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Research Article

CELLULASE PRODUCTION OPTIMIZATION USING CELLULOLYTIC BACTERIA

P. Nisha*

P.G. Department of Biochemistry and Biotechnology, S.S.V. College, Valayanchirangara, Ernakulam, Kerala - 683 556, India.

ABSTRACT

Cellulase enzyme is an important enzyme used in various industries and is very expensive one. Cellulase enzyme is easy to produce from micro organisms. Optimum environmental conditions are varies for each organism for the enzyme production. This study is aimed that, maximum cellulase enzyme production from *Micrococcus sp*bychanging some of the environmental parameters, enzyme activity assayed by Di Nitro Salicylic acid method with glucose as standard. Theparameters studieswere incubation temperature, pH, different concentrations of CMC and incubation time .It was found that, the optimum parameters for higher production of Cellulase enzyme was at 37^{0}C of incubation temperature, pH 8,1.5% of CMC and 72 hr of incubation time,0.9490IU/L.

Keywords: Cellulase, *Micrococcussp,* DNSA,CMC, Enzyme assay.

INTRODUCTION

Cellulase is an enzyme capable to degrade cellulose in the environment.Cellulases degrade cellulose by the enzyme activities such as endoglucanase, which is known ascarboxymethyl cellulose (CMCase) (endo-1,4-β-Dglucanase, EG, EC 3.2.1.4), exoglucanse (known as cellobiohydrolase) (exo-1,4- β –D-glucanase, CBH, EC 3.2.1.91); and β-glucosidase (1,4-β-D-glucosidase, BG, EC 3.2.1.21)[1,2]. Many micro organisms such as bacteria, fungi , actinomycetes and yeasts are capable of producing extracellular cellulase enzyme[3].Number of studies on cellulase enzyme production have been concentrate on fungi than with bacterial strains.[4,5,6,7].Cellulase producing bacteria were isolated from various habitats like soil, hotsprings, organic matters, faeces of ruminants decayed plant materials, and composts to obtain effective enzyme producer[8].Production of cellulase enzyme is very much dependent on Culture and environmental parameters[9] such as temperature, carbon sources , aeration, incubation time, medium ingredients, pH of the medium andcellulose quality[10].

Cellulase is a complex enzyme and has high demand in biotechnological and industrial areas due to its wide range of application.Major applications of cellulases are in textileindustry for 'bio-polishing' of clothes, produce stone washed look of denims and also in household detergents to improve fabric softness and brightness[11].Cellulase could be used in waste water treatment, the pulp and paper industry and in animal feed[12].

Cellulase is also used in various industries like textile, food, detergent and leather industries[4] .Besides these ,which is used in the fermentation of biomass into biofuels[13], fibre modification and they are even used for pharmaceutical applications. Cellulases are used in effluent treatment, wool and dyeing treatment and in cotton preparations .

The aim of this study is to isolate and screening of cellulase enzyme producing bacteria from the polluted water sources. Identify the organism and produce cellulase enzyme using various growth parameters suchasincubation temperature, pH,different concentrations of CMC and incubation time by DNS (dinitro salicylic acid)method to optimize the maximum cellulase production.

MATERIALS AND METHODS

Isolation of organisms

Water samples were collected from polluted water bodies of different places in agricultural fields. Samples were collected in sterile containersand aseptically transfer to the laboratory within an hour. The isolation of organisms was done by Serial dilution total plate count method. Pure cultures of selected colonies were stored in glycerol stock for the further uses.

Screening of cellulose degrading bacteria

Selected colonies were streak plated on CMC agar(Carboxy methyl cellulose -Peptone . 2g, CMC. 2 g , K_2HPO_4 . 4g , Agar .2g , $MgSO_4$ .0.06g , $(NH_2)2SO_4$. 0.50g , Gelatin .0.4g.) plates to screen for cellulase production by qualitative plate assay. After the incubation period of 24hr at 37^0C,CMC agar plates were flooded with 1 % congo red and allowed to stand for 15 min at room temperature and counterstained with 1MNaCl.Clear zone appearingaroundthe growth of bacterial culture indicating positive result of cellulose hydrolysis. The organism able to produce largest clear zonearound was used for the following studies.

Identification of an Organism

The selected organism was identified based on morphological, biochemical and physiological characters according to Bergey's manual of determinative bacteriology.

Crude EnzymePreparation

Pre inoculum was prepared innutrient broth using *Micrococcus sp* ,200µl of pre inoculum was added in to 100ml of sterile CMC broth medium,Incubate the medium at 37^0C for 24hr .1 ml of the broth medium was transferred to micro centrifuge tubes and centrifuged at 4000rpm for 15 min at 4^0C and discard the pellet.Supernatant was used as the crude enzyme source which was stored for further enzyme assay.

Optimization of enzyme production

The influence of environmental factors such as pH, temperature, incubation time and concentration of carboxy methyl cellulose in cellulase production were studied to determine the optimum growth conditions for higher production of an enzyme. The parameters checked were, pH range from 6 to 9 (6, 7, 8 , 9), incubation time (24, 48, 72hrs) and in 5 different concentrations of CMC (0.5, 1, 1.5, 2 , 2.5%).

