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RESEARCH ARTICLE

# Optimization of Process Parameters for Carboxymethyl Cellulase Production under Submerged Fermentation by *Streptomyces lividians*

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## Abstract

Production of carboxymethyl cellulase (CMCase) by *Streptomyces lividans* was detected on CMC agar medium after 4 d of incubation at 28°C, which showed a clear zone of 25 mm around the colony. CMCase production was assayed by measuring the amount of glucose liberated (µmol/mL/min) by dinitrosalicylic acid method. The highest crude enzyme activity (22.37 U/mL) was observed after 72 h of incubation at pH 7.0 and 28°C in a medium that was supplemented with 0.5% medium-viscosity CMC, 1.0% peptone and 0.5% Tween-80. However, enzyme production sharply decreased at 50°C and at pH 5.0. Enzyme production and its activity were also reduced when *S. lividans* was grown in CMC broth supplemented with starch and asparagine as a sole carbon and nitrogen source respectively.

**Keywords:** Carboxymethyl cellulose, *Streptomyces lividans*, dinitrosalicylic acid, peptone, enzyme production.

## Introduction

Every year, cellulose accumulates in large quantities in the form of agricultural, forest and residual wastes (Mandels, 1975). Owing to the abundance and renewability in nature, there has been a great deal of interest in utilizing cellulose as an energy resource and as a feedstock. Cellulose is composed of glucose units linked together by a 1,4-D-β-glycosidic bond (Heck *et al*., 2002). It is a synergistic enzyme used to break up cellulose into glucose or other oligosaccharide compounds and has three major components viz. endo1,4-β-glucanase (EC 3.2.1.4), exo1,4-β-glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) of which endoglucanase is a well-known component. Biotechnological applications of cellulases began in the early 1980s in animal feed followed by food products (Harchand and Singh, 1997). Today, these enzymes account for approx. 20% of the world's enzyme market. In the early years, cellulase research was mainly focused on fungi. Bacterial cellulolysis has recently gained importance as potential source for development of commercial processes because of its high growth rate, high genetic variability, adaptability and high amenability to genetic manipulation (Chowdhury *et al*., 1980; Veiga *et al*., 1983). Actinomycetes comprise an extensive and diverse group of gram-positive, aerobic mycelial bacteria that have an important ecological function in soil cycles. They are widely distributed in soils, where they have an important role in the degradation of lignocelluloses of plant cell walls. Actinomycetes, in particular *Streptomyces* sp., are a widely distributed group of bacteria that have a number of properties that favor them in competition with other saprophytic microorganisms (Williams *et al*., 1983).

*Streptomyces* sp. substantially hydrolyzing crystalline cellulose like avicel have been reported (Schwarz *et al*., 2001). Yet, most of the *Streptomyces* sp. are only able to initiate degradation of crystalline cellulose or to use a soluble form of the polymer (e.g., carboxymethyl cellulose) and cellodextrins (Tomme *et al*., 1995). *Streptomyces flavogriseus*, *S. afghaniensis*, *S. lividans* (Kluepfel *et al*., 1986), *S. reticuli* (Schrempf and Walter, 1995 ), *Streptomyces* sp. strains M7a and M7b (Semedo *et al*., 2000) and *Streptomyces* sp. F2621 (Tuncer *et al*., 2004) have already been reported to produce extracellular carboxymethyl cellulase (CMCase) significantly in optimized culture media using cellulose powder as carbon source under shaking conditions. Culture conditions, therefore, have a prominent role in the formation of CMCase from bacteria (MacKenzie *et al*., 1984; Levin and Forchiassin, 1995). Against these backdrops, this study mainly focused on the enhanced production of CMCase through different media ingredients and culture conditions.

## Materials and methods

*Isolation and identification of Streptomyces:* A total of 125 soil samples were collected from various locations in Manjavadi Kanavai, the highest bamboo forest cover in Dharmapuri District, TN, India. *Streptomyces* isolates were maintained on Bennett's agar slants. All the isolates were used for CMCase screening studies. *Streptomyces* isolate that showed the largest clear zone in CMC agar plate was characterized morphologically and physiologically according to the International *Streptomyces* Project (Shirling and Gottlieb, 1966; Saadown *et al*., 2008).

*Screening for CMCase-producing Streptomyces:* Each *Streptomyces* isolate was suspended in a sterile vial containing 3 mL distilled water to create a spore suspension of $10^7$ spores per mL. A drop (0.1 mL) from the suspension was cultured on the center of CMC agar plates and incubated for 4 d at 28°C. After incubation, the plates were flooded with an aqueous solution of Congo red (0.1% w/v) for 15 min, then washed with 1M NaCl and kept overnight at 5°C. *Streptomyces* colonies showing clear zones against red color of non-hydrolyzed medium were considered as CMCase producers. Positive isolates were tested again for confirmation (Carder, 1986).

