Research Paper

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# Cellulase Producing *Streptomyces niveus* from Slaughter House Waste

Microbiology

**KEYWORDS :** Rumen Fluid, *Streptomyces niveus*, Cellulase, 16S rRNA sequencing, slaughter house waste, Partial purification.

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## ABSTRACT

*Streptomyces niveus a cellulase producer was isolated from the rumen fluid of goat Capra hircus collected from the slaughter house. 14 potential isolates were screened by primary screening from which 7 isolates were found to have appreciable cellulase activity. Among the isolates CP 05 was found to have appreciable cellulase activity. For further study, CP 05 was chosen as its specific enzyme activity in the crude and partially purified sample was found to be 1.41 U/mg and 1.53 U/mg respectively. The isolate was found to be Streptomyces niveus by 16S rRNA analysis optimization of cellulase production was carried out for different parameters.At 50°C the enzyme activity was maximum. The enzyme activity recorded by the crude and partially purified samples was 0.21 and 0.23 U/ml respectively.A maximum enzyme activity of 0.23 U/ml was obtained at pH 9.0 in the crude extract while in the partially purified sample the activity was found to be 0.24 U/ml. At 1.5% of substrate concentration of carboxymethyl cellulose (CMC) the activity of the crude sample was 0.2 U/ml. Maximum enzyme activity of the crude sample was achieved at 120 hours of incubation (0.16 U/ml).*

## 1. INTRODUCTION

Rumen of goat harbors millions of microorganisms that are important for livestock. They utilize nutrients from plant to generate energy. The microorganisms survive in the rumen under various constraints which is associated to be natural or feed related (Das et al. 2012). Various cellulolytic bacteria, fungi and protozoans make up the rumen microorganisms that feed on materials of plant and produce energy as a return to the host which utilizes it (Toyoda et al.2009).

Degradation of cellulosic materials by cellulolytic bacteria is possible due to the enzyme cellulase. Cellulases are widely used in various industrial applicartions that are of importance (Kowsalyaet al.2013). The demand for cellulases has grown more rapidly than ever before and this demand has initiated research on cellulases.

Due to the rising demand for cellulase for its various applications, it has driven many researchers for the production of cellulase from rumen microbes. This project, therefore, was carried out with the aim to collect, screen, characterize and identify potential cellulase producing microbes from the rumen fluid of goat.

## 2. MATERIALS AND METHODS

### 2.1SAMPLE COLLECTION

The rumen fluid of *Capra hircus* (Domestic goat) was collected from the slaughter house near Perambur. The contents of the rumen were collected in a sterile container and stored at 4°C.

### 2.2 ISOLATION OF CELLULOLYTIC BACTERIA

Six fold serial dilutions of the rumen fluid were prepared in different test tubes with autoclaved distilled water. 1ml of the diluted samples were spread on the carboxymethyl cellulose agar plates that was prepared as recommended by Ray et al. (2007) with modifications and incubated at 37°C.

After incubation over 48 hours the zones around the colonies were identified using congo red. The plates were filled with 1% congo red solution and allowed to stay for 15 minutes which was then counterstained with 1M NaCl (Irfanet al. 2012). The largest zone was considered to have the highest activity (Shaikhet al. 2013).

### 2.3 MAINTENANCE OF PURE CULTURE

The colonies which produced zones and which are significant in size were taken and cultured with 1% CMC (Immanuel et al.2006).

### 2.4 CELLULASE ENZYME PRODUCTION

For the preparation of inoculum, the potential isolates were cultured in a 20ml medium whose composition (g/l) was as recommended by Ray et al. (2007). These inoculum were used for the production media whose composition is similar to the inoculums medium except the concentration of carboxymethyl cellulose which is 1% (Shaikh et al. 2013).

### 2.5 PREPARATION OF THE CRUDE ENZYME EXTRACT

The supernatant of the cultured broth that contains the enzyme was considered as the crude extract which was separated by centrifugation process (Shanmugapriya et al. 2012)

### 2.6 ENZYME ACTIVITY ASSAY

#### 2.6.1 TOTAL PROTEIN DETERMINATION

The enzyme was examined for its protein content using Folin phenol reagent, following Lowry's method (Lowry et al. 1951) where BSA was used as standard.

#### 2.6.2 CELLULASE ENZYME ACTIVITY

The cellulase activity was measured by using DNSA (3, 5-dinitosalicylic acid) method (Ghose, 1987), through the determination of the reducing sugars liberated from carboxymethyl cellulose.

