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RESEARCH ARTICLE

PRODUCTION OF SUGARS FROM CELLULOSIC WASTES BY ENZYMATIC HYDROLYSIS

***Pinky Sinha and Sheila Bedi**

Department of Botany, Patna Women's College, Patna, Bihar, India

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ABSTRACT

The present study on the screening of cellulose degrading bacteria, isolated locally from the natural environment; and analysis of their carboxymethylcellulose (CMC) and filter paper activity has been carried out with the rationale to isolate novel strains having significant cellulolytic potential. From the present findings, it was observed that on 4th day the isolate-01 showed maximum CMC activity (9.4 mg/ml of released reducing sugar); followed by isolate-07 (5 mg/ml of released reducing sugar); whereas isolate-03 showed maximum filter paper activity on 6th day (8.0 mg/ml of released reducing sugar); followed by isolate-07 (7.4 mg/ml of released reducing sugar). Thus it can be concluded that isolate-07 has potential for both CMC as well as filter paper activities and can be explored for production of sugar from cellulosic wastes on commercial scale. On the basis of morphology, microscopic observations and biochemical tests, the isolate-07 showed resemblances with Streptomyces species; however, for confirmation 16 S rRNA sequencing is required.

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INTRODUCTION

The importance of cellulose degradation and the use of its byproduct as a source of renewable energy is not a new thing. It all began during the World War II, when the U.S. Army noticed the fast rate at which the cellulosic materials were rotting in the tropical region. As a result, laboratories were set up to find out the cause and solutions to the problem at U.S. Army Natick Development Centre, Natick, Massachusetts, within the Armed Services. A large number of fungi were isolated from the rotten cellulosic materials which produced cellulose degrading enzymes (Reese and Levinson, 1952). With this discovery, the interest shifted from the prevention of deterioration of the cellulosic materials to the development of an enzyme system for hydrolysis of cellulose into glucose, which could be used as a feedstock, for the production of ethanol to alleviate liquid fuel shortages, or for the production of single-cell protein, pharmaceuticals and other compounds.

Ever since the discovery of the enzyme cellulases, the researchers worldwide have focused their attention towards newer microbial isolates, from which the cellulases can be extracted and can be used in different industrial processes. The cellulases have been reported mainly from bacteria, actinomycetes, fungi and yeasts that use different substrates for production of cellulases (Lynd *et al*., 2002).

**Corresponding author: Pinky Sinha*
Department of Botany, Patna Women's College, Patna, Bihar, India
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Cellulolytic microorganisms produce a wide variety of different catalytic and non catalytic enzyme modules which form the cellulases and act synergistically on cellulose to yield soluble glucose. Cellulose utilization by most well-studied microorganisms involves the activities of multiple enzymes, including endo-β-1,4-glucanases, cellobiohydrolases (also called exo- β -1,4-glucanases), and β-glucosidases, that act synergistically to convert crystalline cellulose to glucose (Lynd *et al*., 2002; Warren, 1996). The endoglucanases cleave at random at internal amorphous sites in the cellulose glucan chains, and the exoglucanases act processively to release cellobiose primarily from the chain ends (Teeri, 1997; Warren, 1996). Despite an increased knowledge of microbial cellulolytic systems in the past years, further studies are required to achieve a complete understanding of the mechanism of cellulose degradation by microorganisms and their enzymes. In this perspective, the present study was undertaken to explore the microbial world to enumerate cellulose degrading bacteria and actinomycetes by survey and collection of the samples from agricultural and municipal wastes. The overall aim of the present study was to isolate and investigate novel biomass degrading bacteria and actinomycetes and assessment of their cellulolytic enzyme activity.

MATERIALS AND METHODS

Isolation and purification of the cellulose degrading bacteria and actinomycetes from soil samples: The soil samples, collected locally from the agricultural fields and the

municipal dumps, were serially diluted in sterilized normal saline and the aliquots of the soil solutions were plated on the sterilized solidified Nutrient Agar medium supplemented with Carboxymethylcellulose in the petriplates in aseptic condition. The plates were incubated at 37°C for 24-48 h. The different colonies of bacteria and actinomycetes, thus obtained, were purified and screened for their cellulolytic activities.