*Production of CMCase in submerged culture: Streptomyces lividans* was inoculated in malt extract agar slants and incubated at 30°C for 7 d. Sterile physiological saline (5 mL) containing 1% (v/v) Tween-80 was added to a 7 d old malt extract agar slant culture of *S. lividans* to obtain a spore suspension. The sterile 100 mL cellulase basal salt medium was inoculated with 3% (v/v) spore suspension ($2 \times 10^7$ spores per mL). The culture was incubated at 30°C for 24 h in an orbital shaker incubator at 120 rpm and used as the inoculum for these studies.

*Fermentation conditions:* The cellulase basal salt medium was used to investigate the effects of nutritional and environmental factors on the growth and CMCase excretion of *S. lividans*. The production medium was modified according to the conditions and factors studied. A volume of 50 mL of the production medium was inoculated with 2% (v/v) of the inoculum in a 250 mL Erlenmeyer flask and incubated at the test temperature (10-50°C) in the orbital shaker incubator at 120 rpm for a certain time (0–112 h), as per the study design. Culture conditions were optimized by changing one independent variable at a time while keeping the other variables constant. All the experiments were performed in triplicates. CMCase activity was then assayed by DNS method.

*CMCase assay:* The reaction mixture comprising 0.2 mL crude enzyme solution and 1.8 mL of 0.5% (v/v) CMC in 50 mM sodium phosphate buffer (pH 7.0) was incubated at 50°C in a shaking water bath for 30 min. The reaction was terminated by adding 3 mL dinitrosalicyclic acid (DNS) reagent (solution A: distilled water (1416 mL), 3,5-dinitrosalicyclic acid (10.6 g), NaOH (19.8 g); solution B: Rochelle salt (306.0 g), phenol (7.6 mL), sodium metabisulfite (8.3 g). Solutions A and B was mixed and the tubes were boiled for 5 min. The color developed was measured at 575 nm in a spectrophotometer against the blank containing all the reagents except the crude enzyme (Miller, 1959). Results were calculated as units per mL in which 1 unit of activity was the amount of CMCase required to liberate 1 µmol of reducing sugar per min.

*Effect of temperature and pH on CMCase production:* To find out the optimal temperature for the growth and production of CMCase, we incubated the inoculated flasks at different temperatures namely 10, 28, 37, 45, and 50°C in the orbital shaker incubator (120 rpm for 72 h). To study the effect of pH on CMCase production, we used buffers including citrate buffer (pH 5.0 and 6.0), phosphate buffer (pH 7.0) and Tris buffer (pH 8.0–10.0) at a final concentration of 50 mM to adjust the pH of the cellulase basal salt medium. Following the incubation period, CMCase production was assayed using standard DNS method. The temperature and pH values giving the highest CMCase production were used for further studies.

*Effect of various carbon sources on CMCase production:* To study the effect of various carbon sources on CMCase production, we prepared 50 mL basal salt medium (pH 7.0) in 250 mL Erlenmeyer flasks. Instead of cellulose, the basal salt medium was supplemented with 1% (w/v) of one of the following carbon sources: cellobiose, CMC, fructose, glucose, inulin, lactose, maltose, starch, sucrose, and xylose. The amount of CMCase produced was assayed by standard DNS method.

*Effect of various nitrogen sources on CMCase production:* Instead of yeast extract, the medium was supplemented with one of the following nitrogen sources: asparagine, casein, meat extract, tryptone, peptone, urea, $NH_4Cl$ and $(NH_4)_2SO_4$. The concentration of organic and inorganic nitrogen sources used was 0.5% (w/v) and 0.05% (w/v), respectively. The amount of CMCase produced was quantified by standard DNS method.

*Effect of surfactants on CMCase:* Surfactants such as Tween-20, Tween-40, Tween-80, and Triton X-100 were added at a concentration of 1% (v/v) to the basal salt medium. Controls were prepared in the same way without surfactants.

*Statistical analysis:* All data were given as the mean ± SE (standard error) of triplicates ($n$ = 3). Analysis of variance was performed for all data using data analysis tool of Microsoft XLSTAT 2007. Mean values were separated by the least significant differences at an $\alpha$ value of 0.05.

## Results

By using enrichment methods, we recovered 162 *Streptomyces* isolates from 125 soil samples, which were collected from different locations in the bamboo forest of Manjavadi Kanavai. A total of 119 isolates were able to produce CMCase. *Streptomyces lividans* that showed the largest clear zone (25 mm) on CMC agar plate was used for the production of CMCase (Fig. 1). Mature spore chains of *S. lividans* were short, ellipsoidal, and contained more than 10 spores per chain (Fig. 2a).

Fig. 1. Carboxymethyl cellulase activity staining of *S. lividans* on CMC agar.

