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Original Article

Production of Cellulase from *Micrococcus sp* and effect of growth parameters

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ABSTRACT

Objectives- Isolation of Cellulase enzyme producing bacteria from polluted water sample and optimization of growth parameters such as pH, Incubation temperature, incubation time, Concentration of Carboxy methyl cellulose for more enzyme production .Methods- Isolated bacteria were subjected to screen for Cellulase production on Carboxy methyl cellulose agar .The bacteria showing more clear zone around was selected and identified as *Micrococcus roseus.* The parameters used for the optimization of production were incubation temperature at 27^0C &37^0C , production medium pH range from 6 to 9 (i.e., 6, 7, 8 , 9), in 5 different concentration of Carboxy methyl cellulose (0.5, 1, 1.5, 2 & 2.5%), and incubation time (24, 48, 72hrs). Conclusion - *Micrococcus roseus* produce maximum cellulase at 1% of Carboxy methyl cellulose , 37^0C, pH 7 and at 48hrs. Bacterial cellulase production gain lesser importance especially *Micrococcus sp*, majority of works on cellulase production have been focused on fungi . This work is the easy and not an expensive method to produce cellulase and which has high demand in various industries and high cost in market

Key words: Carboxy methyl cellulose, Optimization, Cellulase, *Micrococcus* , DNS method.

1. INTRODUCTION

For many years, cellulase producing bacteria have been isolated and characterized from variety of sources such as soil, decayed plant materials, hotsprings, organic matters, feces of ruminants and composts.[1] Researchers keep on working for obtaining a new micro organisms

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with higher cellulase activity. [2] Cellulase is an enzyme produced by some of microorganisms including bacteria, yeast and fungi. [3-7] Cellulase is a complex enzyme system and have important role in environment for degradation of cellulose and convert it into useful products.Many important parameters influences the production of cellulase enzymes such as temperature, carbon sources , aeration, incubation time, medium ingredients, pH of the medium and cellulose quality.[8]

The cellulase have attracted considerable attention in recent years due to their great biotechnological, economical and industrial potentials. Cellulases have a wide range of applications such as in food, brewery, wine, pulp and paper, textile, detergent, feed and agriculture. [9] Cellulases are also used in textile industry for 'bio-polishing' of fabrics and making stone washed look of denims and in household laundry detergents for improving fabric softness and brightness .Cellulases are used in cotton preparations, wool and dyeing treatment ,in effluent treatment and in Pharmaceutical industries.

Micrococcus roseus is a gram positive ,non motile cocci ,arranged in tetrads, non spore forming ,pigmented bacteria found in air, water .soil even in our skin. It is a saprophytic or commensal microbes and sometime acts as a opportunistic pathogen.

2. MATERIALS & METHOD

2.1 Isolation and screening of cellulose degrading bacteria

Polluted water samples were collected from different places nearby our college and aseptically transfer to the laboratory immediately. The isolation of an organisms was done by Serial dilution and spread plate count method. Pure cultures of individual colonies were purified and screened for cellulase production 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 by qualitative plate assay. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis. The bacterial colonies having the largest clear zone picked and names AR, which is subjected for identification and optimization of cellulase production.

2.2 Identification

AR was identified based on morphological, biochemical and physiological characters according to Bergey's manual of determinative bacteriology and by 16S rRNA sequencing.

2.3 Preparation of Crude Enzyme

Prepare CMC broth medium, AR was inoculated in the medium. After different times of intervals about 1 ml of the inoculated medium was transferred to micro centrifuge tubes and centrifuged at 4000rpm for 15 min at 4^0C. Supernatant was used as the crude enzyme source which stored for various enzyme assay.

2.4 Optimization of Bioparameters

Effect of temperature on enzyme production and enzyme activity was studied by adjusting the incubation temperature at 27^0C & 37^0C , production medium pH range from 6 to 9 (i.e., 6, 7, 8 , 9) in 5 different concentrations of CMC (0.5, 1, 1.5, 2 , 2.5%), and incubation time (24, 48, 72hrs).

2.5 Cellulose assay by DNS method

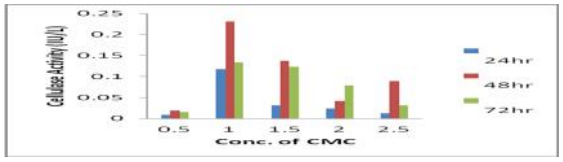
Cellulase activity was assayed by dinitro salicylic acid (DNS) reagent [10] with glucose as a standard .Sugars liberated were determined by measuring absorbance at 540 nm. One unit (IU) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1μmol of glucose per minute under standard assay conditions.

