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**RESEARCH ARTICLE**

# EFFECT OF CULTURAL AND ENVIRONMENTAL PARAMETERS ON GROWTH AND CELLULASE PRODUCTION OF STREPTOMYCES SPECIES ISOLATED FROM MUNICIPAL WASTES

**[1]Prasad, P., [1]Bedi, S. and [2*]Tanuja**

[1]Department of Botany, Patna Women' College-800001, Patna University, Bihar
[2]Department of Botany, B.M.D College, Dayalpur-844502, B.R.A Bihar, University, Bihar

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**ABSTRACT**

This investigation intended to comparatively study and evaluate independent capacities of cellulose degrading native strains of *Streptomyces albospinus* (MTCC 8768) and *Streptomyces somaliensis* (MTCC 8769) isolated from municipal wastes of Patna for growth adaptability and endoglucanase yield, divulging a new insight for a strong suitability of these isolates for large scale production. Both the isolates were able to grow well in all the media tested. However, *S. albospinus* (MTCC 8768) showed luxuriant growth as compared to *S. somaliensis* (MTCC 8769) indicating its wider substrate utilization capacity. The isolates were also able to utilize different carbon sources with exception of lactose. Maximum cellulolytic activity was observed at 37 °C using Congo red test that exhibited a clear zone of 4 mm by *S. albospinus* (MTCC 8768) and 3 mm by *S. somaliensis* (MTCC 8769) around the colonies. Optimum pH was 6.8 at which the amount of reducing sugar released was 9.2 mg/ml by *S. albospinus* (MTCC 8768) and 8.3 mg/ml by *S. somaliensis* (MTCC 8769). *S. albospinus* (MTCC 8768) showed a better adaptability for different media and carbon sources that makes it more suitable to be used for commercial production of the enzyme.



## INTRODUCTION

Cellulosic material is the most abundant renewable carbon source in the world and as reported by HAO *et al*. (2006), cellulose may be hydrolyzed using enzymes to produce glucose, which can be used for the production of useful end products such as ethanol, organic acids and other chemicals. Actinomycetes being one of the known cellulose producers have attracted considerable research interest due to its potential applications 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). Filamentous bacteria of the genus Streptomyces, are able to degrade lignocelluloses found in soil and composts also by the activity of cellulase enzyme (Herai *et al,* 2004; George *et al.*, 2010). According to Fan *et al.* (1987), successful utilization of the renewable resources is dependent on the development of an economically viable process which would include the production of cellulases required for the enzymatic hydrolysis of cellulosic materials. The enzyme production is the major cost in the whole process of enzymatic hydrolysis of cellulose (Ghose and Ghosh, 1979). According to Tholudur *et al.*, (1999), cellulase production in cultures is growth associated and is influenced by various factors and their interactions can affect cellulase productivity. Considerable progress has been made in optimization of culture conditions (Olsson *et al.,* 2003), and mode of cultivation (Weber *et al*., 2005). The production of cellulases using various substrates and nutrients by microorganisms has been reported by Macris *et al.* (1989) and Krishna, (1999). According to Palmer, (1975), the temperature also plays an important role in hydrolysis as it affects the rate of hydrolysis as well as the stability of the enzyme. The pH of an organism's environment has the maximum influence on the bacterial growth. It limits the synthesis of the enzymes responsible for synthesizing the new protoplasm as reported by Dubey and Maheshwari, (2004). Enzymes have an optimum pH within which their activity is maximum and at higher or lower pH values, their activity decreases (Lehinger, 1993). In light of the utility and importance of the cultural and environmental conditions on growth and cellulase production, the present investigation was conducted to screen the selected isolates *S. albospinus* (MTCC No. 8768) and *S. somaliensis* (MTCC No. 8768) for their ability to utilize the nutrients and the various carbon sources for their optimum growth. The influence of temperature and pH on the growth and cellulase production by these strains was also studied. Such processes would help in selection of appropriate medium for production of the required culture and according to Makoi and Ndakidemi, (2008), alleviate shortages of food and animal feeds, solve modern waste

***Corresponding author:** tanujasinghpatna@yahoo.com

disposal problem, and diminish man's dependence on fossil fuels by providing a convenient and renewable source of energy in the form of glucose which can be used for the production of ethanol, organic acids, and other chemicals.