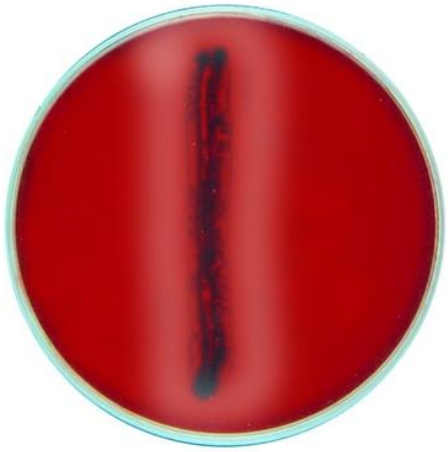


Fig. 2a. SEM showing spore chain morphology and spore surface ornamentation of *S. lividans* (X 5000; 1 µm).

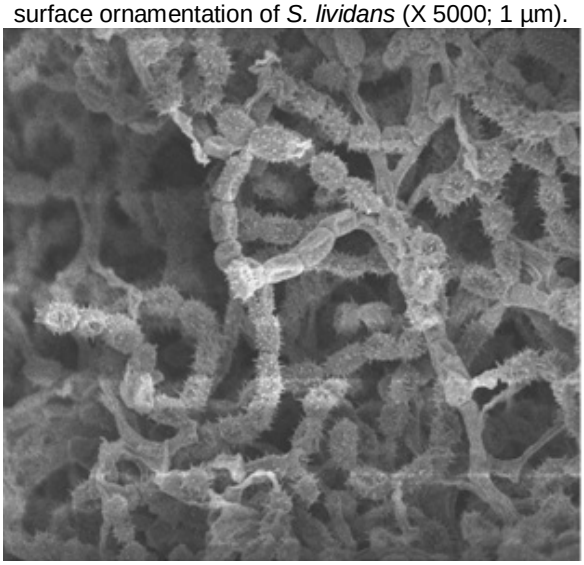
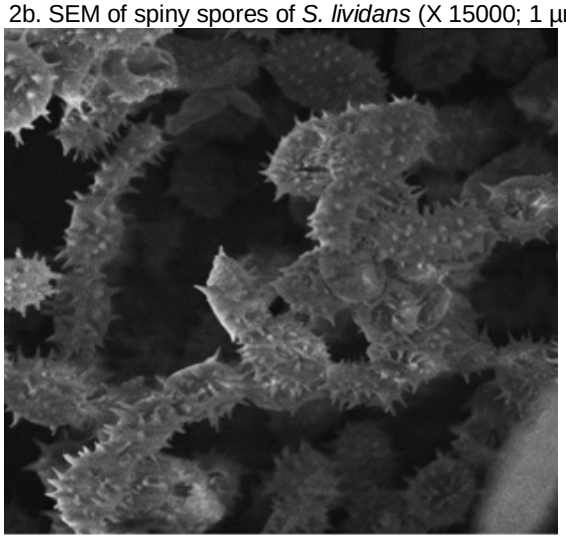


Fig. 2b. SEM of spiny spores of *S. lividans* (X 15000; 1 µm).



Scanning electron microscopy showed *Spirales*-type spore chains with spiny spore sheets. The spiny ornaments were generally straight, but with a short curve at the tip (Fig. 2b). Morphological and physiological characterization of *S. lividans* revealed that it belonged to the gray color series (Fig.3a and b) with a distinctive reverse side color (light brown). Biochemical, physiological and carbon-using characteristics of *S. lividans* are presented in Tables 1 and 2. CMCase production of the isolate gradually raised and reached maximum (8.92-9.62 U/mL) at 64–72 h, after which CMCase activity was found to be slowly decreased (Fig. 4). Microbial biomass was determined by dry-weight method. Dry weight was increased on the first day from 52.5 to 174.69 mg/g cellulose used and reached the maximum at the beginning of the 4$^{th}$ d (361.23 mg) and then decreased. Maximal CMCase activity was observed at 72 h, so in the subsequent experiments, the culture flasks were incubated for 72 h to determine the effect of other parameters on the growth and production of CMCase. The optimum growth and production of CMCase was found at 28°C (Fig. 5). As the fermentation temperature was increased from 10 to 28°C, CMCase activity in the culture supernatant increased from 6.2 to 12.06 U/mL and biomass was increased from 275.12 mg to 439.92 mg/g cellulose used. At 45°C, CMCase activity was lower (11.07 U/mL) and this was parallel with a lower cell growth (400.38 mg) at this temperature.

Fig. 3a. Growth of *S. lividans* on Benett's agar.

Fig. 3b. Close up view of round, umbonate colonies of *S. lividans*.

