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Production and Characterisation of Cellulase by *Streptobacillus* species- APS-8

S.V. Rajagopal[1]*, K. Siva Kumar, A. Uma Sankar[2], M. Rama Rao[3] and S. Sunitha Kumari

[1]Department of Biotechnology, [2]Department of Microbiology, [3]Department of Biochemistry, College of Science, GITAM University, Visakhapatnam - 530 045, India.

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A cellulolytic bacterial species APS-8 was isolated from the soils of Visakhapatnam region. This strain is characterized as Gram positive *Streptobacillus* species. The strain grew well in medium containing Carboxymethylcellulose (CMC), producing maximum enzyme at 37°C and pH 8.0. The activity of extra cellular enzyme was found to be high at temperature 50°C and at pH 5.0. Metal ions like $MgSO_4$, $ZnSO_4$ and $FeCl_3$ enhanced the enzyme activity whereas $MnSO_4$ inhibited the enzyme activity under same assay conditions.

Keywords: Cellulase, Congo red, Carboxymethylcellulose (CMC), Carboxymethylcellulase (CMCase) assay, APS-8.

Life on Earth depends on photosynthesis, which results in production of plant biomass having cellulose as the major component[1]. Cellulose is found in nature almost exclusively in plant cell walls, although it is produced by some animals (e.g., tunicates) and a few bacteria[1-2]. Like starch, cellulose is composed of glucose units; whereas all the glucose molecules in a starch chain share the same orientation ("alpha linkages"), adjacent constituents of a cellulose chain are flipped 180 degrees ("beta linkages"). Due to this molecular arrangement cellulose is tough and fibrous and difficult to break down[3]. Cellulase was identified as one of the key enzyme degrading cellulose[4-5] Cellulase refers to a family of enzymes, which act in concert to hydrolyze cellulose[6]. It is produced chiefly by fungi like *Aspergillus, Trichoderma, Cladosporiu*[7] and bacteria like *Clostridium, Ruminococcus*, *Cellulomonas*[1]. Enzymes have been used since the dawn of mankind in various industrial processes. In this regard cellulases have profound applications. In textile industries it is used for keeping colour brightness of the fabrics for longer time and in stone washing of jeans to make them appear faded in very less time[8-10]. Cellulases are used in the Processing of animal feed, coffee beans and in clarification of fruit juice[8].They are also used to bleach the wood pulp and to recycle the paper[11-12].

One of the most recent developments for the use of cellulases has been the production of ethanol for fuel under the trade name Eoethanol[13]. Screening for cellulolytic micro organisms has been restricted for a long time mainly for molds. In this investigation, a new different cellulolytic bacterium that might be suited for any of such a process was isolated and the characterization of enzyme was done using different parameters.

* To whom all correspondence should be addressed.
E-mail: rajsaladi@yahoo.com

MATERIAL AND METHODS

All chemicals used were of analytical grade and obtained from Merck, Glaxo, Qualigens (Mumbai, India). Culture media was obtained from Hi Media (Mumbai, India).

Composition of Congo red agar medium (gm/ 100ml)

The Congo red agar medium contains Gelatin-200mg, Carboxymethyl Cellulose-188 mg, K_2HPO_4- 50mg, $MgSO_4$- 25mg, Congo red - 20 mg, Agar -2g, the pH was adjusted to 7.0.

Isolation of Cellulolytic Bacterial Species

Soil samples were collected from a depth of 5cm from the moist part underneath a stack of decaying hay into a sterile Ziploc bag and serial dilution was carried out. Each dilution (100µl) was plated separately onto Congo red agar medium and incubated at 37°C for 3 to 7 days[14-19]. After 3-7 days of incubation the cellulolytic organism was identified by a yellow halo against a red background (Fig. 1). The colonies with zone of clearance were selected and plated separately on to a fresh medium to obtain a pure culture[20]. A bacterial strain with large zone of hydrolysis was selected and designated as APS-8.

Cellulase Assay

The enzyme assay was performed according to the Method[21]. An aliquot of 0.5 ml of supernatant was taken in a test tube and mixed with 0.5 ml of 1% CMC in 0.1 M phosphate buffer (pH 6.7) and incubated at 50°C for 30 min and the liberated reducing sugar was determined by DNS method [22] and the Cellulase activity is expressed as micro moles of glucose liberated per minute per ml of the culture filtrate[23].