Carboxy Methyl Cellulose assay

Cellulase assay parameters like pH, temperature and incubation time were checked separately with 0.5, 1, 1.5, 2 , 2.5% of Carboxymethyl cellulose .Cellulase activity was assayed using dinitro salicylic acid (DNS) reagent[14] by estimation of reducing sugars released from CMC solubilised in 0.05 M phosphate buffer at pH 8. 0.5 ml of Crude enzyme added to 0.5 ml of CMC in 0.05 M phosphate buffer and incubated at 50˚C for 30 min. Afterthe incubation time, reaction was stopped by adding 1.5ml of DNS reagent and boiled at 100˚C in water bath for 10 min. By measuring absorbance at 540 nm,sugars liberated were determined. One unit (IU) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1μmol of glucose per minute under standard assay conditions.

RESULTS AND DISCUSSION

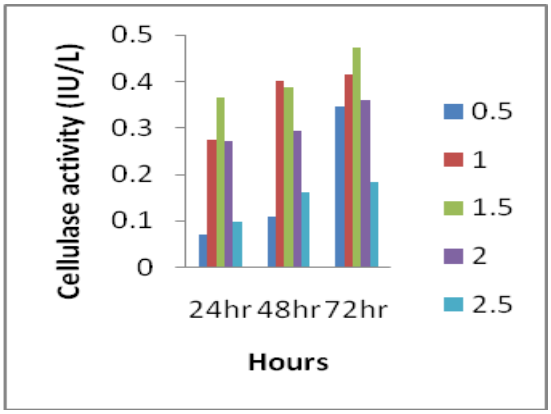
From the result of serial dilution plate count method, 14 different organisms were isolated from the polluted water bodies .All organisms were screened by qualitative plate assay method on Carboxymethyl cellulose and only three organisms were shown zone of clearance around . More clear zone producer was identified as *Micrococcussp* based on Bergey's manual of determinative bacteriology and used as a test organism. Cellulase enzyme producing property of some bacterial species such as *Cellulomonassp*, *Pseudomonassp*, *Bacillus sp and Micrococcussp* were reported[15].

Optimizationresults shown the higher production of cellulase enzyme was0.9490IU/L . pH is the one of the important factor in enzyme production. High acidic and high basic pH values adversely affected enzyme production[16].The pH 6,7,8 and 9 were checked in this study and maximum enzyme production was assayed at pH 8 than the other low and high pH. Mostofthe*Bacillussp* has an optimum pH 5 for higher production of cellulase[17]. Substrate concentration play a major role in production of an enzyme.

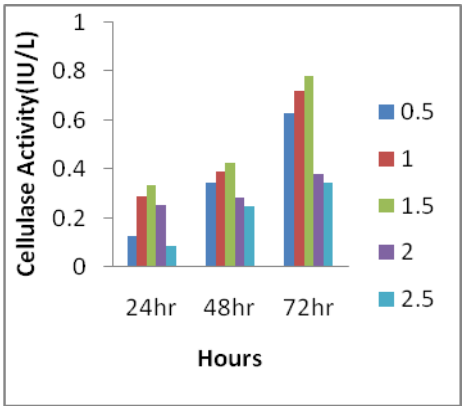
Utilization of CMC as carbon source is best for microbial cellulase production[18]. In present, 5 concentrations, 0.5, 1, 1.5, 2 , 2.5% of Carboxymethyl cellulose were used for study to determine the optimum concentration of Carboxymethyl cellulose and higher enzyme activity. The higher enzyme activity was at 1.5% and enzyme produced more at 72hr of incubation time.Lesser activity was shown in 24hr and 48hr of incubation time. Some of *Streptomyces sp*. has been reported to produce higher cellulase enzyme in 72-120 hr of fermentation[19,20,21]. 37˚C was the optimum

temperature for *Micrococcus sp*formore enzyme production than 27˚C. Optimum temperature for higher cellulase production was 30°C and the lowest yield was achieved at 45 °C[22].*Cellulomonas* ASN2 isolated from soil[23] exhibited its optimum cellulase activity at pH of 7.5 and temperature of 60˚C[24] *Bacillus* strain M9 and NZ showed more enzyme activity at 72 h incubation.From the optimization results, 1.5% of CarboxyMethylCellulose concentration, pH 8, 72 hr of incubation time and 37˚C of incubation temperature are the optimum growth parameters for maximum production of cellulase enzyme production from *Micrococcus sp.* By following this conditions ,could produce cellulase enzyme easily ,inexpensive production and without risks. **(Figure:-1-Cellulase Activity (IU/L) is shown below).**

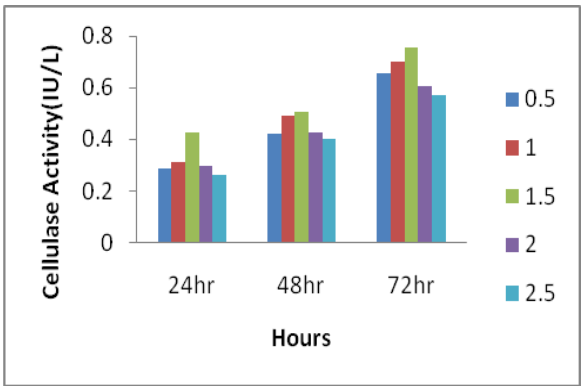
Optical density of each sample with reaction mixture was taken at 540 nm in a spectrophotometer .