Table 1. Biochemical and physiological characteristics of *Streptomyces lividians.*

| Characteristics | Result | Characteristics | Result |
|---|---|---|---|
| Melanin production on | | 20-45°C | + |
| Peptone yeast extract iron agar | - | 50°C | - |
| Tyrosine agar | - | pH 4.0 | - |
| Degradation of | | pH 5.0-9.0 | + |
| L-Tyrosine | - | pH 9.5 | - |
| Adenine | + | Growth in the presence of | |
| Xanthine | - | Lysozyme (0.005%, w/v) | + |
| Hypoxanthine | - | Crystal violet (0.001%, w/v) | + |
| Xylan | + | Crystal violet (0.01%, w/v) | - |
| Casein | + | Phenol (0.1%, w/v) | + |
| Guanine | + | Phenol (0.2%, w/v) | - |
| Gelatin | + | Potassium tellurite (0.001%, w/v) | + |
| Starch | + | Potassium tellurite (0.01%, w/v) | - |
| DNA | - | Sodium azide (0.001%, w/v) | - |
| RNA | + | Sodium chloride (4%, w/v) | + |
| Tween-80 | - | Sodium chloride (7%, w/v) | + |
| Esculin hydrolysis | + | Sodium chloride (8%, w/v) | - |
| Growth at | | Thallus acetate (0.001%, w/v) | + |
| 10°C | + | Thallus acetate (0.01%, w/v) | - |

'-' Negative, '+' Positive.

Table 2. Utilization of different carbon sources by *Streptomyces lividans.*

| Carbon source | Utilization |
|---|---|
| L-Arabinose | ++ |
| Adonitol | + |
| Cellobiose | + |
| D-Fructose | + |
| D-Galactose | ++ |
| D-Glucose | ++ |
| D-Maltose | ++ |
| D-Mannitol | ++ |
| D-Mannose | + |
| D-Inulin | - |
| D-Lactose | + |
| Meso-Inositol | + |
| D-Raffinose | ++ |
| L-Rhamnose | + |
| Melibiose | + |
| D-Sucrose | - |
| D-Xylose | ++ |
| Salicin | + |
| Dulcitol | - |
| Xylitol | - |
| Trehalose | - |
| Dextran | + |
| Sodium acetate | + |
| Sodium citrate | + |
| Sodium malonate | - |
| Sodium pyruvate | + |

'-' Negative, '+' Positive, '++' Strongly positive.

*Streptomyces lividans* was able to grow at a wide range of initial pH (between 5.0 and 9.0). As shown in Fig. 6, maximum CMCase activity was obtained with an initial pH of 7.0. As the pH of the fermentation medium was increased from 5.0 to 7.0, CMCase activity increased from 0.64 to 12.17 U/mL. When the pH of the medium was increased to 8.5, the activity of CMCase in the culture supernatant was reduced to 5.26 U/mL.

Fig. 4. Effect of incubation period on the yield of biomass and CMCase production by *Streptomyces lividans.*

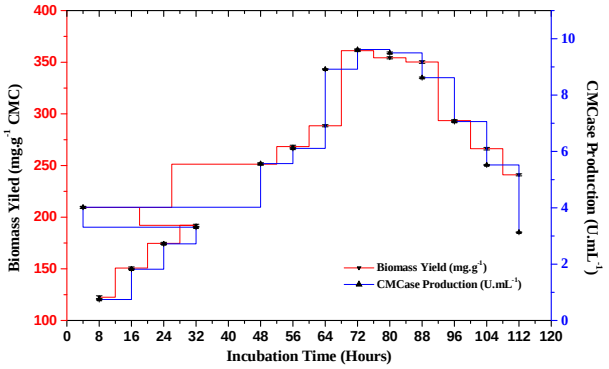



Fig. 5. Effect of incubation tempaerature on the yield of biomass and CMCase production by *S. lividans.*

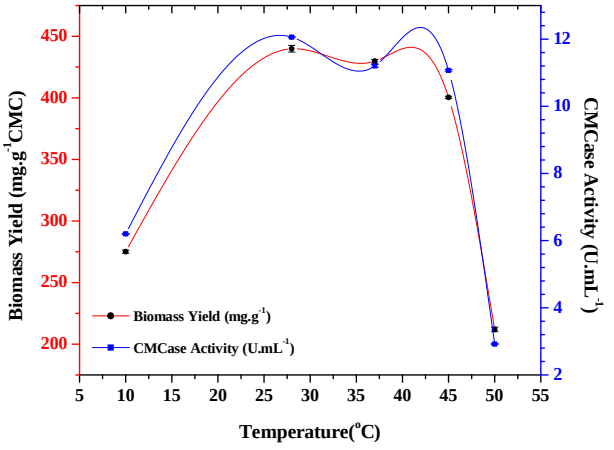


Fig. 6. Effect of initial pH on the yield of biomass and CMCase production by *S. lividans.*