Screening of cellulose degrading microorganisms and their characterization: The isolated microorganisms were screened for their cellulolytic activities with the help of 1% Congo red and 1N HCl. Those showing clearing or solubilizaton zones were selected for further tests for cellulose degradation in quantitative terms. The selected isolates were characterized on the basis of morphological and biochemical parameters by the standard methods (Dubey and Maheshwari, 2004).

Assessment of the cellulolytic enzyme activity in the selected isolates: The cellulolytic potential of the selected cellulose degraders against soluble cellulose carboxymethylcellulose (CMC) and insoluble cellulose (filter paper) was compared by estimating the amount of reducing sugar released during cellulose degradation by DNS method. The selected isolates were inoculated in triplicates in 10 ml of the Nutrient broth medium with cellulose as the substrate for 2, 4, 6 and 8 days at 37°C. The volume of the culture filtrate was measured after 2, 4, 6 and 8 days and the components of cellulase enzyme complex i.e. the endoglucanases and the exoglucanases were assayed.

For CMC activity (endoglucanases activity): CMC activity in culture supernatant was assayed according to the method explained by Wood and Bhat (1998) with some modifications. Two ml of the culture filtrate was incubated with two ml of 0.55% CMC in citrate buffer (pH 6.0) for 2 h at 50°C. The liquid was centrifuged at 10,000 rpm for 15 min at the room temperature. One ml of the supernatant was mixed with one ml of the DNS reagent in a tube. The tube was heated in the boiling water bath for 10 min. After heating, 8 ml of the distilled water was added to it and the optical density was recorded at 540 nm against a control and was compared with the standard curve of glucose to determine the amount of reducing sugar released. The process was done in three sets for each of the isolates.

For filter paper activity (exoglucanases activity): The filter paper activity was assayed according to the method explained by Wood and Bhat (1998) with slight modifications. Two ml of the culture filtrate was incubated with 100 mg of the filter paper discs and two ml of citrate buffer (pH 6.0) for 2 h at 50°C. After incubation, the liquid was filtered and centrifuged at 10,000 rpm for 15 min at the room temperature. The supernatant was used as the crude enzyme. One ml aliquot of the crude enzyme was mixed with one ml of the DNS reagent in a tube. The tube was heated in the boiling water bath for 10 min to allow colour development. After heating, 8 ml of the distilled water was added to it and the colour intensity was measured by the spectrophotometer at 540 nm against a control and was compared with the standard curve of glucose to determine the amount of reducing sugar released. The process was done in three sets for each of the isolates. The control was prepared, by heating one ml of the culture filtrate with one ml of the DNS reagent in boiling water bath for 10 min. After heating, 8 ml of the distilled water was added and the reading was noted down at 540 nm.

RESULTS

Isolation and screening of the isolates: A total of thirty five cellulose degraders were isolated from the soil samples and purified. Among the thirty-five isolates, four efficient cellulose degrading strains were selected on the basis of the diameter of the clear zone surrounding the colonies on the medium. These were labeled as isolate-01, isolate-03, isolate-07 and isolate-18. They were maintained on slants of Nutrient Agar at 4 °C with periodic sub culturing for further use. The selected isolates were critically examined for their colony characteristics and micro morphology by Gram staining. The observations are shown in Table 1.

