Australian Journal of Basic and Applied Sciences, 5(12): 1114-1118, 2011
ISSN 1991-8178

Cellulase Enzyme Production by *Streptomyces* Sp Using Fruit Waste as Substrate

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Abstract: The main purpose of this study to reduced the production cost of cellulase by using alternative carbon source such as lignocellulosic waste and optimized fermentation parameters for high yielding. In the present investigation, isolated the novel cellulase producing actinomycetes, *Streptomyces sp* from decayed fruit waste and optimized the physicochemical parameters for cellulase production. Production of cellulase enzyme by a *Streptomyces sp S7* was detected on cellulose agar medium after 4 days of incubation at 28 °C that exhibited a clear zone of 25mm around the colony. Cellulase production was assayed by measuring the amount of glucose liberated by using the dinitrosalicylic acid assay method. The highest crude enzyme production -was observed at pH 5 and temperature of 40°C in a medium that was supplemented with fruit waste as carbon source. It could be concluded that *Streptomyces sp S7* is a powerful cellulase producer strain under our tested experimental conditions using fruit waste as carbon source.

Key words: Cellulase enzyme production, Streptomyces, Fruit waste, partial characterization.

INTRODUCTION

Increasing demand, rising cost of fossil fuels and global climatic changes have shifted global efforts to utilize renewable resources for the production of alternative energy. Cellulose and hemicelluloses are the major components of plant biomass. Lignocellulosic biomass is a renewable, abundant and inexpensive resource for the bioconversion to biofuels and by products. The complex structure of cellulose poses a major obstacle in the bioconversion of lignocellulose. Cellulose is a linear biopolymer of anhydroglucose units linked by the β-1,4-glycosidic bond. Generally, cellulose exists in crystalline forms connected with amorphous regions.

In nature, a variety of bacteria and fungi produce cellulases to hydrolyze these insoluble polysaccharides to soluble oligomers, and subsequently to monomers (Bedford and Partridge, 2001). However, this conversion is quite difficult owing to the complex structure of plant cell wall designed to resist microbial degradation.Therefore, attempts are being made to find the effective enzyme systems from various cellulolytic microorganisms.Actinomycete's, one of the known Cellulase-producers, has attracted considerable research interest due to its potential applications in recovery of fermentable sugars from cellulose (Putarau 1969; Coral et al. 2002), that can be of benefit for human consumption and to the ease of their growth. They are capable of producing an array of different extracellular enzymes including cellulase, chitinase and xylanase.The present investigation was conducted to isolate streptomyces from the fruit and agricultural waste and to screen them for their ability to utilize cellulose from fruit and agricultural waste. The influence of different culture conditions on production of crude Cellulase by Streptomyces isolate is also studied.

MATERIALS AND METHODS

Isolation and Identification of Actinomycetes:

The fruit waste orange bagasse, banana and mango peel were used in this study. The decayed wastes were collected in the sterile container and were shaken for 1hr on a rotary shaker at 250 rpm to disperse the samples. These samples were diluted and spread plated on a starch casein medium. The plates were incubated for 7 days at 28^{o}C.The colonies were identified by gram staining methods.

Screening of Cellulase producing Actinomycetes:

Carboxymethyl cellulose (CMC) agar medium with 1% of cellulose was prepared and sterilized (autoclave at 121^{o}c for 15 min). This medium was inoculated with loopful of culture at the center of agar plate. Three replicates were used for the each actinomycetes isolates. The inoculated plates were incubated at 28^{o}C for 4 days.After incubation, the zone was identified around the culture by treating with Congo red and NaCl.

Cellulase production by Solid state fermentation:

Production of cellulase enzyme under solid state fermentation using fruit waste as the substrate was carried out using standard techniques.10gm of sterilized substrate was inoculated with actinomycetes culture and 10ml of nutrient solution composed of 0.1% Ammonium sulphate and 0.1% Magnesium sulfate were added. The final

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moisture content of medium was 70%.Cultures were incubated at 28^0Cfor 6 days. After 48 hrs and at the intervals of 24 hr, 40 ml of distilled water was added to the cultures, stirred for 40 minutes, filtered and centrifuged. The supernatant was used as crude enzyme solution.

