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RESEARCH ARTICLE

COMPARATIVE ASSESSMENT OF CELLULOLYTIC POTENTIAL OF TWO *STREPTOMYCES* STRAINS ISOLATED FROM MUNICIPAL WASTES

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ABSTRACT

The study was aimed at screening promising cellulolytic isolates obtained from municipal wastes by using Congo red test made on carboxymethyl cellulose plates. FPase and CMCase activity of the strains were assayed on the basis of release of glucose that was detected using dinitrosalicylic acid method. In the present investigation *Streptomyces albospinus* (MTCC No. 8768) and *Streptomyces somaliensis* (MTCC No. 8769) were selected for their cellulolytic activity on the basis of the Congo red test as these isolates demonstrated larger clear zone of hydrolysis among all the isolates. It was found in *S. albospinus* (MTCC No. 8768), that the amount of exoglucanase decreased from 3.20 to 0.51 (in cellulose units) from 6th day and endoglucanase from 1.87 to 0.99 (in cellulose units) from 4th day, whereas in *S. somaliensis* (MTCC No. 8769), the production of exoglucanases decreased from 2.33 to 0.54 (in cellulose units) from 6th day and endoglucanases from 1.67 to 0.31 from 4th day as the hydrolysis proceeded.. Our findings indicated that the total amount of cellulases secreted by the strain *S. albospinus* (MTCC No. 8768) were more than that secreted by *S. somaliensis* (MTCC No. 8769), and more cellulases were produced in the initial stages of hydrolysis.

Key words: Cellulases, cellulolytic potential, *Streptomyces albospinus* (MTCC No. 8768), *Streptomyces somaliensis* (MTCC No. 8769)

INTRODUCTION

Cellulosic material is the most abundant renewable carbon source in the world. Any process which could efficiently and economically convert cellulosic material to glucose would be of immense industrial significance (Walsh, 2002). Cellulase enzymes provide a key opportunity for achieving tremendous benefits of biomass utilization (Wen et al., 2005). Cellulose may be hydrolyzed using cellulolytic enzymes to produce glucose, which can be used for the production of useful end products (Hao *et. al.*, 2006). In this pretext, cellulolytic microorganisms play an important role in the biosphere by reducing cellulose (Gautam *et. al.*, 2010**).** Actinomycete's, one of the known cellulase-producers, has attracted considerable research interest due to its potential application in recovery of fermentable sugars from cellulose that can be of benefit for human consumption and to the ease of their growth (Jang and Cheng, 2003). Cellulases are a group of hydrolytic enzymes capable of hydrolyzing the most abundant organic polymer i.e. cellulose to smaller sugar components (Jahangeer *et. al*., 2005). The complete enzymatic hydrolysis of cellulosic materials needs different types of cellulases; endo-1, 4-β-glucanase, also referred to as carboxymethyl cellulase or CMCase [EC 3.2.1.4], exo1, 4-β-glucanase [EC 3.2.1.91] and β-1, 4- glucosidase [EC 3.2.1.21] (Yi *et.al.*, 1999). Cellulases have enormous potential in industries and are used in food, beverages, textile, laundry, paper and pulp industries etc (Jahangeer *et. al.*, 2005). The actinomycetes are well known for their ability to produce enzymes to degrade complex and recalcitrant molecules, especially cellulose, lignocelluloses and lignin. In this perspective and considering the importance and application of the cellulases, the present study was aimed to assess the cellulolytic ability of two cellulose degrading strains of *Streptomyces* namely, *Streptomyces albospinus* (MTCC No. 8768) and *Streptomyces somaliensis* (MTCC No. 8769) isolated from municipal wastes.

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MATERIALS AND METHODS

Chemicals

Chemicals used for the preparation of the media were of the highest purity grade and purchased from the local market. The chemicals used were obtained from HiMedia, Loba Chemie, Merck and Qualigens.