Table 1. Morphological characteristics of the selected isolates

Colony morphology	isolate-01	isolate-03	isolate-07	isolate-18
Configuration	Round	Erose	Round	Erose
Margin	Radiating	Smooth	Smooth	Smooth
Elevation	Flat	Slight	Convex	Flat
Surface	Powdery	Slimy	Powdery	Slimy
Gram staining	(+)ve Filamentous	(+)ve Cocci	(+)ve Filamentous	(-)ve Rods

Table 2. Biochemical characteristics of the selected isolates

Biochemical Tests	isolate-01	isolate-03	isolate-07	isolate-18
Amylase Test	+	-	+	-
Caseinase Test	-	-	+	-
Catalase Test	+	-	+	+
Citrate Utilizaion Test	-	-	+	+
Fermentation of Carbohydrates	-	-	-	-
Gelatinase Test	-	-	+	-
Hydrogen Sulphide Test	-	-	+	-
Indole Test	-	-	-	-
Methyl Red Test	-	+	-	+
Nitrate Reduction Test	+	-	+	+
Urease Test	-	-	-	-
Voges- Proskauer Test	-	-	-	-

+ Positive, - Negative

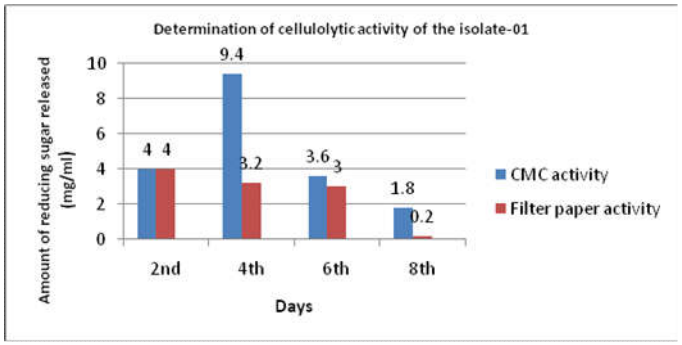

Fig. 1. Amount of reducing sugar released by CMC and filter paper activities

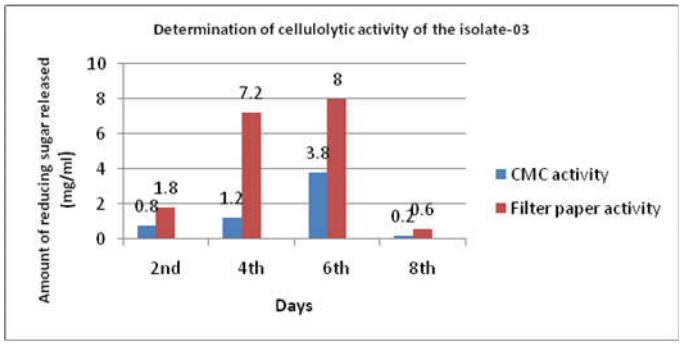

Fig. 2. Amount of reducing sugar released by CMC and filter paper activities

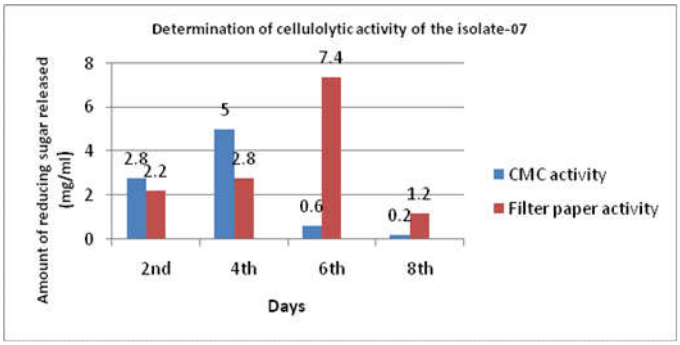

Fig. 3. Amount of reducing sugar released by CMC and filter paper activities

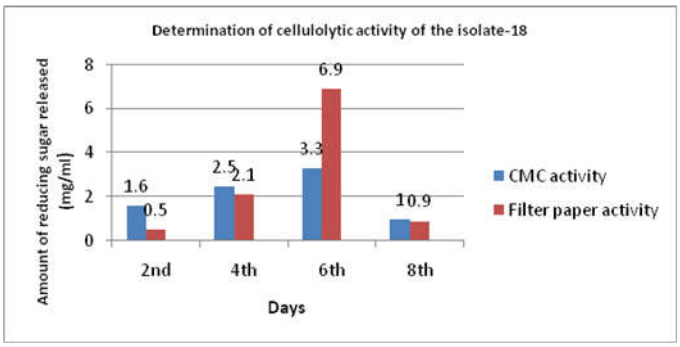

Fig. 4. Amount of reducing sugar released by CMC and filter paper activities

The different biochemical tests *viz.* amylase, caseinase, catalase, fermentation of carbohydrate, gelatinase, hydrogen sulphide, IMViC, nitrate reduction test and urease tests were performed for the characterization of the selected isolates. The results are tabulated in Table 2. The results of these tests were compared to known results for that organism to confirm its identification. For preliminary identification, Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994) and Bergey's Manual of Systematic Bacteriology (Williams *et al*., 1989) have been referred. On the basis of morphology, microscopic observations and biochemical tests, the isolate-01 showed resemblances with *Streptomyces* species; the isolate-03 with *Streptococcus* species; the isolate-07 with *Streptomyces* species; and the isolate-18 with *Pseudomonas* species, respectively. However, for confirmation the isolates are intended to be sent for 16 s rRNA sequencing.

Assessment of the cellulolytic enzyme activity in the selected isolates: The cellulolytic potential of the selected cellulose degraders against soluble cellulose carboxymethylcellulose (CMC) and insoluble cellulose (filter paper) was compared by estimating the amount of reducing sugar released during cellulose degradation by DNS method.