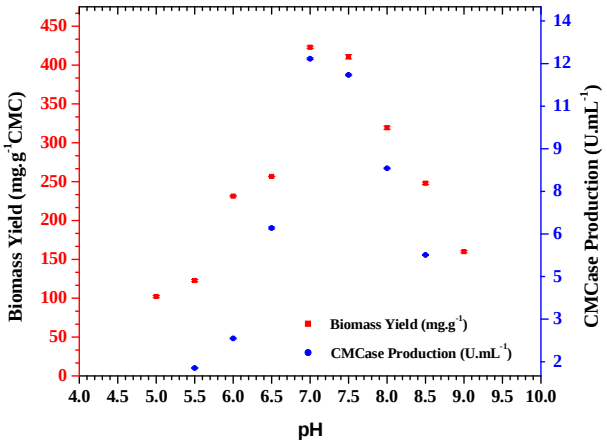
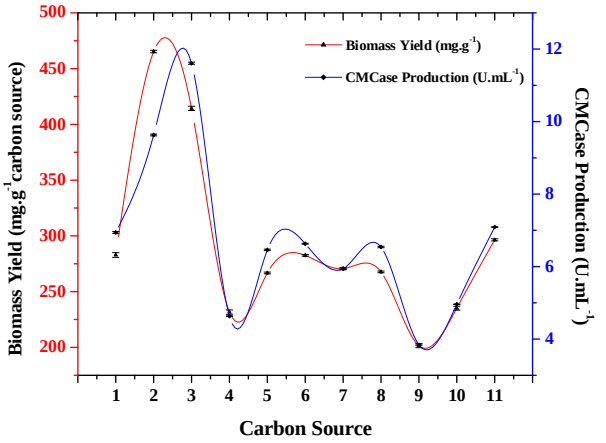



Fig. 7. Effect of different carbon sources on the yield of biomass and CMCase production by *S. lividans.*




1. Cellulose, 2. Glucose, 3. CMC, 4. Fructose, 5. Cellobiose, 6. Inulin, 7. Lactose, 8. Maltose, 9. Starch, 10. Sucrose, 11. Xylose.

The CMCase activity decreased rapidly to 0.83 U/mL when the pH was increased to 9.0. The organism showed good growth in pH range of 6.5-8.0. The maximal cell growth measured was 422.93 mg/g cellulose used at pH 7.0. A comparative study showed that there was a significant variation of growth rate and CMCase production in the culture medium when different carbon sources were used. CMC was the best (11.61 U/mL) among the carbon sources used and the poorest CMCase production was detected with starch (3.84 U/mL). Glucose yielded good cell growth (465.3 mg) but CMCase production was little lower (9.63 U/mL) than CMC (Fig. 7). CMCase produced with various carbon sources was in the following order: CMC > glucose > xylose > cellulose > inulin > maltose > cellobiose > lactose > sucrose > fructose > starch. The best CMCase production was obtained with 0.5% (w/v) medium-viscosity CMC (MV-CMC, 14.47 U/mL). The results showed that MV-CMC stimulated higher yield than high-viscosity CMC. The yield of CMCase decreased in the presence of high concentrations of MV-CMC and the growth became almost stable (Fig. 8).

Fig. 8. Effect of different concentrations of MV-CMC on the yield of biomass and CMCase production by *S. lividians.*

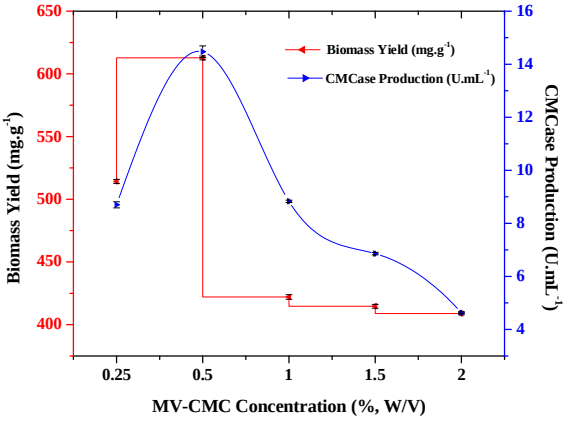
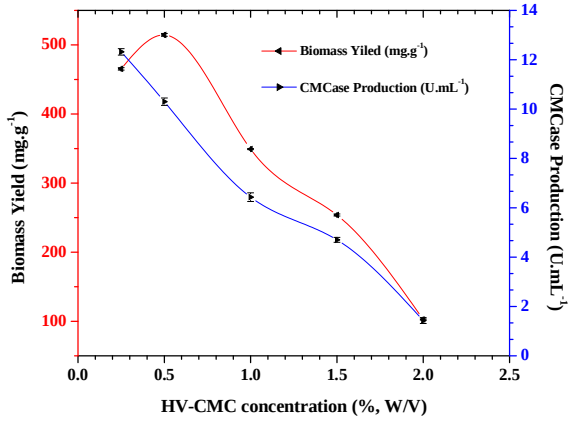
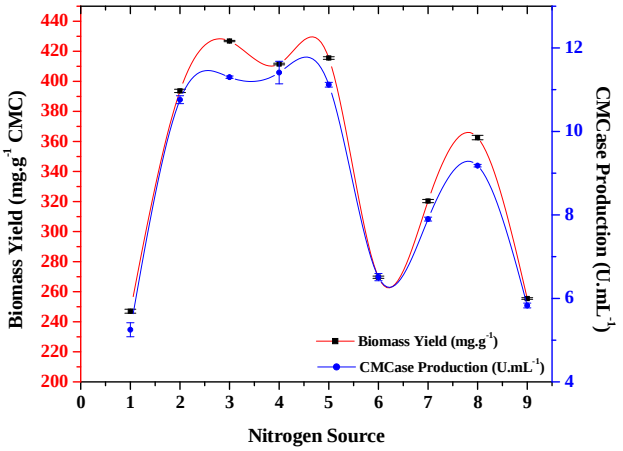