### 2.7 PARTIAL PURIFICATION OF CELLULASE

#### 2.7.1 AMMONIUM SULPHATE PRECIPITATION

80% saturation was attained by adding Ammonium sulphate to the crude enzyme. The mixture was continuously stirred and kept overnight. The mixture was then centrifuged and the pellet was dissolved in appropriate volume of 50mM sodium acetate buffer (pH 5.5) (Lee et al. 2008).

### 2.8 MOLECULAR IDENTIFICATION OF CELLULASE PRODUCING ISOLATE CP 05

#### 2.8.1 ISOLATION OF GENOMIC DNA

The genomic DNA of the isolate CP 05 was extracted by High-salt method and stored in TE buffer at 4°C. The presence of DNA was confirmed by running on a 0.8% agarose gel.

**2.8.2 16S rRNA SEQUENCING**

The isolate CP 05 was identified using 16S rRNA sequencing. The primers, 16S-F (5'GAGTTTGATCATGGCTCAG-3') and 16S-R (5'CTACGGCTACCTTGTTACG-3') were used to amplify the gene where the template was the isolated genomic DNA (Das et al.2012). The amplified gene was checked by agarose gel. The purified PCR product was sequenced. The sequence was analysed by BLAST and the isolate was identified.

**2.9 OPTIMIZATION OF CELLULASE PRODUCTION**

The optimum parameters for cellulase production were determined for the potential isolate. The cellulase production was carried out for different parameters which includes temperature, pH, substrate concentration and incubation period. After fermentation with different parameters the crude extract of each sample was checked for its enzyme activity.

**3. RESULTS**

**3.1 ISOLATION AND PRIMARY SCREENING OF CELLULOLYTIC BACTERIA**

A total of 14 isolates named CP 01- CP14 were obtained from plating the rumen fluid on a minimal agar medium of which 7 were removed due to similar colony and morphological characterization. The remaining 7 isolates were tested on CMC agar plates for their cellulase production. Among the 7 isolates CP 05 and CP 12 were found to be efficient cellulase producers. The zone diameter of CP 05 and CP 12 was found to be 2mm and 1.7mm respectively by congo red assay.

**3.2 SECONDARY SCREENING AND PRODUCTION OF CELLULASE ENZYME**

The potential isolates were then evaluated for their enzyme production in submerged fermentation process. It was found that CP 05 was the most efficient isolate and was chosen for further study.

**3.3 ENZYME ACTIVITY ASSAY**

The total protein content present in the sample was found to be 603 µg/ml. The specific enzyme activity of the crude and partially purified sample measured by DNSA method was found to be 1.41 U/mg and 1.53 U/mg respectively.

**3.4 MOLECULAR IDENTIFICATION OF THE ISOLATE CP 05**

The isolated genomic DNA from the isolate CP05 was used for the amplification of 16S rRNA gene. The amplified product was directly sequenced using forward and reverse primer. The 16S rRNA gene sequence of the isolate CP 05 was compared with 10 different closely related species of Streptomyces sp. available in the GenBank database that showed maximum similarity of 99% with *Streptomyces niveus.*

**3.5 OPTIMIZATION OF CELLULASE PRODUCTION**

The enzyme activity at 50°C was found to be maximum with 0.21 U/ml after which there was a decrease in activity while the partially purified sample showed an activity of 0.23 U/ml at 50°C (Fig-1). Similarly pH is also an important factor that determines the cellulase production. At pH 9.0 a maximum activity of 0.23 U/ml was observed in crude sample whereas for the partially purified enzyme, the activity was 0.24 U/ml at pH 9.0 (Fig-2). Carboxymethyl cellulose plays major role in cellulase production; therefore at a concentration of 1.5% a maximum activity of 0.2 U/ml was obtained (Fig-3). However after 120 hours of incubation a maximum enzyme activity of 0.16 U/ml was obtained (Fig-4).