Enzyme production

Production of cellulose is carried out by inoculating ($1X10^8$ Cells/mL) overnight grown APS-8 culture into a 500 ml flask containing 100ml of production media and incubated at 37° C at 150 rpm in orbital shaker (Remi-Ris-24, Mumbai, India) for 7 days. The production medium contained Gelatin-200mg, CMC- 188 mg, K_2HPO_4 -50mg, $MgSO_4$ -25mg, in 100ml of distilled water, the pH was adjusted to 7.0 with 1.0N NaOH. The growth and the cellulase production were determined for every 24hrs. Enzyme production was tested by CMCase method and the cell density by measuring the optical density at 660nm respectively. At the end of each fermentation period, the whole fermentation broth was centrifuged at 10,000g for 15 min (Plastocraft Super Spin- RV/FM High speed, Mumbai, India) and the clear supernatant was used as crude enzyme.

Effect of different parameters such as - temperature (20°C-90°C), pH (2.0-9.0) and varying salt concentrations (1.0mM-5.0mM) on the production of cellulase by APS-8 were also studied .

Characterization of enzyme

Effect of pH on enzyme activity

The activity of cellulase was tested over a pH range from 2.0-12.0 under standard assay conditions using appropriate buffers (0.1M Acetate pH 2.0, Citrate pH 3.0-5.0), Phosphate pH 6.0-7.0, Tris-HCl pH8.0-9.0, Glycine-NaOH pH 10.0 and Carbonate- Bicarbonate pH11.0-12.0).

Effect of temperature on enzyme activity

The effect of temperature on enzyme activity was studied by performing the standard CMCase assay procedure over a temperature range of 20°C-80°C.

Effect of metal ions on enzyme activity

The effect of different metal ions on cellulase activity was studied by using 0.2 M concentration of metals such as Na^+, Fe^{+3}, Mg^{+2}, Mn^{+2}, Co^+ and Zn^{+2}.

Table 1. Morphological characterization of the APS-8

Colony Morphology	
Configuration	Round
Margin	Entire
Surface	Smooth and shiny
Elevation	Raised
Opacity	Opaque
TEST	APS-8
Gram's Reaction	+ve
Cell Shape	Rods
Size(µm)	2.3-3.5µ in length
Arrangements	In chains
Spores	Absent
Motility	Non motile