Fig. 9. Effect of different concentrations of HV-CMC on the yield of biomass and CMCase production by *S. lividians.*




The high-viscosity medium led to the arrest of growth (Fig. 9). Basal medium contained 0.2% (w/v) yeast extract as nitrogen source. By replacing yeast extract with different organic and inorganic nitrogen sources at 0.5 and 0.05% (w/v) concentrations, we studied the levels of CMCase production. The results showed that all examined organic and inorganic compounds, except asparagine, stimulated the growth and CMCase production (Fig. 10). The highest production of CMCase was obtained with peptone (11.41 U/mL) followed by meat extract (11.30 U/mL) whereas, the lowest production was seen using asparagine (5.25 U/mL). But the highest biomass yield was obtained with meat extract (426.82 mg) followed by tryptone (415.51 mg) and then by peptone (411.52 mg). *Streptomyces lividans* was grown in the presence of different peptone concentrations (0.25-2.0% w/v). Generally, growth and production of CMCase were obtained at all tested concentrations (Fig. 11). The best CMCase production (12.28 U/mL) was achieved with 1.0% (w/v) peptone. The effect of surfactants on CMCase production by *S. lividans* was studied by adding 1% (v/v) of each of the surfactants in the production medium containing 0.5% (w/v) CMC. It was observed that except for Triton X-100, all other surfactants had exerted positive effect on CMCase production.

Fig. 10. Effect of different nitrogen sources on the yield of biomass and CMCase production by *S. lividians.*




1. Asparagine, 2. Casein, 3. Meat extract, 4. Peptone, 5. Tryptone, 6. Yeast extract, 7. $NH_4Cl$, 8. $(NH_4)_2SO_4$, 9. Urea.

Fig. 11. Effect of different concentrations of peptone on the yield of biomass and CMCase production by *S. lividians.*

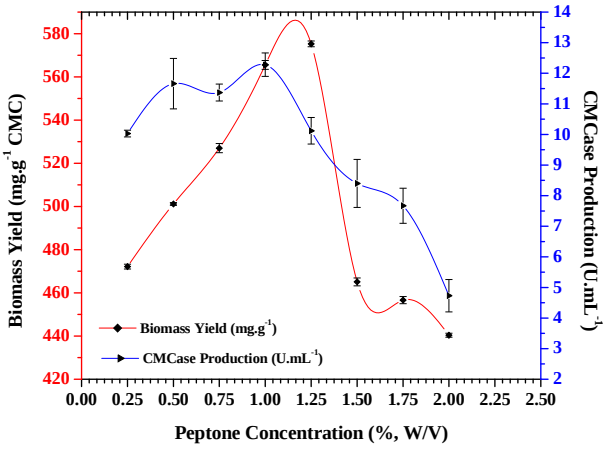



Fig. 12. Effect of surfactants on the yield of biomass and CMCase production by *S. lividians.*

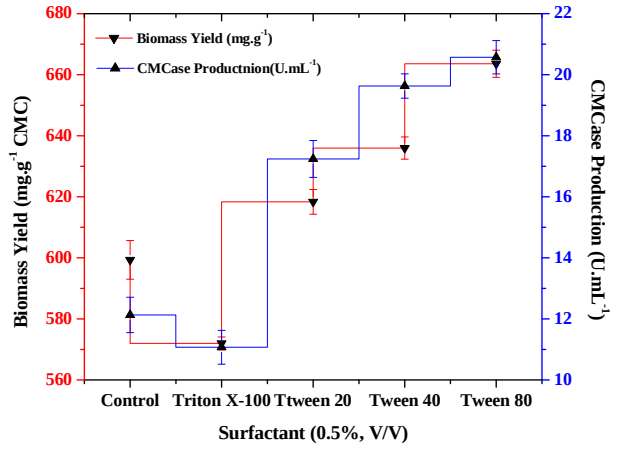
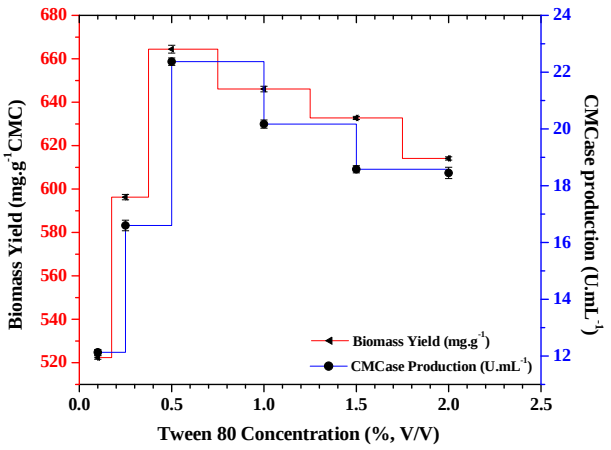



