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Effect of Cellulase Production by Fungi Cultured on Banana Waste

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ABSTRACT

Cellulase production from cellulosic banana waste using Trichoderma harzianum, Aspergillus niger and Fusarium oxysporum was assessed. The wastes were dried, pre-treated with alkali and steam, re-dried and then blended. The powdered wastes were then used as substrates in separate shake-flasks which contained mineral salts medium (MSM) and inoculums of Trichoderma harzianum, Aspergillus niger and Fusarium oxysporum. Fermentations were carried out in flasks containing the MSM, the waste substrate and the inoculum at pH 5.0, 1% substrate concentration and cultured on a rotary shaker at 29±1ºC initially for 5 days to verify cellulase production by the organisms from the waste substrates, then for 7 days or 9 days while varying different fermentation parameters. Cellulase activity and amount of glucose produced by the three test organisms from the waste substrates were determined and compared. The amount of glucose produced was optimized by varying the fermentation parameters: Time, pH, Substrate concentration and Temperature. The results obtained from the fermentations showed that Trichoderma harzianum, produced the highest amount of glucose among the cultures tested. This was produced from banana pulp at pH 4 and temperature of 45ºC on Day 7 of fermentation. The highest amount of glucose produced by Aspergillus niger was also from banana pulp at pH 3 and temperature of 40ºC on Day 5 of fermentation. The highest amount of glucose produced by Fusarium oxysporum was from banana pulp (0.54mg/0.5ml) at pH 4 and temperature of 45ºC on Day 5 of fermentation.

Key words: *Banana peel, Banana pulp, Cellulase and waste*

INTRODUCTION

Cellulose is the principal constituent of the cell wall of most terrestrial plants. The source of cellulose is in plants and it is found as micro-fibrils (2-20 nm in diameter and 100 – 40,000 nm long). These form the structurally strong frame work in the cell walls. Currently, there are two major ways of converting cellulose to glucose: chemical versus enzymatic. Enzymatic hydrolysis of cellulose is an important reaction in nature for it marks the first step in the decay of cellulose, the most abundantly occurring organic material. In the early 1970s, the oil crisis generated interest in using cellulose as a chemical and energy resource.

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One promising approach was to hydrolyze the cellulose to glucose with fungal enzymes and then to ferment the glucose to ethanol which could be used as a liquid fuel[1]. Cellulose is a fibrous, insoluble and crystalline polysaccharide consisting of D-glucose residues linked by β-1, 4- glucosidic bonds. Cellulose is the most abundant biopolymer in nature and can be degraded to glucose through the synergistically hydrolysis of three classes of cellulase, including endo-β-1, 4-glucanase (EC3.2.1.4), exoglucanase or cellobiohydrolase (EC3.2.1.91) and β- glucosidase (EC3.2.1.21)[2]. Glucose from the hydrolysis of cellulose can be easily fermented into useful products such as ethanol, lactic acid, single cell protein and other value added products[3]. Therefore, cellulases are industrially important enzymes having application in diverse industries such textile, paper and pulp and food industry. The previous negative attitude in which wastes were viewed self consciously as valueless and even offensive and for disposal only has been replaced in large part by a positive view in which wastes are recognized as raw materials of potential value[4]. Cellulases are relatively costly enzymes and a significant reduction in cost will be important for their commercial use. Production of cellulases using cheaper substrates is an effective strategy to reduce cost.

In recent years, much work has been carried out towards efficient utilization of agro-industrial residues such as wheat bran, sugarcane bagasse, coconut coir pith and others[5, 6, 7, 8]. Banana peel is an abundant and low cost agricultural waste residue. It is easily available in large quantities. It accounts for about 30% of the weight of the raw fruit and is rich in carbohydrates, protein and various vitamins and mineral elements[9]. However, banana peel does not find any significant commercial application till now and is generally disposed of in open areas, leading to potentially serious environmental problems. It is necessary to explore its industrial reutilization. This study was carried out to explore the feasibility of using banana peel as solid substrate for the production of cellulase. Though banana stalk was tested for cellulase production by *Bacillus subtilis*[8], there is still no evidence on the application of banana peel in cellulase production by *Trichoderma harzianum*. To the best of our knowledge, this is the first report on cellulase production by *Trichoderma harzianum* using banana peel and banana pulp as the substrate.

MATERIALS AND METHODS

Microorganisms and Waste substrates

The organisms used for this study were isolated from three sources: *Aspergillus niger, Trichoderma harzianum* and *Fusarium oxysporum* was isolated from agricultural waste dumping area of in an around Thanjavur District, Tamil Nadu. The microorganisms were identified in the PG and Research Department of Microbiology laboratory, Sengamala Thayaar Educational Trust Women's College, Sundarakkottai, Mannargudi, Tamil Nadu.

