.

Lignocellulose pretreatment.

The different lignocellulose substrates were ground in a Glen Creston model grinder to pass a 600mm sieve. The substrates were then extracted according to the method of Crawford (1978). **Briefly**, the substrates were extracted with hot water at 80°C until all water soluble components were removed (four changes of water were used) and then in sequence with Benzene: Ethanol (1 : 1), 95% ethanol and finally with hot water. The materials so obtained were regarded as extractive-free lignocelluloses. These were dried at 70°C for 24h in a hot air oven. to delignify the lignocellulose substrates, the methods of Toyama and Ogawa (1975) were employed.
Generally, the ectractive-free lignocellulose substrates were autoclaved in 1.0% NaCH solution for 1h at 121°C. The autoclaved lignocellulosic substrates were washed with several changes of distilled water until there was no trace of NaOH. This was confirmed when no pink color developed when few drops of 1% phenolphalein solution was added to the rinse water. The washed lignocellulosic substrates were then dried in a hot air oven at 70°C for 24h and used when needed. These were used as delignified (pretreated) lignocellulosic substrates.

Cellulase Production.

The *Streptomyces* strains: *Streptomyces* strain 21B and *S. setonii* 75Vi2 snowing good growth on tryptone yeast extract agar slants were inoculated into 125ml of the different mineral salts lignocellulose broth (Pridham and Gottlieb, 1948) contained in 250 ml flasks. This was done in triplicate for each organism and each lignocellulose subtrate.

The contents of the fllasks were mixed and incubated at 37°C in a Gallankamp shaker incubator which was operating at the speed of 100 rpm. One milliliter samples were taken periodically (every 24h) for 14 days and assayed for cellulose production

T. fusca Yx was treated in the same manner as described above but the incubation was at 55°C for 12 days.

The mineral salts - cellulose broth consisted of the following: Na_2HPO_4.

$7H_2O$ (4.0g/L); KH_2PO_4 (1.0g/L); NaCl (0.2g/L); $MgSO_4.7H_2O$ (0.02g/L); $CaCl_2$ $2H_2O$ (0.05g/L); Pridham and Gottied's trace elements (1ml/L). vitamin-free casamino acid (2.5g/L); ground delignified (elephant grass or sugar baggase or sawdust) lignocellulose substrates.

Cellulase asay.

The filter paper (FP) assay for cellulase activity was determined according to the method of Mandels *et al.* (1976). Briefly, a 1 x 6 strip of Whatman No. 1 filter paper (50mg) was added to a test tube containing 1.0ml of crude enzyme. The tube was incubated for 1h at 50°C. Dinitrosalicylic acid reagent (30ml) was added to stop the reaction. The tube was placed in a boiling water bath for 15 mins. A blank tube was included (without filter paper) to correct for any reducing sugar present in the enzyme preparation. The optical density of the solution was read in a WPA 5105 spectrophotometer at 550nm. The mg of glucose produced in this test (read off from prepared standard FP-activity curve) is the filter paper activity of the crude cellulase.

Lignocellulose hydrolysis and saccharification efficiency of the cellulase produced.

To determine the susceptibility of the lignocellulose to hydrolysis by the crude cellulose (enzyme), 5g of the pretreated lignocellulosic substrates were weighed out into 250 ml flasks. To each of these flasks, 10.0ml of phosphate buffer (pH 7.2) and 90ml of crude enzyme was added and allowed to hydrolse at 55°C water bath with stirring. A control contained 5g of lignocellulose, phosphate buffer and distilled water to make up the volume to 100 ml and no enzyme.

Samples (1.0 ml) were removed from each flask at different times (0.5, 1, 2, 4,6,8,12,18,24,36, and 48 h) and analyzed for the amount of total reducing sugars present using the DNS method of Summer and Graham (1921); the amount of glucose by the method of somogyi (1952); cellobiose by the method of Dwivedi and Gose (1979), while the amount of xylose was determined using the modified orcinol reaction method of Dwivedi and Gose (1979).

Result

The saccharification efficiency of the cellulases produced by the three organisms while growing on the pretreated lignocellulosic substrates are presented in Table 2 to 4.

Results presented are for 48 h hydrolysis because for this incubation period the highest level of saccharification and the release of the highest quantities of reducing sugars were observed.

The percent saccharification indicated are calculated from the total weight of the original lignocellulose. *Streptomyces* strain 21B cellulose (cellulase activity of 1.3 FPU ml^{-1}) was able to cause 18.4% and 17.2% saccharification of grass and sugar cane bagasse lignocellulose respectively (Table 2). *T. fusca* Yx cellulase (cellulase activity of 1.5 FPUml^{-1}) was able to cause 18.1% and 21.5% saccharification of grass and sugar cane bagasse lignocellulose respectively (Table 3). *S. setonii* 75Vi2 cellulase (cellulase activity of 0.85 FPU ml^{-1}) was able to cause 12.1% saccharification of either grass and sugar cane bagasse lignocellulose respectively (Table 4).