Measurement of Enzyme Activity:

Enzyme activity was assayed using a modified method described by Wood and Bhat with some modifications. 0.2 ml of culture filtrate was added to 1.8 ml of 1% carboxy methyl cellulose prepared in 0.05M sodium citrate buffer (pH 4.8) in a test tube and incubated at 40°C for 30 min. The reaction was terminated by adding 3.0 ml of dinitrosalicylic acid (DNS) reagent and by subsequently placing the reagent tubes in a water bath at 100°C for 15 min. 1 ml of 40% Rochelle salt solution was then added to stabilize the color. Absorbance was recorded at 575 nm against the blank (0.05 M sodium citrate buffer).One unit of enzyme activity was defined as the amount of enzyme that released 1 μM of glucose per min.

Reducing Sugars Content:

Reducing sugars analysis was conducted by using 2 ml of sample which was added to 3 ml of DNS and boiled for 15 min. After cooling, 1 ml of Rochelle salt was added. The absorbance was recorded at 575 nm using a spectrophotometer against the blank of distilled water

Protein Determination:

Protein content was determined according to Lowery *et al* method. In this assay, one ml of crude enzyme supernatant was used and 5 ml reaction mixture was added in a clean dry test tube. The tubes were kept at room temperature for 10 min. Then 0.5ml of Folin reagent was added to the previous mixture. The tubes were incubated for 20 min at room temperature and the absorbance was measured at 720nm

Effect of Temperature and Ph on Enzyme Production:

The effect of pH on enzyme production was studied in buffer over a wide range of pH3-9 (sodium citrate for pH 3.0–6.0, sodium phosphate for pH 6.0–8.0, Tris–HCl for pH 8.0–9.0.) The production media were incubated over atemperture range of 20 -50^0C for 48hrs.

Partial Characterization of Enzyme:

Supernatant solutions from Streptomyces were used as crude enzyme. The temperature profile for cellulase activity was determined by varying the incubation temperature between 20°C and 60^0C. In the same way, cellulase activity was determined in the pH range of 3.0–9.0 using sodium citrate for pH 3.0–6.0, sodium phosphate for pH 6.0–8.0, Tris–HCl for pH 8.0–9.0, To study the cellulase thermal stability, the supernatant was pre-incubated at 50 and 60°C for 2, 4, 6, 8, 16, 20, and 24 h. These experiments were conducted in triplicate, and results expressed as average values.

Result:

Isolation and Screening of Cellulase Producing Actinomycetes:

The aim of this study was to isolate strains of actinomycetes with improved cellulase production. Ten actinomycetes isolates were isolated from decayed fruit wastes and they are identified by colony morphology and Gram staining. Most of the isolates, obtained from fruit wastes, were *Streptomyces sp.* Screening of cellulase producing actinomycetes were conducted using the Congo red test. After 4 days of incubation, all isolates of actinomycetes showed signs of growth on CMC agar and demonstrated positive results in the Congo red test. The *Streptomyces sp S7* gave the clear zone diameter of 25mm. This indicated more cellulose degradation in CMC agar plates cultured with *Stain S7* as compared to plates cultured with other isolates.

Cellulase Production By Solid State Fermentation:

Production of cellulase on solid state fermentation using fruit waste as substrate had been carried out with *Streptomyces sp S7* strain.

Table 1: Reducing sugars and Cellulase produced by isolated *Streptomyces* strain.

Name of the Isolates	Enzyme activity (u/ml)	Reducing sugar(μg/ml)	Protein content(mg/ml)
Streptomyces sp.S7	38	360	0.53

Effect of Ph and Temperature on Enzyme Production:

The effect of temperature on the production of cellulase by *Streptomyces sp S7* was determined at temperatures ranging from20 to 50°C. (Figure1). Though there was considerable amount of enzyme production between 30-50^0C,the optimal enzyme production was noticed at 40^0C.The effect of pH on cellulase enzyme produced from *Streptomyces sp S7* was examined using buffers at varying pH ranging from 4.0 to 9.0 as shown

in (Figure 2). The enzyme shows high activity at a broad range of pH values (pH 4 - 6) with optimal pH at 5.0. The enzyme production reduced to50% at pH 9.