## 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, were prepared in distilled water and 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 preservation of the isolates. Cultural characteristics of the selected strains were observed on 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); modified Cellulose agar replacing carboxymethylcellulose in CMC agar with cellulose; Stanier's basal medium [$(NH_4)_2SO_4$ 1g/l, $K_2HPO_4$ 1 g/l, $MgSO_4$ 0.2 g/l, $CaCl_2$ 0.1 g/l, $FeCl_3$ 0.02 g/l]; Mcbeth medium ($K_2HPO_4$ 1 g/l, $CaCO_3$ 2 g/l, $Na_2SO_4$ 2 g/l, $MgSO_4.7H_2O$ 1 g/l, [$(NH_4)_2SO_4$] 2 g/l, CMC 1.0 %, Agar 15 g/l); Casein Starch Peptone Yeast Malt Extract (CSPY-ME) medium ($K_2HPO_4$ 0.5 g/l, Casein 3 g/l, Maize starch 10 g/l, Peptone 1 g/l, Yeast extracts 1 g/l, Malt extracts 10 g/l, Agar 15 g/l); Starch Casein Agar (Soluble starch 10 g/l, Casein 0.3 g/l, $K_2HPO_4$ 2 g/l, $CaCO_3$ 0.02 g/l, $FeSO_47H_2O$ 0.01 g/l, $KNO_3$ 2 g/l, $MgSO_47H_2O$ 0.05 g/l, NaCl 2 g/l, Agar 18 g/l) [Dubey and Maheshwari, 2004] and Cellulose Congo Red Agar with slight modification ($K_2HPO_4$ 0.50 g/l, $MgSO_4$ 0.25 g/l, cellulose powder 1.88 g/l, Congo red 0.20 g/l, Agar 15 g/l, Gelatine 2 g/l).

### Microorganisms

Two cellulose degrading strains of *Streptomyces* isolated from the municipal wastes and identified 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) were used in the present study. They were maintained on slants of Nutrient Agar (NA) at 4 $^0$C with periodic sub culturing.

### Influence of media on growth of the isolates

The selected strains were inoculated on the slants of CMC agar, modified Cellulose agar, Stanier's Basal medium, Mcbeth medium, Starch Casein Agar, CSPY-ME medium and Cellulose Congo Red Agar; and incubated at 37$^0$C for 7 days. The cultural characteristics were observed.

### Influence of carbon sources on growth of the isolates

The isolates were inoculated separately in mineral salt medium supplemented with 0.5% (w/v) of each of the different carbohydrates as substrates that included dextrose, fructose, sucrose, lactose, mannitol and inositol. The isolates were incubated at 37$^0$C for 7 days. The culture characteristics of the selected isolates on different carbohydrate substrates were observed.

### Influence of temperature on enzyme production

The isolates were serially diluted in sterilized normal saline (0.85%) and direct plating of six fold dilution was done in triplicates on Modified Cellulose agar and CMC agar and incubated at 37$^0$C, 42$^0$C and 55$^0$C for 7 days. The diameter of zone of hydrolysis (in mm) was measured by zone-scale (HiMedia) after every two days.

### Influence of pH on enzyme production

50 ml of CMC broth with pH values 5.0, 5.7, 6.8, 8.0, 9.0 and 11.0 was inoculated separately with the selected isolates and incubated at 37 $^0$C for 7 days. 10 mL of the samples of the culture broth were taken aseptically and centrifuged at 10,000 rpm for 15 minutes at the room temperature. The supernatant decanted was served as a standard enzyme preparation. The endoglucanase activity was measured according to the method described by Wood and Bhat (1998) with slight modifications. 1.0 mL of enzyme solution mixed with 1.0 mL of 0.55% CMC in sodium citrate buffer was incubated 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 by Colorimeter (Systronics). The amount of sugar liberated at each of the selected pH was estimated by using a calibration curve for glucose (1-10 mg/ml).