Sawdust lignocellulose showed the lowest susceptibility to saccharification by cellulase enzymes produced by the three different organisms (Table 2 to 4). The least saccharification efficiency of 3.4% was observed for *S. setonii* 75Vi2 cellulase on sawdust (Table 4).
Results obtained have also shown that it is possible to produce glucose from lignocellulosic materials, using cellulase enzymes produced from these organisms. *Streptomyces* strain 21B released 7.8 mg ml^{-1} and 6.5 mg ml^{-1} of glucose from 5g of grass and sugar cane bagasse lignocellulose respectively (Table 2), while *T. fusca* YX released 6.9 mg ml^{-1} and 8.2 mg ml^{-1} of glucose from 5g of grass and sugar cane bagasse lignocellulose respectively (Table 3).

Discussion.

It is a common aim of research in cellulose production to find a source of cellulase as well as a process that yield total hydrolysis of the cellulose within a reasonable time and economic cost.

The enzyme system of *Trichoderma viride* ITCC 1433, which is a wild type strain and other mutants of this strain have been found to meet these requirements to a great extent (Herr, 1980) So much effort has been *focoussed* on *Trichoderma* and probably to the neglect of other organisms that could be good cellulose producers (Mandels and Andreotti, 1978).

Attempts were made in this study to produce cellulase from two *Streptomyces* strains. One is a new isolate *Strentomyces* strain 21B isolated from soil by Njoku and Antai (1987) and the second is *S. setonii* 75 Vi2 organism which has been previously shown to produce cellulase (Crawford, 1978). A third organism *T fusca* Yx a well known cellulase producer was used for comparison of cellulase producing ability with the new isolate.

The new isolate *(Streptomyces* strain 21B) was found to produce cellulase with 1.3 FPU ml^{-1} when grown on elephant grass lignocellulose in a shake flask culture system. This enzyme activity altghough lower than the 1.5 - 2.0 FPU ml^{-1} cellulase activity reported for *T. viride* QM 9414 (ATCC 26921) the enhanced mutant derived from QM 9123 (Mandels and Sternberg, 1976) is fairly comparable and could possibly do better with genetic manipulation.

It was also observed that cellulase activity of 1.5 FPU ml^{-1} was obtained when *T. fusca* YX was grown on 0.75% sugar cane bagasse lignocellulose. Although *T. fusca* YX is a well known cellulose producer, the cellulase activity obtained in this study was higher than those previously reported by other investigators.
The only possible explanation for this enhanced cellulase production is the type of substrated used. It appears that sugar cane bagasse used. It appears that sugar cane bagasse lignocellulase is a preferred substrate for cellulase production by this organism. The new isolate was able to produce cellulase with 0.9 FPU ml^{-1} when grown on sugar cane bagasse lignocellulose, but produced cellulase with 1.30 FPU ml^{-1} on elephant grass lignocellulose. This clearly shows substrate preference, and tends to indicate that although sugar cane is a type of grass, the organism actually prefers one type of grass to the other as substrate for cellulase production.

Tables 2 to 4 show that the amount of reducing sugars produced and present saccharification of the lignocellulose substrate was dependent on the concentration of cellulase produced by the three organisms. The substrate most susceptible to saccharification was sugar bagasse with 21.5% saccharification. This was closely followed by grass lignocellulose (17.4%). The most resistant substrate (with less than 5% saccharification) was pretreated sawdust lignocellulose. This very low level of saccharification of sawdust lignocellulose by the different cellulase systems was not totally unexpected, because sawdust lignocellulose contains a higher lignin concentration than sugar cane or grass lignocellulose and so is more resistant to degradation. The sawdust used in this study was from coniferous wood which are composed mainly of tracheids, and the lignin is composed mainly of guaicyl lignin, which is more condensed than the guaicyl - p - syringyl lignin in grass (Antai, 1985).

The more condensed lignins are generally more resistant to degradation than the less condensed ones. (Antai & Crawford, 1983).

Although glucose and cellobiose were the primary sugars produced by the hydrolysis process, measureable quantities of xylose were also present in the hydrolysate.

A chi - square test (P = 0.05) of similarity showed that the extent of hydrolysis of sugar cane bagasse lignocellulose by *T. fusca* YX cellulase and grass lignocellulose by *Streptomyces* strain 21B were similar at P = 5% confidence level indicating a clear substrate preference by two organisms.

This study has clearly shown that our new isolate *Streptomyces* strain 21B produces cellulase which is capable of hydrolyzing lignocellulosic material with the resultant release of glucose and other reducing sugars.