Determination of cellulolytic activity of the isolate-01: The isolate-01 was inoculated in triplicates in 10 ml of the Nutrient broth medium with cellulose as the substrate for 8 days at 37°C. The volume of the culture filtrate was measured after 2, 4, 6 and 8 days and it was found to be 8.4 ml, 6.4 ml, 6.0 ml and 5.4 ml, respectively. The components of cellulase enzyme complex i.e. the endoglucanases and the exoglucanases were assayed by taking optical density at 540 nm and comparing with the standard glucose curve to determine the amount of reducing sugar released. The observations are recorded in Figure 1. It was observed that except for initial two days, the amount of reducing sugar released by CMC activity was more than that by filter paper activity and also, the cellulolytic activity of the isolate decreased with time. Maximum amount of reducing sugar released by CMC activity was 9.4 mg/ml on 4th day of inoculation; whereas for filter paper activity, it was on 2nd day (4.0 mg/ml).

Determination of cellulolytic activity of the isolate-03: The isolate-03 was inoculated in triplicates in 10 ml of the Nutrient broth medium with cellulose as the substrate for 8 days at 37°C and the volume of the culture filtrate was found to be 7.8 ml, 6.0 ml, 5.2 ml and 4.8 ml, on 2nd, 4th, 6th and 8th day, respectively. The components of cellulase enzyme complex i.e. the endoglucanases and the exoglucanases were assayed as described earlier. The observations are recorded in Figure 2. It was observed that the amount of reducing sugar released by filter paper activity was more than that by CMC activity and the maximum amount of reducing sugar was released on 6th day by both filter paper activity (8.0 mg/ml) and CMC activity (3.8 mg/ml).

Determination of cellulolytic activity of the isolate-07: The process described earlier was repeated for the isolate-07 and the volume of the culture filtrate was found to be 8.2 ml, 6.0 ml, 5.6 ml and 5.4 ml, on 2nd, 4th, 6th and 8th day, respectively. The components of cellulase enzyme complex i.e. the endoglucanases and the exoglucanases were assayed as described earlier. The observations are recorded in Figure 3. It was observed that initially the CMC activity was more (2.8 mg/ml on 2nd day and 5.0 mg/ml on 4th day, respectively) but from 6th day onwards, filter paper activity increased (7.4 mg/ml on 6th day and 1.2 mg/ml on 8th day, respectively). In both cases, the activity increased initially and decreased later on.