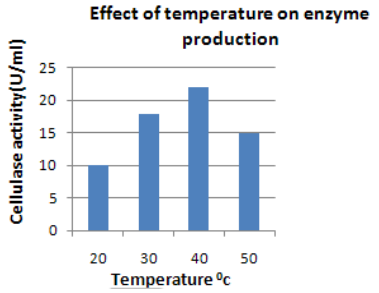

Fig. 1: Effect of temperature on enzyme production.

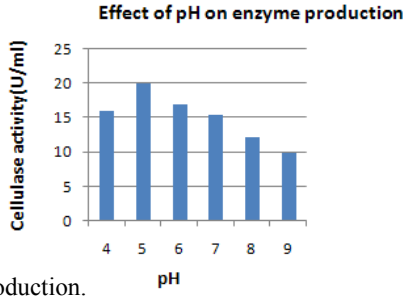

Fig. 2: Effect of pH on enzyme production.

Partial Characterization of Cellulase:

Temperature profile for cellulase activity was determined by varying the incubation temperature between 20^0C and 60^0C.Optimal activity was observed at 50°C (Figure 3) and then enzyme activity reduced to 75% and 50% at 40° and 60°C, respectively. In the same way, cellulase activity was determined in the pH range of 3.0-9.0, incubated at 50^0C. The pH profiles demonstrate that 50% cellulase activity is maintained over a wide pH range (4.0–8.0), with optimal activity occurring at pH 5.0 (Figure 4).

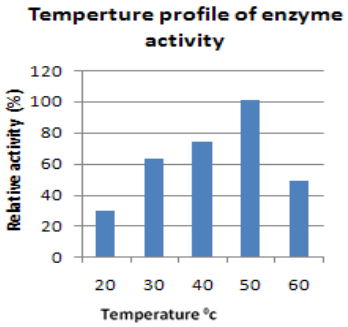

Fig. 3: Temperature profile of cellulase enzyme Activity.

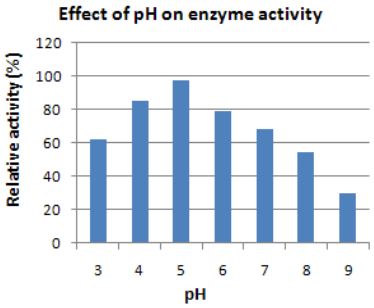

Fig. 4: Effect of pH on cellulase enzyme activity.

Thermo stability experiments are shown on (Figure 5). Shows that crude enzyme was able to retain 78% residual activity at 50°C for 2 h, and 50% after 5h incubation, the half-life of crude enzyme being 6 h at 50°C and 1.5 h at 60°C. Our results strongly suggest that the cellulase in this supernatant is thermo tolerant and as such could be considered appropriate for many biotechnological processes.

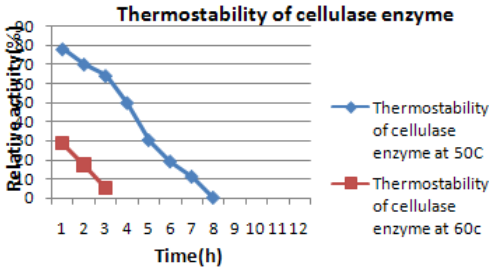

Fig. 5: Thermostability of cellulase enzyme produced by Streptomyces sp.

Discussion:

Streptomyces species have always been a source of thousands of bioactive compounds. Enzymes are one of the important products of this unusual group of bacteria. *Streptomycetes* sp. with potential cellulolytic activity is subjected to produce cellulase in liquid culture.

In the present work, ten actinomycetes isolates were isolated from decayed fruit wastes and they are identified by colony morphology and Gram staining. Most of the isolates, obtained from fruit wastes, were *Streptomyces sp.* We have shown that *Streptomyces sp.S7* produces cellulases using low-cost residues, such as fruit waste. Screening of actinomycetes strains was conducted by using the Congo red test. Since the sole carbon source in CMC agar was cellulose, the result of the test was strong evidence that cellulase was produced. *Streptomyces sp S7* gave the highest ratio of clear zone diameter to colony diameter. This indicated more cellulose degradation in CMC agar plate cultured with *Streptomyces sp S7* as compared to plates cultured with the other strains.