## RESULTS

### Influence of media on growth of the isolates

Morphological observations of the isolates *S. albospinus* (MTCC No. 8768) and *S. somaliensis* (MTCC No. 8769) on media CMC agar, modified Cellulose agar, Stanier's Basal medium, Mcbeth medium, Starch Casein Agar, CSPY-ME medium and Cellulose Congo Red Agar are recorded in Table-1 and Table-2, respectively. *S.albospinus* (MTCC No. 8768**)** showed luxuriant growth on all the media tested except Starch Casein Agar (SCA) and Casein Starch Peptone Yeast Malt Extract (CSPY-ME) on which the growth was moderate and poor, respectively. The colour of colony in all the tested media was white except Cellulose agar and CSPY-ME on which it was dirty white. The texture of the colony was powdery in all media used except SCA and CSPY-ME on which it was dry. No pigmentation was observed in any of the tested media as shown in Fig.1 (a). All the tested media except the Cellulose Congo Red agar were favourable for *S. somaliensis* (MTCC No. 8769) and supported luxuriant growth. The colour of colony on all the tested media was white except Cellulose agar on which it was orange. The texture of the colony was powdery on all media used except SCA on which it was slimy. Orange coloured pigmentation was observed on the reverse side of the colony on Cellulose agar (Table-2).

### Influence of carbon sources on growth of the isolates

Morphological observations of the isolates *S. albospinus* (MTCC No. 8768) and *S. somaliensis* (MTCC No. 8769) on

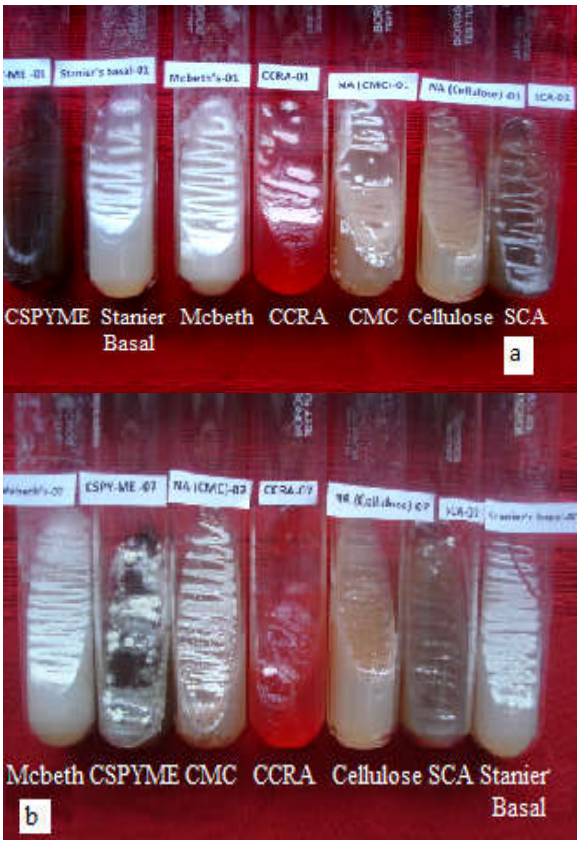


**Fig.1. Growth of isolates on different test media**
(a) *S. albospinus* (MTCC No. 8768); (b) *S. somaliensis* (MTCC No. 8769)