The waste substrates used were in this study were Banana peel (bpe) and Banana pulp (**bpu**). Banana fruit was washed and peeled. The banana peel was drained to remove excess water. The banana pulp was prepared from the after maceration of banana fruit. The excess of water was removed from the pulp. The pulp was then washed in water to remove excess juice. Both the peel and pulp were dried in the oven at 70ºC for 3 days. After drying, the banana wastes were pounded into small pieces using a mortar and pestle. A modified method of Ali *et al.*[10] which involved alkali and steam treatments was used in the pretreatment of the waste substrates.

The pounded pieces of the samples were autoclaved for one hour at 121ºC with 5% (w/v) NaOH (20 ml per gram of substrate) in separate conical flasks for delignification. The autoclaved bpe and bpu waste substrates were filtered through muslin cloth. They were then washed thoroughly with water and neutralized with 1M HCl. The wastes were finally washed with distilled water and dried at 70ºC. After drying, the treated wastes were ground in a blender for 10 minutes each.

Enzyme assays

The three selected test fungi were cultivated using the submerged culture technique and later maintained on PDA slants. A 10ml three to four day old spore suspension was made from PDA slants of each culture using sterile distilled water. The fermentation media used was Mary

Mandels' mineral salts solution and it was used along with different carbon and nitrogen sources. The medium (M1) contained the following (per L) Cellulose,10g ; Peptone,1g ; (NH4)2SO4, 1.4g ; KH2PO4, 2g ; CaCl2, 0.3g ; MgSO4.7H20, 0.3g ; Urea, 0.3g ; *trace metal solution (2.5g FeSO4; 0.98g MnSO4.H2O ; 1.76g ZnSO4.H2O ; 1.83g CoCl2.6H2O dissolved in 495ml of distilled water and 5 ml of conc. HCl), 1ml ; pH 4.8[11]. Trace metal stock solution (1ml is used per L).

Mineral salts glucose medium was prepared and approximately 2.8×106 spores/cells of each culture were inoculated into 500 ml flasks containing 100 ml of medium each. The spores/ cells were counted using a Neubauer counting chamber. The flasks were incubated for 24 hours at 29±1ºC on a Gallenkamp (England) rotary shaker at 250rpm to develop the inoculum.

The waste substrates provided the carbon sources in the fermentation media. These were combined with M1 to give Mineral salts glucose medium (MSGM), Mineral salts banana peel medium (MSbpeM) and Mineral salts banana pulp medium (MSbpuM).

All the media mentioned above were prepared separately and dispensed in conical flasks. They were sterilized in the autoclave at 121ºC for 15 minutes.

The pH of the fermenting media containing the waste substrates at a level of 10g/L was adjusted to 5.0. The suspension of germinated spores was inoculated at a level of 10% (v/v) into the production medium in the flasks. These were incubated at 29±1ºC on a shaker at 100rpm. Glucose production in the medium was measured on Day 5 of fermentation[12, 13] .

Cellulase activity was determined colorimetrically by measuring the increase in reducing groups by the hydrolysis of a carboxymethylcellulose (CMC) substrate[11]. The procedure followed the 0.5ml assay described by Jeffries[11].

Samples were withdrawn from the culture at 2-day intervals over a period of 7-9 days and the supernatant that resulted following centrifugation at 3000 rpm for 15 minutes to remove solids, were assayed for total reducing sugars using DNS method of Miller[14]. Enzyme solutions were diluted in 0.05M citrate buffer, pH 4.8. The enzyme diluted in buffer and one percent CMC (0.5ml each) was mixed well and incubated for 30 minutes at 50ºC. Three milliliters of the DNS was added and the tubes were placed in boiling water bath for five minutes. The tubes were cooled and the reducing sugar, glucose was determined[11]. The sample, enzyme blank, glucose standard and control were boiled together and absorbance was read at 540nm using a spectrophotometer. A control (substrate and buffer) was used to set the spectrophotometer at zero absorbance. During the course of the experiments, the absorbance of the sample tube, corrected by subtraction of the enzyme blank was translated into glucose during the reaction using a glucose standard. The linear glucose standard was used to translate the absorbance values of the sample tubes into glucose i.e. mg glucose produced during the reaction. For a 30-minute assay, 1mg of glucose equals 0.185 unit

Determination of Optimal Conditions for Enzyme Production

Effect of varying time: Cellulase activity was measured at regular intervals while fermentation was observed at 29±1ºC for a period of 9 days and the period of maximum enzyme production was determined.