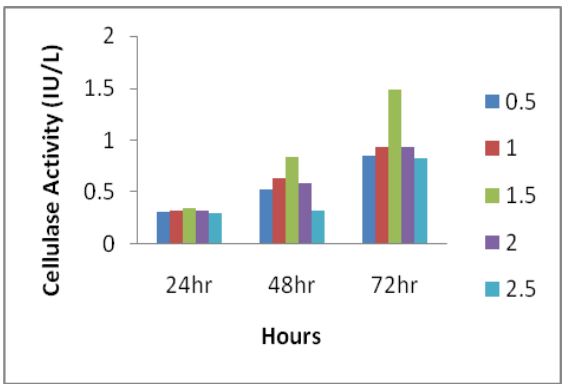
At pH-7, 27°C



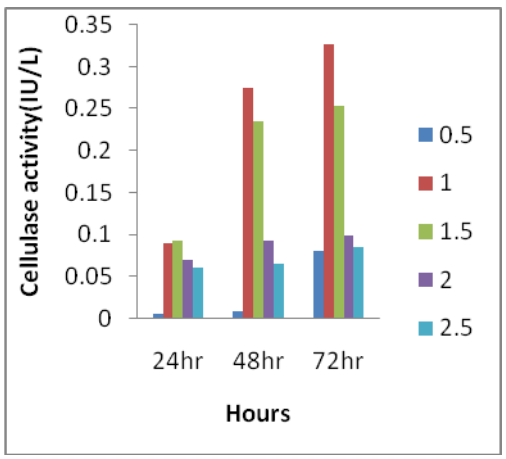
At pH-7, 37°C



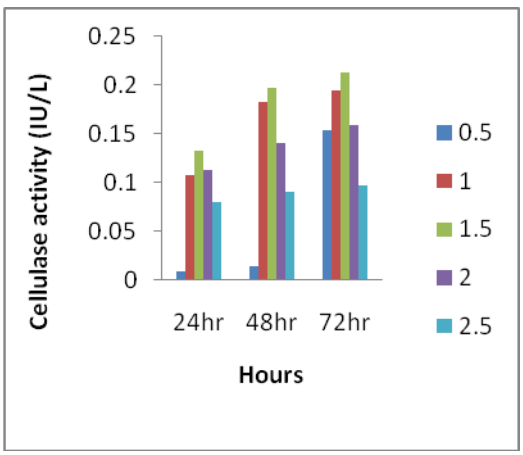
At pH-8, 27⁰C



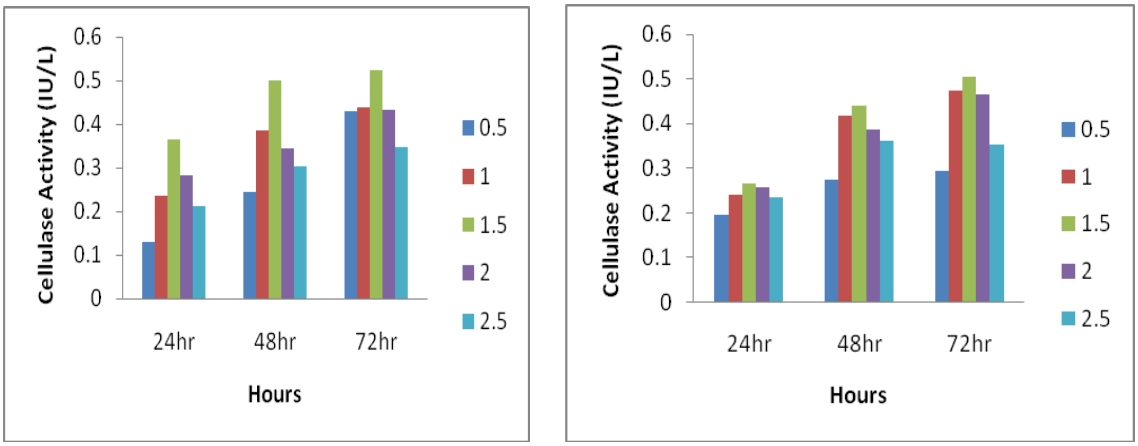
At pH-8, 37⁰C



At pH-6, 27⁰C



At pH-6, 37⁰C



At pH-9, 27^{0}C **At pH-9, 37^{0}C**

Fig. 1: Cellulase Activity (IU/L)

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