Media

Media used during the course of the present investigation, unless otherwise mentioned, were sterilized by autoclaving at 15 p.s.i. for 15 min. Nutrient Agar (Peptone 5 g/l, Beef extract 3 g/l, Sodium chloride 5 g/l, Agar 15 g/l) was used for isolation and preservation of cellulose degraders; CMC agar (carboxymethylcellulose 0.5 g/l, $NaNO_3$ 0.1 g/l, K_2HPO_4 0.1g/l, $MgSO_4$ 0.05g/l, yeast extract 0.05g/l, agar 15 g/l) [Kasana *et. al*., 2008] and Modified Cellulose agar replacing carboxymethylcellulose in CMC agar with cellulose for cellulose degrading efficiency test; and Citrate buffer (pH 6), 0.55% CMC in buffer and Dinitrosalicylic acid (DNSA) reagent for cellulase assay.

Microorganisms

Two promising cellulose degrading strains of *Streptomyces* were isolated from the municipal wastes by direct plating of six fold serial dilutions of samples. Isolate selection was a consequence of the production of clear zones on CMC agar when treated with Congo red dye followed by 1N HCl for 15-20 minutes. The isolates were identified according to the standard cultural, biochemical and physical characteristics (Cappuccino & Sherman, 2005) and confirmed by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh as *Streptomyces albospinus* (MTCC No. 8768) and *Streptomyces somaliensis* (MTCC No. 8769). Experimental isolates were maintained on slants of Nutrient Agar (NA) at 4 ^{0}C with periodic sub culturing.

Enzyme preparation

The strains were inoculated in triplicates in 50 mL of sterilized CMC broth medium at pH 7.0 and incubated for 7 days at 37^{0}C for the production of enzyme. 10 mL of the samples of the culture broth were taken aseptically at every two days interval and centrifuged at 10,000 rpm for 15 minutes at the room temperature. The supernatant decanted was served as a standard enzyme preparation.

Enzyme assay procedure

Determination of Filter paper cellulase (FPase/ Exoglucanase) activity

The exoglucanase activity was assayed according to the method explained by Wood and Bhat (1998) with some modifications. The FPase activity was measured by mixing 0.1 mL of enzyme solution with 50 mg of Whatman No. 1 filter paper discs and 1.0 mL of citrate buffer (pH 6.0), pH 7.0 at 37 ^{0}C for 60 min. The reaction was stopped by adding 1.0 mL 3, 5-dinitro salicylic acid (DNSA) reagent. The mixture was boiled for 10 min and 8.0mL of distilled water was added. Its optical density at 540 nm was determined using UV/Vis spectrophotometer (Thermo Scientific). The FPase activity was measured by using a calibration curve for glucose. One unit of FPase was defined as the amount of enzyme that released 1µmol of glucose per mL. The control was prepared, by heating one ml of the culture filtrate with one ml of the DNSA reagent in boiling water bath for 10 minutes. After heating, 8 ml of the distilled water was added and the reading was noted down at 540 nm.

Determination of Caboxymethyl-cellulase (CMCase/ Endoglucanase) activity

Endoglucanase activity in culture supernatant was determined according to Wood and Bhat (1998) with slight modifications. The CMCase activity was measured by mixing 1.0 mL of enzyme solution with 1.0 mL of 0.55% CMC in sodium citrate buffer, pH 7.0 at 37 ^{0}C for 60 min. The reaction was stopped by adding 1.0 mL 3, 5-dinitro salicylic acid (DNSA) reagent. The mixture was boiled for 10 min and 8.0mL of distilled water was added. Its optical density at 540 nm was determined spectrophotometrically. The CMCase activity was measured by a similar procedure as described for FPase activity.

RESULTS

Screenig and characterization of the isolates

All the bacterial isolates obtained, two cellulose degrading strains of *Streptomyces* designated as 01 and 07 were selected on the basis of Congo red test. These strains expressed maximum zone of clearing on the CMC agar plate. On Gram's staining and light microscopy observation, the isolates 01 and 07 showed Gram-positive filaments indicating that they belonged to actinomycetes group. Biochemical analysis of isolate 01 showed positive results for amylase test, catalase test, nitrate reduction test and negative for caseinase, citrate utilization, sugar fermentation, gelatinase, indole, methyl-red, urease and Voges-Proskauer tests (Table-1). Isolate 07 showed positive results for amylase test, caseinase test, catalase test, citrate utilization test, gelatinase test, hydrogen sulphide test, nitrate reductase test and negative for fermentation of carbohydrates, indole test, methyl red test, urease test and Voges-Proskauer test (Table-1). Microscopic and biochemical characteristics of these selected isolates were used for identification according to Bergey's manual of Determinative Bacteriology (Holt *et al*., 1994). Isolate 01 was identified as *Streptomyces albospinus* and isolate 07 as *Streptomyces somaliensis* and were further confirmed by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh to be *Streptomyces albospinus* (MTCC No. 8768) for isolate 01 and *Streptomyces somaliensis* (MTCC No. 8769) for isolate 07.

