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Research Article

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Isolation and identification of *Cellulomonas cellulans* from silver fish and characterization of cellulase enzyme

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ABSTRACT

Cellulase enzyme has the ability to hydrolyze the cellulosic materials and play a vital role in the field of Genetic Engineering and Biotechnology. The current research enzyme producing strain was isolated from the gut of the Silver fish. The organism was confirmed as Cellulomonas cellulans. The enzyme was produced using CDA broth and the production of cellulase was about 2.5ml/100ml broth. Further assay of enzyme was done and the enzyme assay was found to be 0.160 mg glucose/min/mg of protein. The molecular weight of the enzyme was determined by SDS PAGE method, the molecular weight of cellulase enzyme was found to be 35 KD, 18KD, 12 KD.

Key words: cellulase, *Cellulomonas cellulans*, Silver fish, CDA, cellulosic

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INTRODUCTION

The microbial cellulose utilization is examined at higher levels of aggregation encompassing the structure and composition of cellulosic biomass, taxonomic diversity (etc). Cellulose utilizing microorganisms are present in soil and in the guts of animal. The carbon cycle is closed by the action of these cellulose utilizing microorganisms. In animals, cellulose acts as a major source of dietary protein [1]. A wide variety of bacteria and fungi utilizes the cellulose and degrades it. Fungi like *Fusarium, Aspergillus sp* and bacteria like *Clostridium thermocellium, Ruminococcus albus* and *Actinomycetes* belong to the genera *Cellulomonas sp*. Cellulose – Cellulose is the most abundant polymer in the biosphere. It occurs in plant biomass and associated with the structural substances such as lignin and hemicellulose. Cellulose is an unbranched polymer of 1000-1 million D-glucose units linked together with β-1, 4 glucosidic bonds. It has a molecular weight of 2, 00,000-20, 00,000 daltons [2]. The cellulosic materials include cotton, wood, baggasse, corncob, animal wastes and waste paper products [3]. Cellulase enzyme is the major factor responsible for the hydrolysis of cellulosic materials. A wide range of bacteria and fungi are capable of degrading cellulose by the action extracellular enzyme cellulase. Some organisms form a multi protein complex called cellulosome, which lies on the external surface of the cell.

EXPERIMENTAL SECTION

Isolation of *Cellulomonas cellulans*
The live silver fish was used as a sample for the present studies. The sample was collected from old book house and this was transferred to sterile screw cap tubes. 1 ml of sterile distilled water was added. The silver fish was smashed

to extract the intestinal organism. A loopful of sample was streaked on czapek's dox media containing cellulose as substrate. These plates were incubated at 37°C for 4-7 days.

Identification of *Cellulomonas cellulans*
For the identification of test strain *Cellulomonas cellulans* isolated from silver fish following preliminary and various biochemical tests were done as per Bergy's manual of determinative Bacteriology [4]. Colony morphology on CDA plate appeared as round, smooth, white, opaque and convex colonies.

Cellulase purification – 1 ml of test strain containing ($100x10^9$) wells were transferred to 100 ml of CDA broth and incubated for 4-6 days at 37°C. 10 ml of broth was taken and centrifuged at 12,000 rpm at 4°C for 15-20 min. The pellet was discarded and supernatant was taken and ammonium sulphate was added at 60% saturation. The contents were centrifuged at 10,000-12,000 rpm for 20 min. The supernatant was discarded and the precipitate was dispensed in eppendorf tube. The contents of the eppendorf tube were transferred to dialysis bag. Dialysis procedure was performed to purify the protein from salt contaminants [5].

Estimation of Cellulase activity by DNS method and Gel diffusion method

Estimation of reducing sugars by DNS method

Preparation of standard sugar - The standard sugar Glucose was prepared by adding 100mg in 100ml of distilled H_2O the con is 1 mg/ml.

Preparation of Working Standard - 0.45 ml of 1% of CMC solution was pipetted out at a temperature of 55°C and 0.05 ml of enzymes extract. A set of test tubes were taken 0.2, 0.4, 0.6, 0.8 and 1 ml of standard sugar solution was added. In one tube working standard was taken. The mixture was incubated at 55°C for 15 min. Keep the enzyme mixture substrate in water bath and 0.5 ml DNS reagent was added and the mixture was heated in water bath for 15 min. While tubes are warm add 1.0 ml potassium sodium tartrate solution. Cool to room temperature; add water to make 5ml volume. Measure the absorbance at 540nm. Standard graph was plotted and enzyme activity was determined [5].

Gel diffusion method
Prepare an agar gel contain 1.7% agar and 1% CMC. The media was poured into the petridish and punched the wells with narrow cork borer. The extracted, purified enzymes were placed in the well and incubated for 2 hours at 30°C. Plates were flooded with 10 ml of 0.1 % aqueous solution of Congo red. Dye was poured off and flooded the plates with 10 ml of 5M Nacl [6].

Determination of molecular weight by SDS polyacrylamide gel electrophoresis
SDS PAGE (10% gel) was done to separate the extracted protein sample and determined its molecular weight using the procedure described [7]**.**

RESULTS AND DISCUSSION

Isolation and Identification of strain *Cellulomonas cellulans* - *Cellulomonas cellulans* isolated from the gut of silver fish was streaked on cellulose czapek dox medium and Growth was observed as white, opaque smooth colonies. Gram staining reported as gram positive coccobacilli catalase positive and it was able to hydrolyse the starch and on peptone Glucose yeast extract agar produced circular, convex, yellow-whitish and glistening colonies which confirmed the strain as *Cellulomonas cellulans* [8].