different carbon sources that included dextrose, fructose, sucrose, lactose, inositol and mannitol are recorded in Table 3 and Table 4 respectively. *S. albospinus* (MTCC No. 8768) showed luxuriant growth on dextrose, fructose, sucrose, inositol and mannitol substrates. The growth was poor on lactose. The colour of the colonies was white and the texture powdery on all carbon sources used. The peach coloured pigment started to appear on the reverse side of the colony from 6$^{th}$ day as shown in Fig. 2 (a-f). *S. somaliensis* (MTCC No. 8769) showed luxuriant growth on sucrose and inositol. The growth was moderate on dextrose, fructose, mannitol and poor on lactose substrates. The colour of the colonies was white and the texture powdery on all carbon sources used except lactose on which it was dry. The orange coloured pigment started to appear on the reverse side of the colony from 6$^{th}$ day as shown in Fig. 3(a-f).

### Influence of temperature on enzyme production

The selected isolates were plated in triplicates on Modified Cellulose agar and CMC agar and incubated at 37$^0$C, 42$^0$C and 55$^0$C for 7 days and the diameter of zones of hydrolysis (in mm) was observed. The diameters of clear zone around colonies on Modified Cellulose agar were 4.0 mm for the *S. albospinus* (MTCC No. 8768) and 3.0 mm for *S. somaliensis* (MTCC No. 8769) at 37$^0$C. At 42$^0$C, it was 3.0 mm and 2.0 mm for *S. albospinus* (MTCC No. 8768) and *S. somaliensis* (MTCC No. 8769), respectively. The same on CMC agar were 3.0 mm and 2.0 mm for both the isolates at 37$^0$C and 42$^0$C respectively. None of the isolates showed hydrolysis at 55$^0$C on either of the media (Table 5).

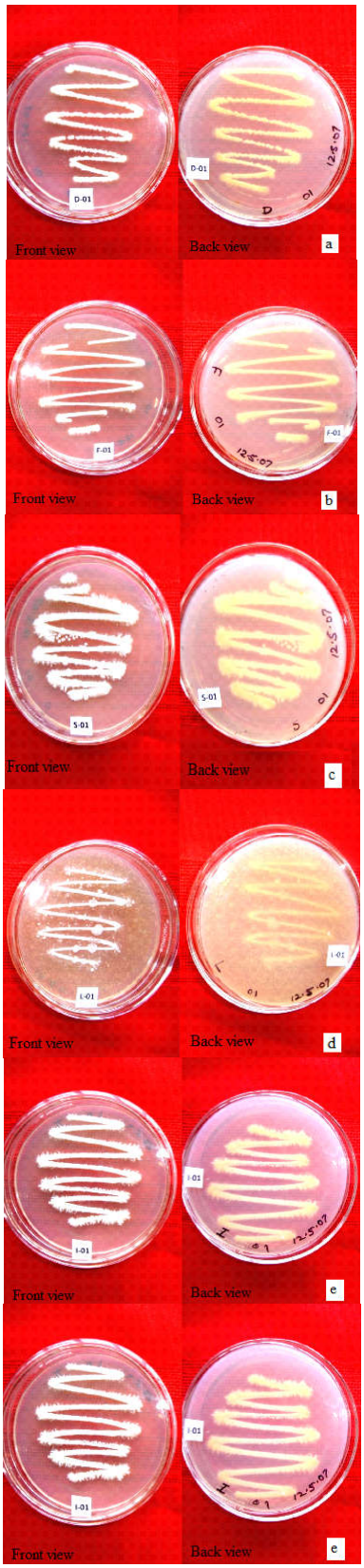


**Fig. 2. Front view and back view of growth of *S. albospinus* (MTCC No. 8768) on different carbon sources (a) Dextrose, (b) Fructose, (c) Susrose, (d) Lactose, (e) Inositol and (f) Mannitol.**

### Influence of pH on enzyme production

The selected isolates were separately inoculated in CMC broth having pH ranging between 5.0 and 11.0 and incubated at