Fig. 13. Effect of different concentrations of Tween-80 on the yield of biomass and CMCase production by *S. lividians.*




When Tween-80 was used, CMCase activity in the culture medium was increased 1.69 (20.57 U/mL) folds when compared to that of control (12.13 U/mL). As shown in Fig. 12, the highest biomass production was obtained with Tween-80 (663.60 mg). The highest CMCase production was obtained with Tween-80 followed by Tween-40 and then by Tween-20. Different concentrations of Tween-80 (0-2% v/v) were added to the production medium to define the optimal concentration of Tween-80, needed to induce maximum CMCase yield. The obtained results showed that an increase of Tween-80 corresponded to a corresponding increase in biomass and CMCase yield (Fig. 13). The highest CMCase yield was obtained in medium containing 0.5% (v/v) Tween-80 (22.37 U/mL).

## Discussion

Soils of bamboo forest, which are rich in lignocellulosic materials. In these soils, actinomycetes are adapted to use different plant polymers as carbon sources by hydrolyzing cellulose, xylan and pectin through enzymatic activity (Semedo *et al.*, 2000). The highest percentage among the other fiber hydrolytic enzymes was of the CMCase. Such a high percentage was also reported (180 *Streptomyces* isolates screened) and found that nearly all the isolates were able to hydrolyze CMC (Wachinger *et al.*, 1989). Selection of those isolates that are able to produce the three fiber hydrolytic enzymes (CMCase, xylanase and pectinase) makes the use of these isolates more efficient. Such a way, the production of these enzymes from a single microorganism makes its industrial and biotechnological application more feasible and economical (Beg *et al.*, 2000). Several actinomycetes reported in the literature have been able to produce cellulolytic enzymes, most of them belonging to the genus *Streptomyces*, such as *S. antibioticus*, *S. flavogriseus*, *S. lividans*, *S. Reticuli*, and *S. hygroscopicus*. Most of the *Streptomyces* cellulases are active against amorphous or soluble cellulose; therefore, being able to produce endoglucanases but unable to degrade microcrystalline cellulose, which requires the presence of exoglucanase.

The main final product of both enzymes is cellobiose, which is the substrate for β-glucosidase. However, *S. reticuli* (Walter and Schrempf, 1996) and *S. hygroscopicus* (Ishaque and Kluepfel, 1980) produce endoglucanases, exoglucanases and β-glucosidases, which allow efficient and synergistic degradation of cellulose to glucose. In another study, maximum expression of CMCase activity was obtained after 120 h incubation of *Streptomyces* sp. (Zhang *et al*., 2006). The CMCase was 11.8 IU/mL with specific activity of 357 IU/mg protein. Similar incubation time (120 h) was reported by other authors (Jand and Chen, 2006). The decrease of CMCase production after 120 h incubation time may be due to catabolite repression by glucose, since the maximum amount of glucose was accumulated at 120 h. The isolate grew with pellet morphology, thereby avoiding the high-viscosity problems encountered with filamentous growths when scaled-up to large industrial bioreactors (Semedo *et al*., 2000). The findings of this study are also in line with another study, which indicated that the temperature optima for CMCase productivity of *Streptomyces* sp. BRC1 and BRC2 was 26°C (Chellapandi and Jani, 2008). Similarly, *Streptomyces* sp. F2621 (Tuncer *et al*., 2004) and *S. albogriseus* (Van Zyl, 1985) also showed maximum cellulase activity at 26 and 30°C, respectively. But the optimal temperature for cellulase activity was in the range of 40-55°C for several *Streptomyces* species including *S. lividans*, *S. flavogriseus*, and *S. nitrosporeus* (McCarthy, 1987). The optimum temperature for CMCase production by *Streptomyces* sp. T3-1 was 50°C (Jang and Chen, 2003), whereas that for cellulase production by *S. reticuli* was 55°C (Schrempf and Walter, 1995).