The effect of environmental factors such as Temperature and pH were found to be important parameters that influenced enzyme activities and production. The optimal temperature for cellulase enzyme production by *Streptomyces sp S7* was found to be around 30 to 45^{0}C and the highest production obtained at 40^{0}C.The result also showed that enzyme production was maximum at pH 5 with fruit waste as substrate.

Cellulase enzyme from *Streptomyces sp S7* was found active over a pH range of 4-8 with maximum activity at pH 5. Theberge *et al.* (1992) showed that the optimum pH for endoglucanase from a strain of *Streptomyces lividans* was 5.5. Jaradat *et al*. (2008) also found that cellulase enzyme from *Streptomyces sp*. (strain J2) was active over a pH range of 4 - 7 with maximum activity at pH 6.

The maximum cellulase activity of *Streptomyces sp S7* was recorded at 50°C and the optimum range is 40-60°C. Furthermore, McCarthy (1987) reported an optimal temperature for cellulase activity in the range of 40 - 55°C for several *Streptomyces* species including *Streptomyces lividans, Streptomyces flavogrisus*, and *Streptomyces nitrosporus*. Indeed, our results about pH and temperature profiles, and also the thermo stability characteristics have shown that our strain is promising. Although the commercial preparation is more resistant at very high temperatures (above 60^{0}C), the pH profile is very similar, especially in the range of 2–9.

Conclusion:

Cellulase enzyme production accounts for 40% of cost in bioethanol production. To reduce the cost of production, lignocellulosic substrate is used instead of synthetic cellulose due to their reasonable cost, high enzyme production capacity etc. The reduction in cost paves an economically easy way of ethanol production. It is an important issue to deal with the residue both the comprehensive utilization of lignocellulosic resources and for the prevention of environmental pollution.

The microorganism *Streptomyces sp S7* used in this study was able to grow and to produce cellulase using fruit waste as sources of carbon. The optimum of pH and temperature of enzyme production were of 5.0 and 40°C, respectively, and the cellulase retained 50% of activity after 5h at 50°C. The results herein obtained make this strain and these low cost substrate worthy of further investigation, and potentially feasible for biotechnological applications in different areas.

REFERENCE

Baig, M.M.V., V.P. Mane, D.R. More, L.P. Shinde, M.I.A. Baig, 2003. "Utilization of Agricultural Waste of Banana: Production of Cellulases by Soil fungi", *J. Environ. Biol.,* 24: 173-176.

Bhikhabhai, R., G. Johansson, G. Pettersson, 1984. "Isolation of cellulolytic enzymes from *Trichoderma reesei* QM 9414". *J Appl Biochem.,* 6: 336-345.

Carrard, G., A. Koivula, H. Soderlund, P. Beguin, 2000. "Cellulose-binding domains promote hydrolysis of different sites on crystalline cellulose". *Proc Natl Acad Sci U SA.* 97: 10342-10347.

Castellonos, O.F., A.P. Sinitsyn, E.Y. Vlasenko, 1995. "Comparative evaluation of hydrolytic efficiency toward microcrystalline cellulose of *Penicillium* and *Trichoderma* cellulases". *Biores. Technol*., 52: 119-124.

Doppelbauer, R., H. Esterbauer, W. Steiner, R. Lafferty and H. Steinmuller, 1987. "The use of cellulosic wastes for production of cellulases by *Trichoderma reese"i. Appl. Microbiol. Biotechnol.,* 26: 485-494.

Gama, F.M. and M. Mota, 1997. "Enzymatic hydrolysis of cellulose (I): relationship between kinetics and physico-chemical parameters". *Biocatalysis and Biotransformation*, 15: 221-236.

Gruno, M., P. Väljamäe, G. Pettersson, G. Johansson, 2004. "Inhibition of the *Trichoderma reesei.* cellulases by cellobiose is strongly dependent on the nature of the substrate". *Biotechnol Bioeng,* 86: 503-511.