Effect of varying pH: The pH of the fermentation media were adjusted to various values ranging from 2.0-6.0 with 0.1N NaOH or 0.1N HCl. The pH was determined using the pH meter

Effect of varying substrate concentration: Different concentration of the waste substrates ranging from 1.0% to 5.0% were used in the fermentation media.

Effect of varying Temperature: The fermentation was carried out at different temperatures ranging from 30ºC to 45ºC.

Optimization experiments were carried out and each of the organisms were grown on each of the substrates and hydrolyzed using parameters that produced maximal activity of the enzyme from all the earlier experiments. In accord with the International Union of Biochemistry, one enzyme unit equals 1micromole (μ) of substrate hydrolyzed per minute[15].

RESULTS AND DISCUSSION

Table 1: Fermentation of Banana wastes substrates by Test Fungi

Waste Substrate	Glucose Produced mg/0.5 ml)		
	Trichoderma harzianum	***Aspergillus niger***	***Fusarium oxysporum***
Banana Peel	0.49	0.41	0.15
Banana Pulp	0.40	0.45	0.42

Fermentation conditions: Substrate concentration 1%, pH: 10%, temp: 29±1ºC, time: 5 days

Table 2: Effect of Substrate Concentration on the Fermentation of Banana waste by test fungi

Substrate Concentration	Glucose Produced (mg/0.5 ml)					
	Trichoderma harzianum		*Aspergillus niger*		*Fusarium oxysporum*	
	bpe	bpu	bpe	bpu	bpe	bpu
1 %	0.27	0.29	0.20	0.24	0.17	0.21
2 %	0.29	0.31	0.27	0.26	0.25	0.258
3 %	0.31	0.35	0.24	0.27	0.30	0.293
4 %	0.29	0.35	03.24	0.26	0.24	0.324
5 %	6.17	0.32	0.25	0.27	0.29	0.294

Fermentation conditions: pH: 5.0: temp: 30ºC, time: 5 days.

Table 3: Effect of pH on the Fermentation of Banana waste by test fungi

Waste Substrate	pH 2.00	pH 3.00	pH 4.00	pH 5.00	pH 6.00	pH 7.00
Trichoderma harzianum						
Banana Peel	0.210	0.178	0.199	0.243	0.312	0.296
Banana Pulp	0.150	0.191	0.252	0.338	0.356	0.235
Aspergillus niger						
Banana Peel	0.156	0.152	0.219	0.240	0.208	0.246
Banana Pulp	0.182	0.172	0.236	0.314	0.289	0.292
Fusarium oxysporum						
Banana Peel	0.142	0.145	0.152	0.199	0.152	0.142
Banana Pulp	0.142	0.172	0.214	0.210	0.235	0.187

(Fermentation conditions: Substrate concentration: 1 %, Temp: 29±1ºC)
Values are expressed as mean

Table 4: Effect of Substrate Concentration on the Fermentation of Banana waste by test fungi

Waste Substrate	1%	2%	3%	4%	5%
Trichoderma harzianum					
Banana Peel	0.272	0.278	0.334	0.314	5.754
Banana Pulp	0.296	0.312	0.362	0.365	0.332
Aspergillus niger					
Banana Peel	0.209	0.278	0.249	0.252	0.269
Banana Pulp	0.242	0.292	0.279	0.265	0.272
Fusarium oxysporum					
Banana Peel	0.178	0.259	0.318	0.285	0.312
Banana Pulp	0.219	0.252	0.285	0.325	0.296

(Fermentation conditions: Temp: 29±1ºC.)
Values are expressed as mean

Table 5: Effect of Temperature on the Fermentation of Banana waste by test fungi

Waste Substrate	35°C	40°C	45°C	50°C
Trichoderma harzianum				
Banana Peel	0.279	0.243	0.299	0.358
Banana Pulp	0.285	0.249	0.312	0.362
Aspergillus niger				
Banana Peel	0.209	0.199	0.287	0.315
Banana Pulp	0.236	0.205	0.317	0.327
Fusarium oxysporum				
Banana Peel	0.179	0.190	0.257	0.265
Banana Pulp	0.204	0.213	0.258	0.295

Values are presented as mean

Table 6: Fermentation of Banana wastes using optimized fermentation parameters using the test fungi

Waste Substrate	*Trichoderma harzianum*	*Aspergillus niger*	*Fusarium oxysporum*
Banana Peel	0.449	0.365	0.287
Banana Pulp	0.556	0.372	0.295