Enzyme assay

The results of FPase and CMCase

In our present investigation for the strain *S. albospinus* (MTCC No. 8768) , the optical density at 540 nm on the 2nd, 4th, 6th and 8th day for the Filter paper activity was 0.031, 0.080, 0.056 and 0.034 respectively; and for the CMC activity 0.031, 0.040, 0.053 and 0.024 respectively. The same for the control was 0.005, 0.022, 0.036 and 0.024 respectively (Table 2). For the strain *S. somaliensis* (MTCC No. 8769) , the optical density at 540 nm on the 2nd, 4th, 6th and 8th day for the Filter paper activity was 0.041, 0.045, 0.074 and 0.037 respectively; and for the CMC activity 0.044, 0.058, 0.030 and 0.027 respectively. The same for the control was 0.027, 0.026, 0.026 and 0.026 respectively (Table 3).

Table 1. The biochemical activities of the selected isolates

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

+ Positive, - Negative

Table 2. *Streptomyces albospinus*: Optical Density at 540 nm

Day	Control	Filter paper activity	CMC activity
2nd	0.005	0.031	0.031
4th	0.022	0.080	0.040
6th	0.036	0.056	0.053
8th	0.023	0.034	0.024

Table 3. *Streptomyces somaliensis*: Optical Density at 540 nm

Day	Control	Filter paper activity	CMC activity
2nd	0.027	0.041	0.044
4th	0.026	0.045	0.058
6th	0.026	0.074	0.030
8th	0.026	0.037	0.027

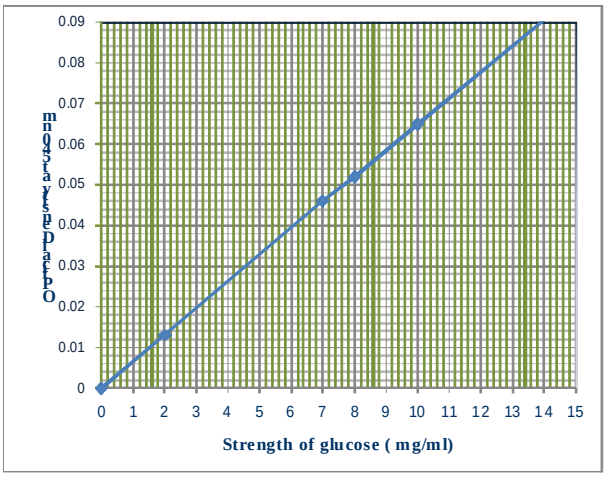

Fig. 1. Standard glucose curve
on the x-axis: glucose concentration in mg/ml;
on the y-axis: Optical Density at 540 nm
Scale: on the x-axis: 5 small div. = 1 mg/ml;
on the y-axis: 5 small div. = 0.01 O.D. at 540 nm

Fig.1. shows the standard curve of glucose (1-10 mg/ml) and with this curve, the amount of sugar obtained by the isolates was estimated. *S albospinus* (MTCC No. 8768) secreted 1.87 units, 3.20 units, 1.03 units and 0.51 units of exoglucanases on the 2nd, 4th, 6th and 8th day respectively; and the endoglucanases secreted on the 2nd, 4th, 6th and 8th day were 1.87 units, 0.99 units, 0.87 units and 0.06 units respectively as shown in Fig.2. *S. somaliensis* (MTCC No. 8769) secreted 1.00 units, 1.00 units, 2.33 units and 0.54 units of exoglucanases on the 2nd, 4th, 6th and 8th day respectively; and the endoglucanases secreted on the 2nd, 4th, 6th and 8th day were 1.23 units, 1.67 units, 0.31 units and 0.06 units respectively as shown in Fig. 3.