Henriksson, G., G. Johansson, G. Pettersson, 2000. "A critical review of cellobiose dehydrogenases". *J Biotechnol.,* 78: 93-113.

Henrissat, B., 1991. "A classification of glycosyl hydrolases based on amino acid sequence similarities". *Biochem J.,* 280: 309-316.

Henrissat, B., H. Driguez, C. Viet, M. Schülein, 1985. "Synergism of cellulases from *Trichoderma reesei* in the degradation of cellulose". *Bio/Technology.,* 3: 722-726.

Ilmen, M., A. Saloheimo, M.L. Onnela, M.E. Penttilä, 1997. "Regulation of cellulase gene expression in the filamentous fungus *Trichoderma reesei"*. *Appl Environ Microbiol.,* 63: 1298-1306.

Jaradat, Z., A. Dawagreh, Q. Ababneh and I. Saadoun, 2008." Influence of culture conditions on cellulase production by *Streptomyces* sp. (Strain J2)". Jordan J. Biol. Sci., 1: 141-146.

Lee, S.B., I.H. Kim, D.D.Y. Ryu, H. Taguchi, 1983. "The structural properties of cellulose and cellulase reaction mechanism". *Biotechnology and Bioengineering,* 25: 33-51.

McCarthy, A.J., 1987. "Lignocellulose degrading actinomycetes." FEMS Microbiol. Lett., 46: 145-163.

Mandels, M. and J. Weber, 1969. "Production of cellulases". *Adv. Chem. Ser.,* 95: 391-414.

Mandels, M., R. Andreotti, and C. Roche, 1976. "Measurement of saccharifying cellulase". *Biotechnol. Bioengng Syrup.,* 6: 21-33.

Mulakala, C., P.J. Reilly, 2005. " *Hypocrea jecorina* (*Trichoderma reesei*) Cel7A as a molecular machine: A docking study". *Proteins.,* 60: 598-605.

Nazhad, M., L. Paszner, J.N. Saddler and L.P. Ramos, 1995. "Structural constraints affecting the initial enzymatic hydrolysis of recycled paper". *Enzyme and Microbial Technology*, 1: 68-74.

Nidetzky, B., M. Hayn, R. Macarron, W. Steiner, 1993. "Synergism of *Trichoderma reesei* cellulases while degrading different celluloses". *Biotechnology Letters.,* 15: 71-76.

Okeke, B.C., S.K.C. Obi, 1995. "Saccharification of agro wastes materials by fungal cellulases and hemicellulases". *Biores. Technol*., 51: 23-27.

Persson, I., F. Tjerneld and B. Hahn-H/igerdal, 1991. "Fungal cellulolytic enzyme production: a review". *Process Biochem.,* 26: 65-74.

Ruy, D. and M. Mandels, 1980. "Cellulases: biosynthesis and applications". *Enzyme Microb. Technol.,* 2: 91-102.

Theberge, M., P. Lacaze, F. Shareck, R. Morosoli and D. Kluepfel, 1992. "Purification and characterization of an endoglucanase from *Streptomyces lavidans* 66 and DNA sequence of the gene". Applied Microbiol., 58: 815-820.

Vlasenko, E.Y., O.F. Castellanos, A.P. Sinitsyn, 1993. "Susceptibility of different cellulose containing materials to hydrolysis by cellulolytic enzymes". *Prikl. Biokhim. Mikrobiol*., 29: 834-843.

Wang, D.I.C., G.C. Avgerinos, I. Biocic, S.D. Wang and H.Y. Fang, 1983. "Ethanol from cellulosic biomass". *Philosophical Transcripts of the Royal Society of London B. Biological Science*, 300: 323-333.

White, A.R., R.M. Brown, Jr., 1981. "Enzymatic hydrolysis of cellulose: Visual characterization of the process". *Proc Natl Acad Sci U SA.* 78: 1047-1051.

Wood, T.M. and K.M. Bhat, 1988. "Methods for measuring cellulase activities". *Methods in Enzymology*, 160(9): 87-112.