Values are presented as mean

The three test organisms are capable of producing cellulase as evidenced by the production of glucose (Table 1). Optimal glucose production from *T. harzianum* was observed on Day 7 of fermentation, Day 5 for *A. niger* and Day 3 for banana peel and Day 5 for banana pulp for *F.oxysporum.* Banana pulp released higher amount of glucose from the hydrolysis than banana peel (Table 2). Hydrolysis rates decline with time due to depletion of the more amorphous substrates, product inhibition and enzyme inactivation[15]. Caritas and Humphrey[16] and Narasimha[17] also gave similar time course reports of maximum glucose yield on 5th day of fermentation using *A. niger.* Effect of pH on glucose production from the two waste substrates by the three microorganisms was shown on Table 3. This supports the findings of Lee *et al.,*[18] who reported that CMCase, Avicelase and FPase activities exhibit a pH optimum of approximately 4, while the pH optimum of β-glucosidase was between pH 5 and 6.

The effect of substrate concentration was shown on Table 4. Further increase in cellulose concentration beyond the level that gave the optimum glucose did not result in proportionate increase in glucose yield Haapela *et al.,*[19] and Jeffries[11] reported that maximum endoglucanase activity was recovered on the medium with cellulose at 10 g/l. Mandels and Reese[20] also reported that maximal yields of cellulase were obtained on one percent substrate (cellulose, lactose, cellobiose and glucose) using *T. viride* and *Myrothecium verrucia.* These reports support the findings of this study as substrate concentration of 10 g/L gave the highest amount of glucose from *T. harzianum* on banana pulp.

Since the substrates contain different minerals apart from carbon which may serve as nutrient supplements, increase in substrate concentration leads to increase in these nutrients this may adversely affect the cell concentration. The increase in glucose production until the optimum that was obtained was due to the availability of cellulose in the medium; while a decrease in production beyond optimum concentration is explained to be as a result of an inhibitory effect of accumulated cellobiose and cellodextrins of low degree of polymerization to the growth medium. It might also be due to the specific binding of the enzymes with the substrates[21]. Low glucose production after optimum very probably highlights sugar depletion from the substrates into the medium[22].

Effect of Temperature was shown on Table 5. The optimum temperature for the synthesis of enzymes for saccharification of agro waste in all cases to enzymatic hydrolysis can be attributed to lignin content of the material. Pretreatment of lignocellulosic material enhances enzyme activity and maximum

saccharification was achieved within the range 30-45^{o}C coinciding with the characteristics of mesophiles[23].

Optimum glucose from the waste substrates using *T. harzianum,*was produced at 45ºC on Day7 but at pH 5.0 and 3% substrate concentration for banana peel and pH 4 and 1% substrate concentration for banana pulp.

Optimum glucose from the waste substrates using *A. niger* was produced at pH 3, 2% substrate concentration on Day 5 but at 45ºC for banana peel and 40ºC for banana pulp.

Optimum glucose from the waste substrates using *F.oxysporum* was produced at pH 3, 3% substrate concentration, 45ºC on Day3 for banana peel and pH 4, 4% substrate concentration and 45ºC on Day 5 for banana pulp. The exo-β-1, 3-glucanases produced by *Fusarium oxysporum* yield glucose as the end product, whereas endo-β-1, 3-glucanase releases a mixture of oligosaccharides with glucose as the minor product. Because β-1, 3-glucan is the main structural polysaccharide responsible for the strength and rigidity of the yeast cell wall, β-1, 3-glucanases have been suggested to play a role in important morphogenetic processes involving the controlled autolysis of β-1, 3 glucan. During vegetative growth, several endo- and exo-1, 3- β- glucanases are synthesized, some of which are secreted only to remain entrapped in the cell wall whereas others are released to the surrounding medium[18].

In conclusion, this study revealed that banana peel and pulp, which are examples of domestic and industrial agro-wastes, produce large amounts of cellulase enzymes when hydrolyzed by cellulolytic microorganisms and instead of being left behind for natural degradation can be utilized effectively under these conditions, to produce cellulase.

