EFFECT OF CARBON SOURCES ON CELLULASE PRODUCING ACTIVITY OF BACTERIAL ISOLATES

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Abstract- Cow dung and municipal solid wastes have been selected as cellulolytic bacterial habitants. Nine bacterial strains designated as cd1, cd2, cd3, cd4, mw1, mw2, mw3, mw4 and mw5 were isolated and screened the cellulolytic activity on Berg’s media containing 1%carboxymethyl cellulose (CMC) and cellulose substrate. Six strains showed the halo zone formations which indicate the cellulolytic activity. Among cd strains, cd3 was more effective on cellulolytic activity. On the other hand, mw4 was also potentially more effective. For both carbon sources, they showed the highest halo zone diameters with 30mm and 15mm. The two strains have been studied for quantitative determination of cellulolytic activities by DNS method. CMC and cellulose were used as inducer substrates for cellulase enzyme. In CMC broth, the enzyme producing of cd3 and mw4 showed the reducing sugar formation 1.7mg/ml and 1.677mg/ml when the crude enzyme react with 1% cellulose solution. In cellulose broth, they showed the reducing sugar formation in 0.562mg/ml and 0.415mg/ml. The optimal incubation period for cellulase enzyme producing was 36hr and 60hr for cd3 and mw4 in CMC broth but 84hr and 60hr were the optimal incubation period in cellulose broth. The selected strains were more favour CMC than cellulose for the producing activity of cellulase enzyme.

Keywords- Bacteria, Cellulose, CMC, DNS, Enzyme, Quantitative, Screening

I. INTRODUCTION

Cellulose is considered as one of the most important sources of carbon on this planet and its annual biosynthesis by both plants and marine algae occurs at a rate of 0.85×1011tonnes per annum [1]. The cellulose is composed of D-glucose units linked together to form linear chain via β-1, 4-glycosidic linkages [2]. Cellulose may be hydrolyzed using enzymes to produce glucose, which can be used for the production of ethanol, organic acids and other chemicals [3]. The cellulase system constitute three major enzymes; i.e., endoglucanase (endo- 1,4-β-D-glucanase, EC3.2.1.4), exo - glucanase (exo - 1 , 4 - β-D – glucanelllobiohydrolase, EC3.2.1.91), and β-glucosidase (β-D-glucoside glucanohydro- lase, EC 3.2.1.21), which act synergistically towards the complete breakdown of cellulose. Endo-glucanases make nicks within the cellulose biomolecule thereby exposing their reducing and non-reducing ends, cellobiohydrolases release cellobiose units—a disaccharide of two glucose molecules linked by a β-1,4 linkage—the repeating units of cellulose from the chain ends; and finally β-glucosid- dases act on cellobiose to liberate glucose [4]

Cellulase enzymes produced chiefly by microbial sources, starting from prokaryotic organisms like bacteria, and protozoans to eukaryotic organisms that catalyze the cellulyolysis. However, there are also the cellulases produced by animal sources and plant materials. Cellulases are inducible enzymes which are synthesized by microorganisms during their growth on cellullosic materials [5]. Bacteria has high growth rate as compared fungi has good potential to be used in cellulase production. For many years, cellulose degrading bacteria have been isolated and characterized for obtaining more effective cellulases from variety of sources such as soil, decayed plant materials, hot springs, organic matters, feces of ruminants and composites [6]. Cellulases contribute to 8% of the worldwide industrial enzyme demands. The cellulase market has been estimated in the United States to be as high as US $ 400million per year. In the period 2004 -2014 an increase of approximately 100 % in the use of cellulase as a specialty enzyme is expected [7]. Cellulase enzymes provide a key opportunity for achieving tremendous benefits of biomass utilization through the bioconversion of the most abundant cellulosic wastes into the simplest carbohydrate monomer, glucose. cellulase enzymes, cellobiohydrolase and endoglucanase, have wide application in the textile paper and pulp and food industries. The most well-known application is the use of cellulases in bio stoning [8]. It was aimed to develop bacterial strains which can produce cellulase enzyme for the industrial applications. In the present investigation, nine bacterial isolates from cow dung and municipal solid wastes were screened for cellulolytic activities and effect of carbon sources on quantitative measurement were done by using Dinitrosalicylic acid method.

II. MATERIALS AND METHODS

A. Collection of Sample

The samples for the isolation of bacteria were collected from Mandalay Urban Area and Mandalay Technological University Campus, Myanmar. The samples are cow dung and municipal solid wastes.
Sampling was done by taking all possible aseptic measures and was stored at 4°C.

B. Isolation of Cellulolytic Bacteria

Cellulolytic bacterial strains were isolated by using serial dilution. The medium used for the isolation of bacteria contains cellulose 1%, 2g NaNO3, 0.5g MgSO4, 0.05g K2HPO4, 0.01g FeSO4, 0.02g CaCl2, and 0.02g MnSO4 and Distilled water 1L. Plates were incubated for 72 hours at 30°C. Bacterial colonies were purified by repeated streaking. The purified colonies were preserved at 4°C for further screening for cellulase activity.