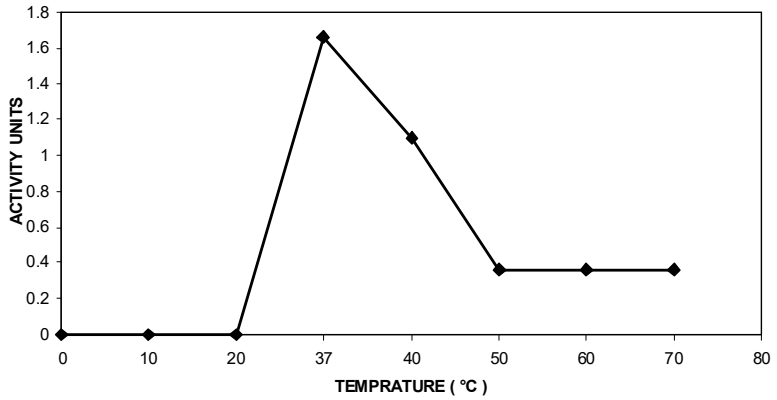

Fig. 3. Effect of temperature on cellulase production

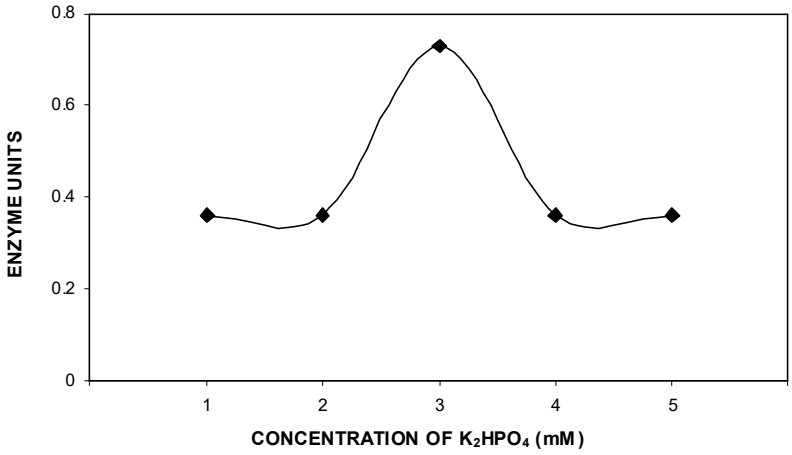

Fig. 4. Effect of varying salt concentration on cellulase production

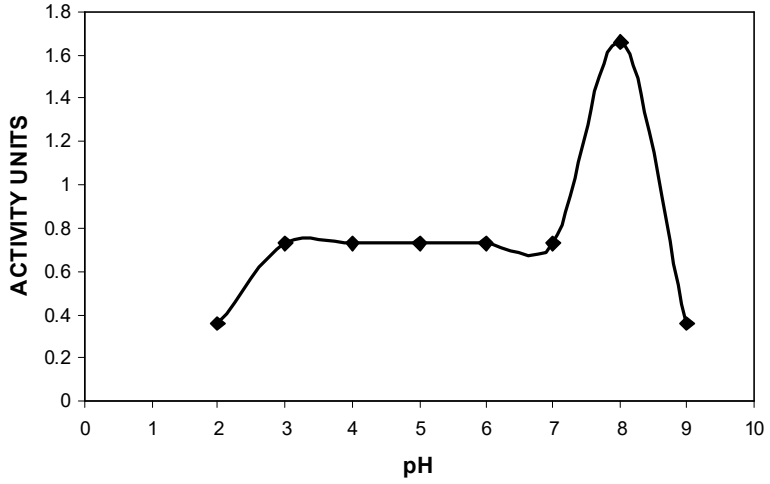

Fig. 5. Effect of pH on cellulase production

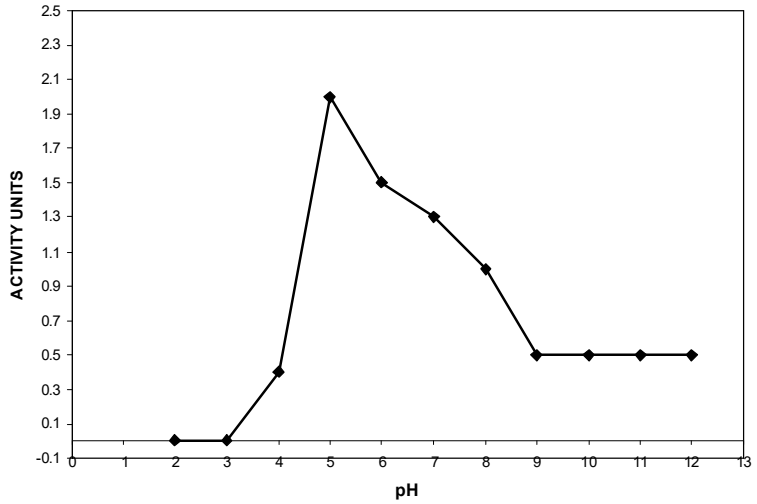

Fig. 6. Effect of pH on cellulase activity

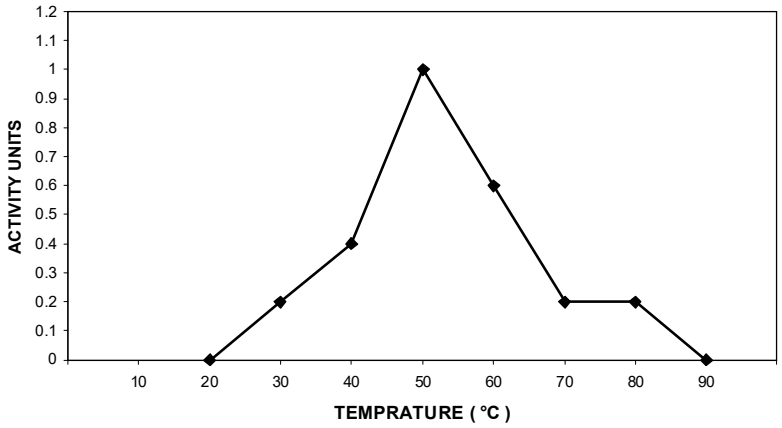

Fig. 7. Effect on temperature on cellulase activity

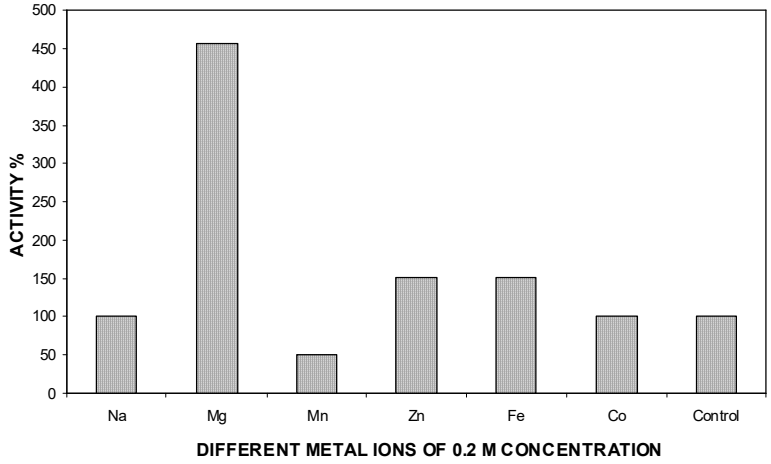

Fig. 8. Effect on metal ions on cellulase activity

enzyme activity significantly. Wher as, $ZnSO_4$ and $FeCl_3$ found to enhance the enzyme activity moderately. However, $MnSO_4$ inhibited the activity by 50%.

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