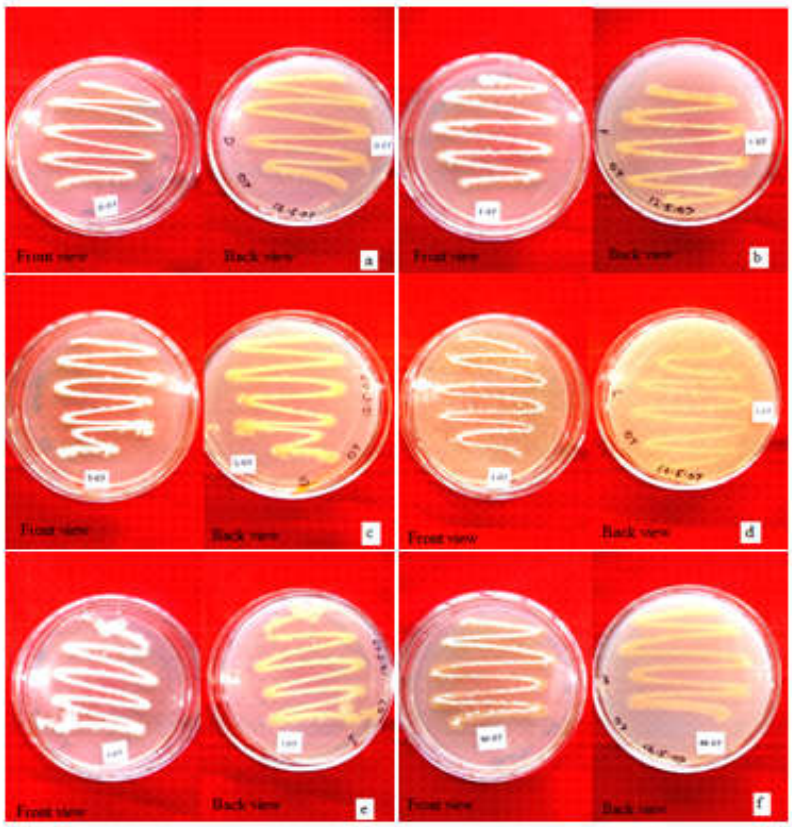

**Fig. 3. Front view and back view of growth of *S. somaliensis* (MTCC No. 8769) on different carbon sources,**
(a) Dextrose, (b) Fructose, (c) Susrose, (d) Lactose, (e) Inositol and (f) Mannitol.

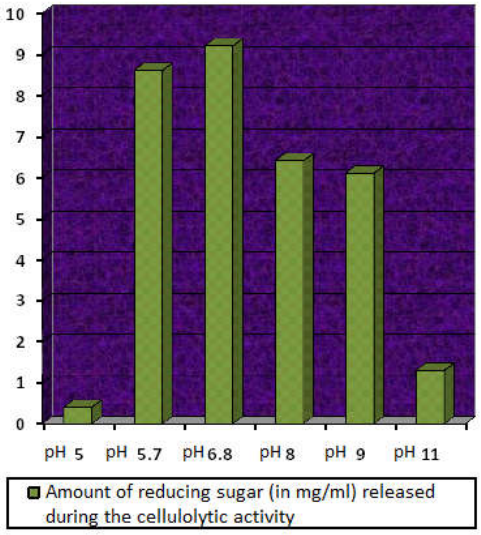


Fig. 5. Amount of reducing sugar released by *S. albospinus* at different pH of the medium.

**Fig. 4. Optical Density of the different strengths of glucose solution (1-10 mg/ml) at 540 nm.**
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.