C. Screening of Cellulolytic Bacteria

Pure cultures of bacterial isolates were individually transferred to cellulose and CMC agar plates. After incubation for 48 hours, the plates were flooded with 1% congo red dye and allowed to stand for 15 minutes at room temperature. One molar NaCl was thoroughly used for counterstaining the plates. The clear zone formed by the isolates indicated their cellulase activity.

D. Production of Cellulase

Cellulase enzyme was produced using Berg’s medium with following composition CMC 1%, NaNO3 0.2%, MgSO4 0.05%, K2HPO4 0.005%, FeSO4 0.001%, CaCl2 0.002% and MnSO4 0.002%. 150ml of autoclaved production medium inoculated with 2% of culture broth was incubated on water bath shaker at 120rpm at 37°C for 5 days. Bacterial cultures were harvested with 12 hours incubation time interval by centrifugation at 5000rpm for 20 minutes. The culture supernatants were used for the assay of extracellular enzyme.

E. Assay of Cellulase

Cellulase activity was assayed by determining the reducing sugar produced during enzymatic reaction by DNS method. 0.5ml of culture supernatant was added into 1ml of 0.05 M citrate buffer (pH 4.8) solution in test tubes containing various concentrations of cellulose substrate. The reaction mixture was incubated at 50°C for 60min in a water bath shaker at 80-85 rpm. And then 3ml of DNS reagent was mixed to the reaction mixture and this mixture was boiled for exactly 5 minutes to terminate the reaction in a vigorously boiling water bath. After that cool in a cold water bath, record the absorbance with spectrophotometer at 540nm against the blank without enzyme filtrate.

F. Effect of Carbon Sources for Cellulase Production

The Berg’s medium containing 1% cellulose instead of CMC was also used for cellulase enzyme production. The flasks were inoculated with 2% inoculum and incubated at 37°C in water bath shaker for 5 days. The assay was carried out at 12 hours incubation time interval.

III. RESULTS AND DISCUSSION

In this study, four bacterial strains were isolated from cow dung and termed as cd1, cd2, cd3 and cd4. On the other hand, the bacterial strains isolated from municipal waste were named as mw1, mw2, mw3, mw4 and mw5. Screening of bacteria for cellulase producing activity was conducted by using Berg’s medium containing different concentrations of CMC and cellulose. After the incubation, the plates were flooded with 1% congo red dye and, after 15 minutes the dye was discarded and the plates were again flooded with 1M NaCl for 15 minutes. Among nine bacterial strains, five strains showed cellulase activities with halo zone formation. In the case of cd strains, cd3 showed the highest diameter of about 30mm at every concentration of cellulose and CMC. To compare cellulase activities from different sources, cd3 and mw4 were chosen for further studies although mw4 did not have the zone diameter as much as cd3.

Cellulase activity can be expressed by estimating reducing sugars during enzymatic reaction by DNS colorimetric method using 0.3%, 0.5%, 1% cellulose as substrate. The reducing sugar concentrations were calculated according to glucose standard curve (Fig. 2). Nutrient sources were found to be the important factor for the cellulase production. Since carbon is considered as the primary nutrient for the bacteria, carbon sources of CMC and cellulose were utilized for the cellulase production. Maximum production of cellulase in the form of reducing sugar 1.7mg/ml for cd3 and 1.677mg/ml for mw4 was observed when CMC was served as the carbon source. In addition optimum incubation time for cd3 and mw4 were observed at 36 hours and 60 hours when CMC was served as carbon source. But, reducing sugar concentration was decreased to 0.562mg/ml at 84 hours for cd3 and 0.415mg/ml at 60 hours for mw4 after using cellulose as carbon source. Moreover enzyme concentration was estimated by using different concentration of cellulose as substrate. Among tested concentration of cellulose, 1% cellulose was the suitable concentration for both cd3 and mw4. Moreover we observed that both isolates cd3 and mw4 were more favoured CMC than cellulose as a carbon source for cellulase enzyme production.

Fig. 1 Halo zone formation of isolates on Berg's medium containing 0.5% CMC
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CONCLUSION

Nine bacterial strains were isolated from cellulose waste materials, cow dung and municipal waste. Only six bacterial isolates showed the cellulolytic activity. From the results, it has been revealed that CMC was more suitable for cellulase enzyme production than cellulose substrate. The optimum incubation period for cd3 and mw4 were 36 hours and 60 hours for enzyme production in Berg’s media supplemented with 1% CMC. According to the present result, substrate enzyme ratio should be increased. In quantitative measurement, the bacterial strain cd3 produced more cellulolytic enzyme than mw4.

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