Determination of cellulolytic activity of the isolate-18: The process was repeated for the isolate-18 and the volume of the culture filtrate was found to be 7.8 ml, 6.4 ml, 6.0 ml and 6.0 ml, on 2nd, 4th, 6th and 8th day, respectively. The components of cellulase enzyme complex i.e. the endoglucanases and the exoglucanases were assayed as described earlier. The observations are recorded in Figure 4. It was observed that initially the CMC activity was more (1.6 mg/ml on 2nd day and 2.5 mg/ml on 4th day, respectively) but on 6th day, filter paper activity increased (6.9 mg/ml). In both cases, the activity increased initially and decreased later on.

DISCUSSION

In the light of the utility and importance of cellulose degraders for the sustainable development, the present investigation has been carried out with the rationale to isolate and purify the efficient strains of cellulose degrading bacteria from wastes. Keeping this fact in mind, the soils from the specific sites were explored for the isolation, identification and partial characterization with respect to morphological and biochemical activities. The investigation clearly indicated the presence of a wide variety of cellulose degrading microorganisms in municipal and agro wastes. This may be attributed to the presence of abundant cellulosic materials in the wastes in the form of domestic and crop residues. The amount of exoglucanase and endoglucanase secreted by the selected isolates 01, 03, 07 and 18 on 2nd, 4th, 6th and 8th day has been estimated in the present investigation. It was found that there was a gradual increase in the production of cellulase enzyme by the isolate till the 4th day and thereafter a decrease was registered in cellulase production. Likewise the production of

cellulase by the isolates 03, 07 and 18 started decreasing after 6th day. This may be explained by considering the fact that when the cellulase acts on cellulose, the most susceptible portions are rapidly digested and the residue becomes increasingly resistant to enzyme attack (Mandels and Weber, 1969). Besides, the end products also act as inhibitors in the process of biodegradation of cellulose thus slowing down the process.

Conclusion

From the present findings, it was observed that on 4th day the isolate-01 showed maximum CMC activity (9.4 mg/ml of released reducing sugar); followed by isolate-07 (5 mg/ml of released reducing sugar); whereas isolate-03 showed maximum filter paper activity on 6th day (8.0 mg/ml of released reducing sugar); followed by isolate-07 (7.4 mg/ml of released reducing sugar). Thus it can be concluded that isolate-07 has potential for both CMC as well as filter paper activities and can be explored for production of sugar from cellulosic wastes on commercial scale. The investigation may further be continued with the isolation of more new varieties of cellulose degraders to enrich the list of efficient cellulose degraders. Also, development of microorganisms for cellulose conversion via consolidated bioprocessing can be pursued.

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REFERENCES

Dubey, R.C. and Maheshwari, D.K. 2004. *Practical Microbiology*, S. Chand & Company Ltd., New Delhi. pp.352

John G. Holt, Noel R. Krieg, Peter H.A. Sheath, James T. Sterley, Stanley T. Williams 1994. *Bergey's Manual of Determinative Bacteriology.* (9th Ed).

Lynd, L. R., P. J. Weimer, W. H. van Zyl, and I. S. Pretorius 2002. Microbial cellulose utilization: Fundamentals and Biotechnology. *Microbiol. Mol. Biol. Rev*. 66: 506-577.

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

Reese, E.T. and H.S. Levinson 1952. A comparative study of the breakdown of cellulose by microorganisms. *Physiol. Plant.* 5: 345-366.

Stanley T. Williams, M. Elisabeth Sharpe, John G. Holt 1989. *Bergey's Manual of Systematic Bacteriology*. Vol. 4, Ed. 1.

Teeri, T. T. 1997. Crystalline cellulose degradation: new insight into the function of cellobiohydrolases. *Trends Biotechnol*. 15:160-167.

Warren, R. A. J. 1996. Microbial hydrolysis of polysaccharides. *Annu. Rev. Microbiol*. 50: 183-212.

Wood, T.M. and Bhat, K.M. 1998. Method for Measuring CellulaseActivities. In: Methods in Enzymology Cellulose and Hemicellulose, Wood, W.A. and Kellogg, J.A. (Eds.). *Academic Press,* New York, pp: 87-112.

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