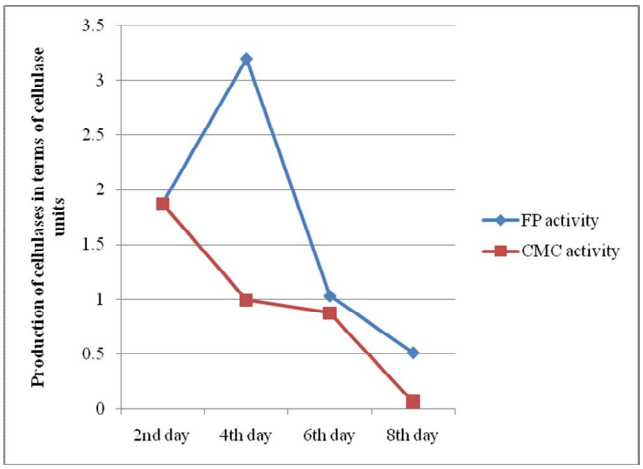

Fig. 2. Production of exoglucanases (FP activity) and endoglucanases (CMC activity) by *Streptomyces albospinus* (MTCC No. 8768)

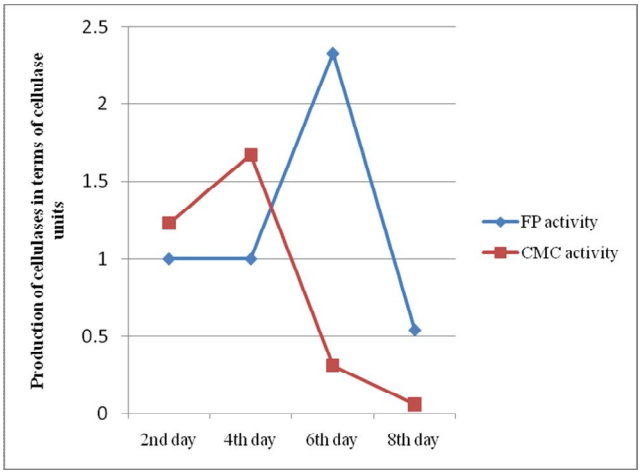

Fig. 3. Production of exoglucanases (FP activity) and endoglucanases (CMC activity) by *Streptomyces somaliensis* (MTCC No. 8769)

DISCUSSION

A wide variety of bacteria are known for their production of hydrolytic enzymes with streptomycetes being the best known enzyme producers (Vinogradova and Kushnir, 2003). Two *Streptomyces* isolates identified as *S. albospinus* (MTCC No. 8768) and *S. somaliensis* (MTCC No. 8769) recovered from the municipal wastes, produced cellulolytic enzymes. The purpose of this study was to compare the cellulase enzyme production by these strains. In the present investigation, the amount of cellulase secreted by the isolates at an interval of two days was compared. It was found that initially there was a gradual increase in the production of cellulase enzyme by the isolates and thereafter a decrease was registered in cellulase production. It was also observed that in the initial stages of hydrolysis the endoglucanases produced was either equal to the exoglucanases as in *S. albospinus* (MTCC No. 8768), each 1.87 units or more as in *S. somaliensis* (MTCC No. 8769) that produced 1.00 units exoglucanase and 1.23 units endoglucanase as shown in Fig.2. and Fig. 3.respectively. But in the later stages of hydrolysis the production of exoglucanases was more than the endoglucanases. This result

is considerably similar to that of Mandels and Weber (1969) who reported that when the cellulase acts on cellulose, the most susceptible portions are rapidly digested and the residue becomes increasingly resistant to enzyme attack. According to Van Dycke (1972), the end product acts as inhibitor to the process of cellulose hydrolysis and the decline in hydrolysis are the early removal of more assessable amorphous cellulose, resulting in an increase in the proportion of more resistant crystalline cellulose and the denaturation of adsorbed cellulases (Howell and Mangat, 1978). To our knowledge, there is no record of cellulolytic activity of *S. albospinus* (MTCC No. 8768) and *S. somaliensis* (MTCC No. 8769); therefore, we could not compare the cellulolytic activities of these strains with other researches. Comparing the total amount of the cellulase enzyme complex secreted by the two strains, the strain *S. albospinus* (MTCC No. 8768) was found to secrete more cellulase enzyme than *S. somaliensis* (MTCC No. 8769). However, further characterization would be helpful in using these potential cellulose degraders for commercial purpose and landfill bioremediation.

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