37 $^{0}$ C for 7 days. For the strain *S. albospinus* (MTCC No. 8768), the optical density at 540 nm on 7$^{th}$ day for endoglucanase activity was 0.003, 0.056, 0.060, 0.042, 0.040 and 0.009 at pH 5.0, 5.7, 6.8, 8.0, 9.0 and 11.0, respectively. With the help of standard curve of glucose (1-10 mg/ml) shown in Fig.4, the amount of reducing sugar obtained at pH 5.0, 5.7, 6.8, 8.0, 9.0 and 11.0 was found to be 0.4 mg/ml, 8.6 mg/ml, 9.2 mg/ml, 6.4 mg/ml, 6.1 mg/ml and 1.3 mg/ml, respectively as illustrated in Fig. 5. The optimum pH for cellulase activity was found at pH 6.8. The enzyme activity gradually increased when increasing the pH up to the optimum followed by a gradual fall in activity. For *S. somaliensis* (MTCC No. 8769), the optical density at 540 nm on 7$^{th}$ day for endoglucanase activity was 0.002, 0.043, 0.054, 0.042, 0.042 and 0.041 at pH 5.0, 5.7, 6.8, 8.0, 9.0 and 11.0, respectively. With the help of standard curve of glucose the amount of reducing sugar obtained at pH 5.0, 5.7, 6.8, 8.0, 9.0 and 11.0 was found to be 0.2 mg/ml, 6.8 mg/ml, 8.3 mg/ml, 6.4 mg/ml,

6.4 mg/ml and 6.3 mg/ml, respectively as illustrated in Fig. 6. The optimum pH for cellulase activity was found at pH 6.8. The results showed that the enzyme production and activity.

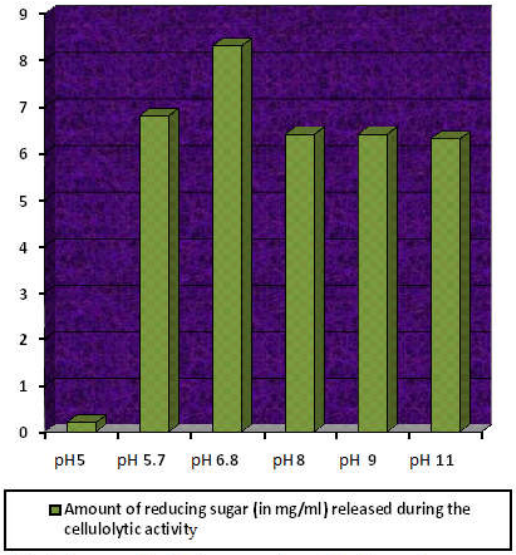


Fig. 6. Amount of reducing sugar released by *S. somaliensis* at different pH of the medium.

**Table 1. Cultural characteristic of *S. albospinus* (MTCC No. 8768) on different media**

| Medium | Growth of the colony | Colour of the colony | Texture | Pigments |
|---|---|---|---|---|
| CMC | +++ | White | Powdery | x |
| Cellulose agar | +++ | Dirty white | Slimy | x |
| Stanier's basal medium | +++ | White | Powdery | x |
| Mcbeth medium | +++ | White | Powdery | x |
| Starch Casein Agar | ++ | White | Dry | x |
| CSPY-ME | + | Dirty white | Dry | x |
| Cellulose Congo Red Agar | +++ | White | Powdery | x |

- No growth; ++ Moderate growth; + Poor growth; +++ Luxuriant growth

**Table 2 Cultural characteristic of *S. somaliensis* (MTCC No. 8769) on different media**

| Medium | Growth of the colony | Colour of the colony | Texture | Pigments |
|---|---|---|---|---|
| CMC | +++ | White | Powdery | x |
| Cellulose agar | +++ | Orangish | Powdery | Orange |
| Stanier's basal medium | +++ | White | Powdery | x |
| Mcbeth medium | +++ | White | Powdery | x |
| Starch Casein Agar | ++ | White | Slimy | x |
| CSPY-ME | +++ | Dirty white | Powdery | x |
| Cellulose Congo Red Agar | + | White | Powdery | x |

- No growth; ++ Moderate growth; + Poor growth; +++ Luxuriant growth.