3. RESULTS & DISCUSSION

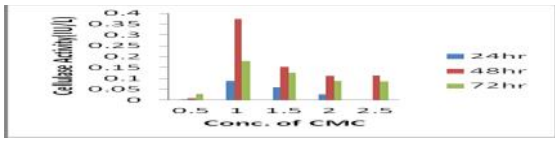
In this study, the cellulase producing bacterial strain was isolated from polluted water bodies nearby our college and cellulolytic organisms were screened by qualitative plate assay method. One of the organism

was used as a test organism and identified as *Micrococcus roseus* (AR) based on Bergeys manual determinative bacteriology and molecular characterization. Cellulolytic property of some bacterial genera such as *Cellulomonas* species, *Pseudomonas* species, *Bacillus* species and *Micrococcus* species were reported. [11]

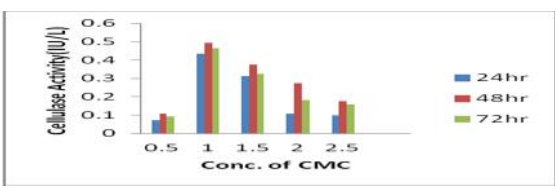
Optimization of an enzyme production and yields were assessed at different incubation period (24hr, 48hr, 72hr) , temperature (27^{0}C & 37^{0}C) and at pH (6-9) by using substrate as CMC (Fig - 1) . *Micrococcus roseus* (AR) shows higher activity was 0.68295 IU/L obtained at pH 7 and lower cellulase activity was at high and low pH . The crude cellulases from *Cellulomonas* ASN2 isolated from soil [12] exhibited its optimum activity at pH of 7.5 and temperature of 60˚C. In optimization AR producing more cellulase at 37^{0}C than 27^{0}C and shows maximum activity at 1% of CMC and at 48 hr of incubation time. Different *Streptomyces sp*. has been reported to produce maximum cellulase after 72-120 hr of fermentation. [13-15] Similar studies reported by Sheikh Nizamudeen and Bajaj, (2009), [16] *Bacillus* strain M9 and NZ showed more cellulase enzyme activity at 72 h incubation.



pH6,27^{0}C



pH 6, 37^{0}C



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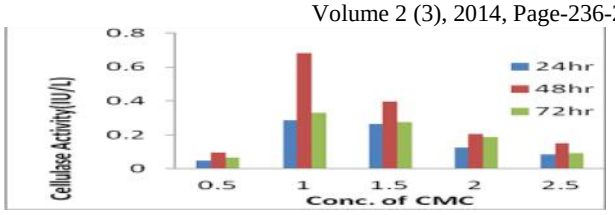
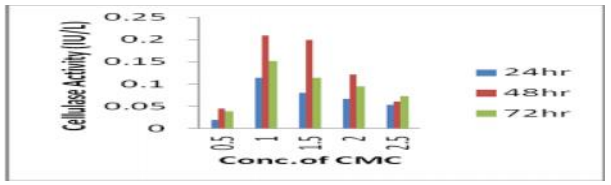
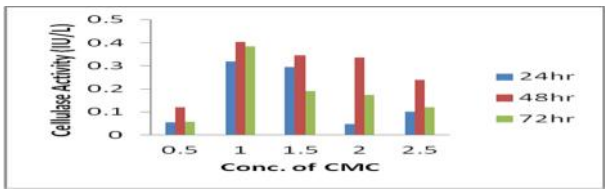


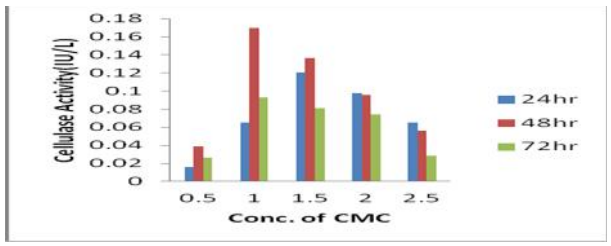
pH 7,37^{0}C



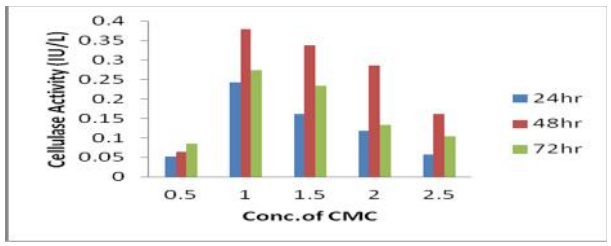
pH 8, 27^{0}C



pH 8, 37^{0}C



pH 9, 27^{0}C



pH 9, 37^{0}C

Fig 1: Graphical Representation of Cellulase Activity(iu/l) in Variable temp and pH

4. CONCLUSION

Lesser studies have been conducted by researchers on bacterial production of cellulase. The optimum conditions for maximum cellulase enzyme production of *Micrococcus roseus* is at 1% of Carboxy methyl cellulose , 37^{0}C, pH 7 and at 48hrs. By using these growth conditions ,industrial production of cellulase is reliable with *Micrococcus roseus* in future with cheapest input rate. *Micrococcus roseus* is non pathogenic and easily growing organism under these characteristics it is suitable for large scale and small

scale production .Another important factor is that, few bacteria may able to produce cellulase like *Cellulomonas, Bacillus*, etc, and cellulase production optimization by *Micrococcus roseus* is not much studied.

5. ACKNOWLEDGEMENT

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