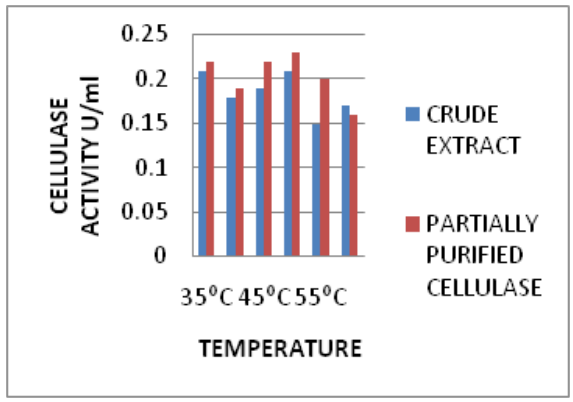


**Fig-1 Effect of temperature on cellulase production by *Streptomyces niveus***

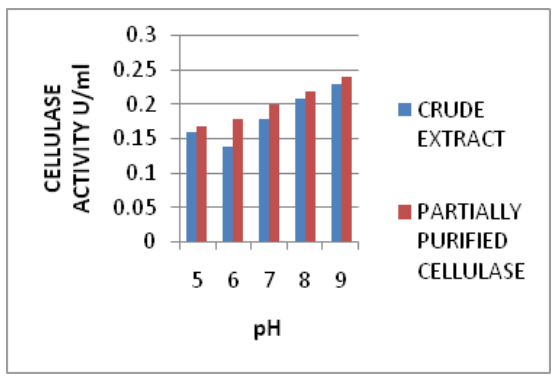


**Fig-2 Effect of pH on cellulase production by *Streptomyces niveus***

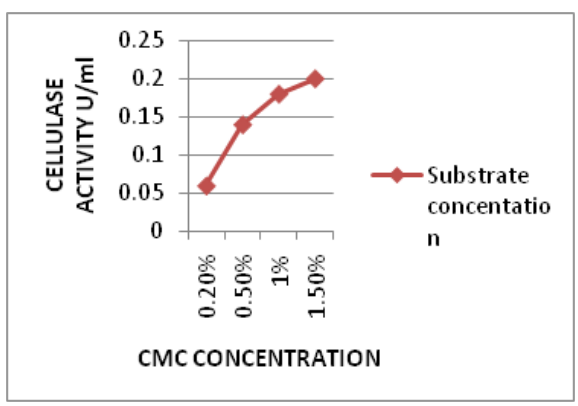


**Fig-3 Effect of substrate concentration on cellulase production by *Streptomyces niveus***

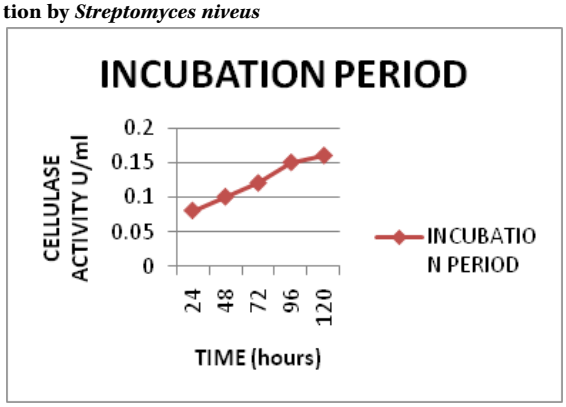


**Fig-4 Effect of incubation period on cellulase production by *Streptomyces niveus***

**4. DISCUSSION**

In this study *Stretomyce niveus* which is a cellulolytic bacterium was isolated from the slaughter house waste i.e rumen fluid. Similarly cellulase producers Bacillus sp and Pseudomonas sp were isolated from municipal and industry wastes by Shaikh et al. (2013). Bacteria producing cellulase from cow dung were also reported by Shanmuga priya et al. (2012). Soil is a natural source of bacteria, from which cellulase producing *bacillus subtilis* was isolated and reported by Kowsalya et al. (2013).

While optimizing cellulase production by *Stretomyce niveus*, it produced maximum activity of 0.21 U/ml at temperature of 50ºC, while the Bacillus sp isolated by Shaikh et al. (2013) showed an activity of 3.5 U/ml at the same temperature of 50°C. The cellulase producers isolated from cow dung showed highest activity at 40ºC (Shanmuga priya et al. 2012).

pH is also an important factor in cellulase production where *Streptomyces niveus* showed highest activity at pH 9.0 while the Cellulomonas sp isolated by Irfanet al. (2012)recorded optimum activity at pH 7.5. *Trichoderma viridae* which produces cellulase presented an optimum activity at pH 8.0 as reported by Iqbalet al (2011).

Carboxy methyl cellulose (CMC) is the subatrate widely used to investigate cellulase producers. 1.5% substrate concentration was utilized effectively by *Streptomyces niveus*. Similarly the Pseudomonas sp utilized0.5% of the substrate and showed maximum activity Shaikhet al. (2013).

*Streptomyces niveus* is a slow growing bacterium hence the effect of incubation period on its cellulase production was 120 hours. While the Bacillus sp and Pseudomonas sp isolated by Shaikhet al. (2013) produced maximum activity at 96 hours.

*Streptomyces niveus* was found to be a potential cellulase producer and it can be used for industrial application after *in vitro* experiments.

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