REFERENCES

1. Mandels, M., Hontz, L., Nystrom, J., Enzymatic Hydrolysis of Waste Cellulose. *Biotechnol. Bioeng.* **16:**1471-1493 (1974).
2. Sehnem, NT., Bittencourt, LR., Camassola, M., Dillon, A.J.P., Cellulase production by *Penicillium echinulatum* on lactose. *Appl. Microbiol. Biotechnol.* **72:** 163-167 (2006).
3. Chandra, M., Kalra, A., Sangwan, NS., Gaurav, SS., Darokar, M.P., Sangwan, R.S., Development of a mutant of *Trichoderma citrinoviride* for enhanced production of cellulases. *Bioresour. Technol.* **100:** 1659-1662 (2009).
4. Pranner, J., Environmental Microbiology and Waste Utilization, G/AMV proceedings (ed. Emejuaiwe), Academic press, London, 67-69 (1979).
5. Hao, X.C., Yu, X.B., Yan, Z.L., Optimization of the medium for the production of cellulase by the Mutant *Trichoderma reesei* WX- 112 using response surface methodology. *Food Technol. Biotechnol.* **44:** 89-94 (2006).
6. Muniswaran, P.K.A., Charyulu, N.C.L.N., Solid substrate fermentation of coconut coir pith for cellulase production. *Enzyme Microb. Technol.* **116:** 436-440 (1994).
7. Gutierrez-Correa, M., Tengerdy, R.P., Production of cellulase on sugar cane bagasse by fungal mixed culture solid substrate fermentation. *Biotechnol. Lett.* **19:** 665-667 (1997).
8. Krishna, C., Production of bacterial cellulases by solid state bioprocessing of banana wastes. *Bioresour. Technol.* **69:** 231-239 (1999).
9. Li, R.M., Chen, R., Xiao, Z.C., The analysis of the nutritional components of banana peel. *J. Zhanjiang Norm Coll*. **22:**42-45 (2001).
10. Ali, S., Sayed, A., Sarker R.T., Alam, R Factors affecting Cellulase Production by *Aspergillus terreus*. *World J. Microbiol and Biotechnol* **7:**62-66 (1991).
11. Jeffries, T.W., Production and Applications of Cellulase Laboratory Procedures, 1-10 (1996).
12. Srivastava, S.K., Gopalkrishnan, K.S., Ramachandran, K.B., The Production of β-Glucosidase in Shake-flasks by *Aspergillus wentii. J. Ferment. Technol.* **65(1):** 95 -99 (1987).
13. Bukoye, M.A., Use of Cellulosic wastes for Cellulase Production by *Aspergillus niger-CS9 and Penicillium digitatum-YF*50. M.Sc dissertation. Department of Microbiology, University of Ilorin. (2001).
14. Miller, G.L., Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugars. *Anal. Chem.* **31 :**426-428 (1959).

15. Ghose, T.K., Measurement of Cellulase Activities. *Pure and Appl Chem.* **59(2):**257-268 (1987).
16. Caritas, U.O., Humphrey, C.N., Effect of Acid Hydrolysis of *Garcinia kola* (bitter Kola) pulp waste on the production of CM-cellulase and β-glucosidase using *Aspergillus niger*. *African Journal of Biotechnology*, **5(10):**819-822 (2006).
17. Narasimha, G., Sridevi, A., Buddolla Viswanath, Subhosh Chandra, M., Rajasekhar Reddy, B., Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger*. *African Journal of Biotechnology*, **5(5):**472-476 (2006).
18. Lee, R., Lynd, Paul, J., Weimer, Willem H., van Zyl, Isak S., Pretorius Microbial Cellulose Utilization: fundamentals and Biotechnology. *Microbial mol. Biol Rev.* **66(3):** 506-577 (2002).
19. Haapela, R., Parkkinen, E., Susminen, P., Unko, S., Production of extracellular enzymes by Immobilized *Trichoderma reesei* in shake flask cultures. *Appl. Microbiol. Biotechnol*. **43:** 815-821 (1995).
20. Mandels, N., Reese E.T., Induction of Cellulase in Fungi by Cellobiose. Pioneering Research Division, Quartermaster Research and Engineering Center, Natick, Massachusetts. **79:** 816-826 (1959).
21. Gilkes, N.R., Langsford, M.T., Wakarchuk, W., Kilburn, D.G., Miller, R.C., Warren, R.A.J., The Cellulase system of *Cellulomonas flmi*. *Journal of General Microbiology*. **130:**1367-1376 (1984).
22. Brien, D.J.O., Craig, J.C. (Jr) Ethanol production in a continuous fermentation/membrane pervaporation system. *Applied Microbiology and Biotechnology*, **44(6):** 700 (1996).
23. Baig, M.M.V., Baig, M.L.B., Baig, M.I.A., Majeda, Y., Saccharifycation of Banana Agro waste by Cellulolytic Enzymes. *African Journal of Biotechnology*, **3(9):**447-450 (2004).