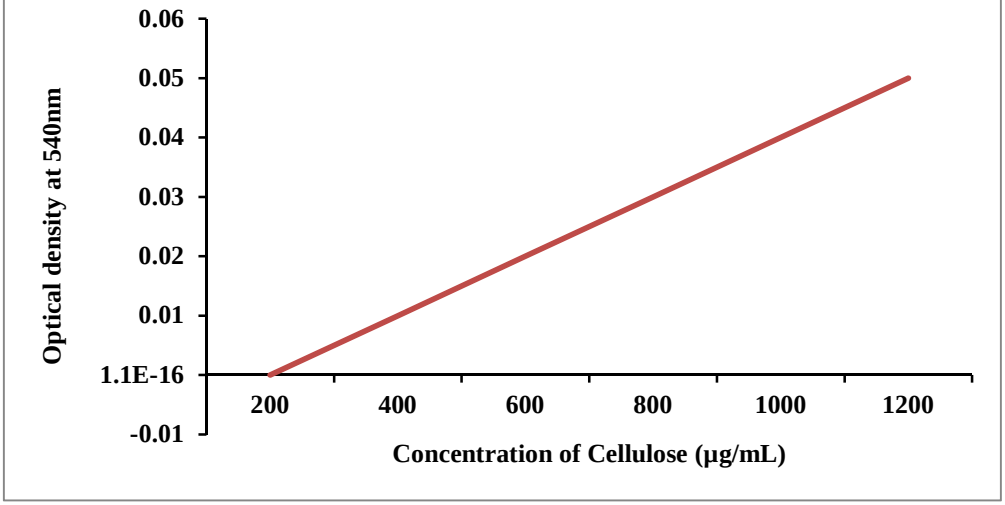
The enzyme produced was further purified from the 7^{th} day broth culture by crude procedure like ammonium sulphate precipitation and dialysis method. The test result showed enzyme production about 2.5ml/100ml broth which was collected and stored as 1ml aliquot in eppendroff tube.
Estimation of Enzyme by Assay method

TABLE 1 - GLUCOSE ESTIMATION OF *CELLULOMONAS CELLULANS*

S.No.	Volume of working standard (ml)	Conc. Of working standard (µg/ml)	Volume of DNS (ml)	Volume of potassium sodium tartarate (ml)	Volume of distilled water (ml)	Optical density at 540 nm
B	-	-	0.5	1.0	3.5	0.00
S_1	0.2	200			3.3	0.01
S_2	0.4	400			3.1	0.02
S_3	0.6	600			2.9	0.03
S_4	0.8	800			2.7	0.04
S_5	1	1000			2.5	0.05
Cellulose in CDA	0.5	500			3.0	0.06

The amount of glucose released is 0.160 mg glucose/min/mg of protein

Fig 1. Glucose Estimation of *Cellulomonas cellulans*



Cellulase production has attracted a worldwide attention due to the possibility of using this enzyme complex for conversion of abundantly available renewable lingocellulosic for production of carbohydrate for numerous industrial applications. Although different fungi and bacteria have been used for the production of cellulase and xylanases, cellulose system of the *Cellulomonas* are increasing in usage in industries. Since high activities of cellulase are reported during growth on cellulosic substrates. In the present study, an attempt was made to isolate the *Cellulomonas spp.* from the intestinal juice of silver fish. One isolate the *Cellulomonas* was isolated on CDA plates as white coloured colonies in streaking. The isolate was further identified by Grams staining, motility, catalase and starch hydrolysis. The strain showed Gram positive, catalase, positive and zone of clearance on starch plates, over layed with 1% iodine solution. The report [9] of the strain isolated from intestinal fluid of the silver fish *Lepisma spp.* in anoxygenic condition in broth culture using whattman 42 filter paper. The microbes appeared as white colonies on a solid medium. Morphologically the bacterium is a Gram positive, non spore forming rod which was identified as *Cellumonas spp*. Enzyme activity was determined by the Dinitrosalicylic acid (DNS) method. The test result showed that the enzyme activity was 0.160 mg of glucose released per min per mg of protein [Table 1, Figure 1]. Similar study was conducted [3]. SDS-Polyacrylamide (10%) Gel was used for separation of purified enzyme using the procedure described by Davis *et al*., 1968. The molecular weight of cellulase enzyme was found to be 35 KD, 18 KD and 12 KD. Cellulase enzyme purified from isolate *Cellulomonas* spp. showed efficient activity by invitro methods which can be produced in a higher amount and can be used in various biotechnology field.

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REFERENCES

[1] LR Lynd; PJ Weimer; WH van Zyl; IS Pretorius, *Microbiol Mol Biol Rev.*, **2002,** 66(4), 739.
[2] T Wang; T Liu; Z Wu, *Acta Biochim Biophys Sin.*, **2004**. 36(10), 667-672
[3] TV Ojumu; BO Solomon; E Betiku; SK Layokun; B Amigun, *African Journal of Biotechnology.*, **2003,** 2(6), 150-152.
[4] JG Holt, NR Krieg, PHA Sneath, JT Staley, ST Williams. Bergey's Manual of Determinative Bacteriology, 9th edition, Williams and Wilkins, Baltimore, **1994**.
[5] S Sadasivam. Biochemical methods, revised second edition, New Age International Publishers, New Delhi, **1996**; 220-227.
[6] T Kauri; DJ Kushner, *FEMS Microbiology Letters.*, **1985,** 31(5), 301-306.
[7] BJ Davis, *Annals of the New York Academy of Sciences.*, **1968,** 28(121), 404-427.
[8] M Ramasamy; M Meyers; J Bevers; H Verchtest, *Journal of Applied bacteriology.*, **1981**, 51, 475-481.
[9] N Chakraborty; GM Sarkar; SC Lahiri, *The environmentalist.*, **2000**, 20(1), 9-11.