Higher amounts of cellulase were produced by *Streptomyces* species at pH 7.0 (Ishaque and Kluepfel, 1980). Similarly, *Streptomyces* sp. F2621 and *S. albogriseus* also showed maximum cellulase productivity with initial pH of 6.5-7.0 (MacKenzie *et al*., 1984). The higher levels of cellulase from *S. nitrosporeus* obtained between the pH range 6.7 and 7.2. CMCase produced by *Streptomyces* sp. J2 was found to be active over a pH range of 4.0-7.0 with maximum activity at pH 6.0 (Van Zyl, 1985; Jaradat *et al*., 2008). This result is considerably similar and show that the optimum pH for endoglucanase from a strain of *S. lividans* was 5.5 (Theberge *et al*. 1993). However, the results appeared to contradict with the previous results that an alkaline novel *Streptomyces* species isolated from East African soda lakes have an optimal pH of 8.0 (Solingen *et al*., 2001). Cellulase synthesis of *S. reticuli* was regulated by inductive and repressive action of metabolizable sugars on catalytic domain (Schrempf and Walter, 1995). Concentrations of cellotriose and cellotetrose for *Streptomyces* sp. (Godden *et al*., 1989) and of glucose and cellobiose for *Streptomyces* sp. BRC1 and BRC2 (Chellapandi and Jani, 2008), were investigated as inducers for cellulase production.

It has been reported that the biosynthesis of cellulase is induced when the media are incorporated with cellulose and its derivatives (Messner *et al*., 1991; Kubicek *et al*., 1993). Cellulase induction mainly depends on the presence of low levels of cellulase in the uninduced organisms. The basal cellulase activity would digest cellulose-releasing oligosaccharides that could enter the cell and trigger expression of cellulases (Carle-Urioste *et al*., 1992). The results showed that MV-CMC stimulated higher yield than high-viscosity CMC. This is probably due to high viscosity of the medium, which decreases the oxygen supply to the cells. High viscosity leads to retard cell division, resulting in low production metabolism and CMCase excretion (Zhang *et al*., 2006). The obtained data showed that the organic nitrogen compounds stimulated higher growth and CMCase production than inorganic compounds. This finding probably could be attributed to the lack of amino acids in inorganic compounds. However, the maximum CMCase yield was observed with peptone, which may function as a source of certain essential amino acids to enhance enzyme production (Rakshit and Sahai, 1989). The highest cellulase activity was recorded with *S. omiyaensis* A2 when beef extract was used as a nitrogen source and the lowest when asparagine was used (Alam *et al*., 2004). It was observed that 7.86 U/mL CMCase was produced within 84 h when meat extract was used as a nitrogen source from a strain of *Streptomyces* sp. BRC1 (Chellapandi and Jani, 2008). But in a study, conducted on *Streptomyces* sp. J2, the highest level of cellulase production was achieved when $NH_4Cl$ was added to the CMC medium whereas the lowest yield was observed when yeast extract and asparagine were used as nitrogen sources, yielding relative activities of 35% and 55%, respectively (Jaradat *et al*., 2008). These results are in close agreement with that 0.6% (w/v) peptone which induced the highest CMCase production in the cases of *Chaetomium globosum* and *Trichoderma reesei* (Rakshit and Sahai, 1989; Umikalsom *et al*., 1997). The CMCase yield was decreased in *C. globosum* when the concentration of peptone was increased above the optimal concentration. An excess of peptone in the culture medium may induce proteases that hydrolyze CMCase (Umikalsom *et al*., 1997).

## Conclusion

Among different media ingredients optimized, detergents in production medium showed a reasonable inductive response on CMCase production. *S. lividans* was studied for evaluating its potential of CMCase formation, giving a new insight for a strong suitability of these isolates for CMCase production in large-scale fermentation media. The results also show that the optimized environmental and cultural conditions are prominent needs for producing extracellular CMCase by this soil isolate. This strain is promising for industrial application as it grows quickly in broth condition in simple and low-cost process to enhance production yield and secreted

enzymes are frequently required for industrial applications. Therefore, it is considered as potential industrial candidate for effective saccharification process. Apart from nutrient and environmental factors, surfactants are also considered as good inductive sources for production media optimization. Surfactants are used to increase the permeability of cell membrane by which it enhances membrane transport and excretion of extracellular enzymes in the production media (Okeke and Obi, 1993). However, the lower stimulatory effect was observed with Triton X-100. This may be due to a decrease in oxygen supply, resulting in a diminution of growth (Pardo, 1996). In accordance with the present study results, another study has reported that Tween-80 at a concentration of 0.22% (v/v) was the optimal concentration for the production of cellulase by *Nectria catalinensis* (Pardo, 1996). A maximum CMCase was produced (11.93 U/mL) at 84 h by *Streptomyces* sp. BRC1 and BRC2, when the production medium was supplemented with Tween-80 (Chellapandi and Jani, 2008). To induce a higher cellulase production, 0.1% and 0.2% (v/v) Tween-80 were added to the cellulase production media of *T. reesei* strain QM-9414 and *S. flavogriseus*, respectively (Krishna *et al*., 2000). The use of Tween-80 increased the production level of endoglucanase up to 10 times in *S. reticuli* (Walter and Schrempf, 1996).

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