This ability is comparable to what has been reported for the fungal cellulase systems (Kosaric *et al.*, 1980· Bailey and Ratto, 1983).

summary

The hydrolysis of lignocellulosic substrates by crude cellulase produced by a new isolate *Streptomyces* strain 21B was studied. The saccharification efficiency of the cellulase produced by this isolate compared favourably with that of two well known cellulase producers. *T. fusca* YX and *S setonii* 75Vi2. *Streptomyces* strain 21B cellulase (cellulase activity of 1.3 FPU ml^{-1}) was able to cause 18.4% and 17.2% saccharification of grass and sugar cane bagasse lignocellulose respectively, while *T. fusca* YX cellulase (cellulase activity of 1.5 FPU ml^{-1}) was able to cause 18.1% and 21.5% saccharification of grass and sugar cane bagasse lignocellulose respectively. *S. setonii* 75Vi2 cellulase (cellulase activity of 0.85 FPU ml^{-1}) was able to cause 12.1% saccharification of both grass and sugar cane bagasse lignocellulose.

When *Streptomyces* strain 21B cellulase was used to hydrolyse 5g of grass lignocellulose 7.8 mg of sugar was formed. And when *T. fusca* YX cellulose was used to hydrolyse 5g of sugar cane baggase lignocellulose 8.2mg sugar was formed. Glucose was always the principal product, while little quantities of cellobiose and xylose were also formed in each case.

Table 1

Filter paper activity (FPU ml^{-1}) of crude cellulase produced by three organisms as a result of their growth on lignocellulose substrates.[a)]

Lignocellulose Substrate	Filter paper activity (FPU ml^{-1})[b)]		
	S. viridosporus T7A	*S. setonii*	*T. fusca* YX
Grass	1.30	0.85	1.20
Sugar cane bagasse	0.90	0.61	1.50
Sawdust	0.20	0.18	0.85

a) Length of incubation period was 14 days for *Streptomyces* strain 21B and *S. setonii* 75Vi2, and 12 days for *T. fusca* YX, being the period for optimum cellulase production by each organism.

b) One Filter paper unit is the number of milligram (mg) of glucose produced by the enzyme after 1h incubation at 55°C.

Table 2

Degree of saccharification of the three lignocellulose substrates and the amount of sugars released after 48h hydrolysis with cellulase from *Streptomyces* strains 21B.

Enzyme activity used (FPU)ml^{-1}	Substrates a)	Saccharification at 48h hydrolysis (%) b)	Composition of sugars at 48h hydrolysis (mg/ml)[c]			
			Glu.	Zyl.	Cell.	Total
1.3	Grass	18.4	6.5	0.4	0.9	7.8
1.3	Sugar cane bagasse	17.2	4.3	0.7	1.5	6.5
1.3	Sawdust	4.9	1.1	0.2	0.4	1.7

a) Five grams of pretreated lignocellulose substrates were used during the hydrolysis.

b) The % saccharification (Degree of saccharification, D.S.) was calculated using the method of Vallender and Erikson (1985)

c) Glu. = Glucose Xyl. = Xylose Cell = Cellobiose.

Table. 3

Degree of saccharification of the three lignocellulose substrates and the amount of sugars released after 48h hydrolysis with cellulase from *T. fusca* YX.

Enzyme activity used (FPU ml^{-1})	Substrates [a]	Saccharification at 48h hydrolysis (%) [b]	Composition of sugars at 48h hydrolysis (mg/ml)[c]			
			Glu.	Xyl.	Cell.	Total
1.5	Grass	18.1	4.8	0.9	1.2	6.9
1.5	Sugar cane bagasse	21.5	5.4	1.2	1.6	8.2
1.5	Sawdust	5.5	1.5	0.4	0.4	2.3

a) Five grams of pretreated lignocellulose substrates were used during the hydrolysis.

b) The % saccharification (Degree of saccharification, D.S.) was calculated using the method of Vallender and Erikson, (1985).

c) Glu. = Glucose Xyl. = Xylose Cell = Cellobiose

Table 4.

Degree of Saccharification of the three lignocellulose substrates and the amount of sugars released after 48h hydrolysis with cellulase from *S. setonii* 75Vi2.

Enzyme activity used FPU ml^{-1})	Substrates[a]	Saccharification at 48h hydrolysis (%)b)	Composition of Sugars at 48h hydrolysis (mg ml^{-1})[C]			
			Glu.	Xyl.	Cell.	Total
0.85	Grass	12.1	2.6	0.7	0.9	4.2
0.85	Sugar cane bagasse	12.1	2.6	0.7	1.3	4.6
0.85	Sawdust	3.4	0.7	0.2	0.3	1.2

a) Five grams of the lignocellulose substrates were used during the hydrolysis.

b) The % saccharification (Degree of Saccharification, D.S.) was calculated using the method of Vallender and Erikson (1985).

c) Glu. = Glucose; Xyl. = Xylose; Cell = Cellobiose.

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