**Table 3. Culture characteristics of *S. albospinus* (MTCC No. 8768) on different carbohydrate substrates**

| Carbohydr | Growth | Colour of the colony | Texture of the colony | Pigments (on reverse side) |
|---|---|---|---|---|
| Dextrose | +++ | White | Powdery | Peach |
| Fructose | +++ | White | Powdery | Light Peach |
| Sucrose | +++ | White | Powdery | Peach |
| Lactose | + | White | Dry | Peach |
| Inositol | +++ | White | Powdery | Peach |
| Mannitol | +++ | White | Powdery | Peach |

- No growth; ++ Moderate growth; + Poor growth; +++ Luxuriant growth.

**Table 4. Culture characteristics of *S. somaliensis* (MTCC No. 8769) on different carbohydrate substrates**

| Carbohydr | Growth | Colour of the colony | Texture of the colony | Pigments (on reverse side) |
|---|---|---|---|---|
| Dextrose | ++ | White | Powdery | Orange |
| Fructose | ++ | White | Powdery | Orange |
| Sucrose | +++ | White | Powdery | Orange |
| Lactose | + | White | Dry | x |
| Inositol | +++ | White | Powdery | Orange |
| Mannitol | ++ | White | Powdery | Orange |

- No growth; ++ Moderate growth; + Poor growth; +++ Luxuriant growth

**Table 5. Effect of different temperatures on zone of hydrolysis (in mm) on CMC and Modified Cellulose agar**

| Isolate | CMC agar | | | Cellulose agar | | |
|---|---|---|---|---|---|---|
| | 37ºC | 42ºC | 55ºC | 37ºC | 42ºC | 55ºC |
| *S. albospinus* (MTCC No. 8768) | 3.0 | 2.0 | x | 4.0 | 3.0 | x |
| *S. somaliensis* (MTCC No. 8769) | 3.0 | 2.0 | x | 3.0 | 2.0 | x |

x: no hydrolysis

## DISCUSSION

Recent developments in biochemistry, genetics and protein, as well as in the structure–function relationships of cellulases from bacteria and fungi, have led to speculation and anticipation of their enormous commercial potential in biotechnology and research (Hao *et al.*, 2006). Enzyme production is closely controlled in microorganisms and therefore, to improve its productivity these controls can be exploited and modified. Optimization of fermentation media ingredients and environmental factors for enzyme production is a more convenient and effective strategy, compared to other recent approaches like molecular techniques, to manifest the physiological characteristics to synthesis endoglucanase (Van Zyl, 1985). To establish a successful fermentation process, it is necessary to make the microorganism for overproduction of the desired metabolite (Gautam *et al.*, 2011). Culture condition, therefore, play a prominent role on formation of endoglucanase from bacteria (Levin and Forchiassin, 1995). In the present study, it is investigated how the different media ingredients and culture conditions would make an influence on native strains of *Streptomyces*, isolated from municipal wastes, for enhancing endoglucanase yield. The isolates *S. albospinus* (MTCC No. 8768) and *S. somaliensis* (MTCC No. 8769) were grown on different media and it was found that they were able to grow well in all the media tested as shown in Table 1 and 2. They were also able to utilize different carbon sources with very few exceptions like the lactose (Table 3 and 4). These findings supported their wide range adaptability to different media and carbon sources. Both *Streptomyces* strains were comparatively studied for evaluating their independent capacities on cellulase formation, divulging a new insight for a strong suitability of these isolates for endoglucanase production on large scale fermentation. An increased endoglucanase enzyme activity by these isolates was found at $37^0$C which decreased with increasing temperature (Table 5). Both the isolates showed maximum enzyme production and endoglucanase activity at neutral pH as shown in Fig. 5 and 6 as compared to acidic and alkaline environment. The results emphasize on the optimization of environmental and cultural conditions that would be beneficial in commercialization of this process. Findings indicated the wide range adaptability of both the isolates to different media and carbon sources, however *S. albospinus* (MTCC No. 8768) as compared to *S. somaliensis* (MTCC No. 8769) showed a wider adaptability as well as production of endoglucanase and thus comparatively was more suitable to be used for commercial production of the enzyme.

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