ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
November 2016 Vol.:7, Issue:4

Isolation, Screening and Optimization of Cellulase Enzyme from Local Garden Soil

IJPPR
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ISSN 2349-7203
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Billore Vandita[1*], Sharma Shaligram[2], Sharma Pooja[3]

[1] Department of Biological Sciences, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.*

[2] Department of Neurosciences, Georgia State University, Georgia, Atlanta

[3]Biocatalyst Institute, Indore, M.P., India.

Submission: 26 October 2016
Accepted: 1 November 2016
Published: 25 November 2016

HUMAN JOURNALS

www.ijppr.humanjournals.com

Keywords: Cellulase, CMC media, Maltose, Cellulose, pH, Temperature

ABSTRACT

Cellulose is the most common organic polymer representing the major tons of total annual biomass production through photosynthesis. It is the most abundant bi-polymer on earth and the dominating waste material from agriculture. Cellulose from such major land plants as forest trees and cotton is assembled from glucose which is produced in the living plant cell from photosynthesis. It is being used as raw material in the manufacture of nitrocellulose (cellulose nitrate) which is used in smokeless gunpowder and as the base material for celluloid used for photographic and movie films. The cellulase producing bacteria were isolated from garden soil. Isolates were obtained by the primary screening technique which was showing maximum cellulase activity. When congo red test was applied, some bacterial isolates showed positive results with clear zone of hydrolysis ranging from 1-7mm. Bacterial colony were rod-shaped and are gram positive. While on optimization enzyme activity gradually increases as the pH value increased from 5- 6.5. It was also found that production of cellulose is significantly influenced by different carbon sources. Hence cellulose was most effective as a sole carbon source for cellulase enzyme production. The enzyme cellulase was found to be significantly stable up to 40^0C. Along with this, it was also observed that cellulase yield also depends on concentration of substrate, 1.5 % cellulose was found best among different substrate used. Optimization of growth conditions and process have been attempted to a large extent in improving cellulase production.

INTRODUCTION

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1→4) linked D-glucose units. Cellulose, a polymer of glucose residues connected by β-1, 4 linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature. Cellulose is the structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form biofilms. Cellulose is the most common organic compound on Earth. About 33% of all plant matter is cellulose (the cellulose content of cotton is 90% and that of wood is 40–50%). The biodegradation of cellulose has important consequences for the cultivation of arable soils, under conditions of restricted oxygen supply, microbial populations and metabolism change significantly with the result phytotoxic concentrations of acetic acid accumulate (Lynch, 1977, 1978, 1980; Lynch & Gunn, 1980; Lynch *et al.*, 1980). The degradation of cellulose by micro-organisms is a major component of the carbon and energy flux in soil. Lignocellulosic crop residues, such as cereal straw, provide the principal input of cellulose to arable soils (Lynch, *et al.* 1979) Cellulosic materials, including the agro-industrial wastes, can be converted into commercially important products such as ethanol, methane, glucose syrups and single cell proteins (Ryu and Mandel, 1980). The production of *cellulases* required for the enzymatic hydrolysis of cellulosic materials (Fan *et al.*, 1987; Smiths *et al.*, 1996). *Cellulases* are inducible enzymes synthesized by a large diversity of microorganisms including both fungi and bacteria during their growth on cellulosic materials (Kubicek *et .al* 1993; Sang Mok *et. al* 2001). *Cellulase* production is also influenced by several other factors, such as carbon, nitrogen and phosphorus sources, the ratio of carbon to nitrogen provided, trace elements, pH and aeration rate (Philippidis *et .al* 1994).

Efficient and highly discernible result (Montenecourt, *et al* 1977; Shimokawa *et al* 2009) plate screening methods are a prerequisite which gives a more rapid *Cellulases* have been utilized for the preparation of plant producers in the preliminary screening. Production of cellulases is regulated by 3. Dominguez, J., (2004). State of the art and new the speed of accumulation of products (Goyal *et al.* 1991). Earthworms are beneficial effects producing potential of the earthworm gut bacterial on soil environment such as modification of soil physical isolates which can be further optimized for the maximum properties and impact on decomposition of soil organic cellulase production and it can be used for further matter (Lee *et al* 1992). This research was performing to improve the performance of cellulases to make

them more effective so that less enzyme is needed. Optimization of growth conditions and processes have been attempted to a large extent in improving cellulase production.

MATERIALS AND METHODS

Micro-organisms are usually the most convenient sources and they can be obtained from various natural environments. Bacteria have an attractive potential for the exploitation of Cellulases and hemicellulases due to their rapid growth rate, enzyme complexity and extreme habitat variability. For the present study, soil from the park (in front of Blossom Pharma Biotech Institute & Research Centre) was chosen as the source of cellulolytic microorganism. The glassware's were cleaned and sterilized in the autoclave. The media components were weighed and dissolved in 80 ml Distilled water. After this, the pH of media was adjusted and then agar was added. The flask was covered by cotton plug and wrapping was done by using wrapping paper. The CMC agar media was then sterilized in the autoclave.

Test tubes were taken and filled with 9ml & one with 10 ml of distilled water. The soil suspension was prepared by added 1g soil in 10 ml sterilized distilled water inside the laminar. 1 ml soil suspension was transferred into the first dilution i.e. 10^{-1} with the help of a pipette. Then 1ml of suspension was transferred from 10^{-1} into the second dilution i.e. 10^{-2} and the process continues up to 10^{-10} dilution. Then 1ml Qty of the 2^{nd}, 4^{th}, 6^{th}, 8th & 10^{th} dilutions was then transferred into Petri plates. The prepared CMC agar media was then transferred to these plates using pour plate technique. The plates were then incubated for 48 hrs at 37^{0} C.

Fig 1 Culture of Colonies obtained by serial dilution

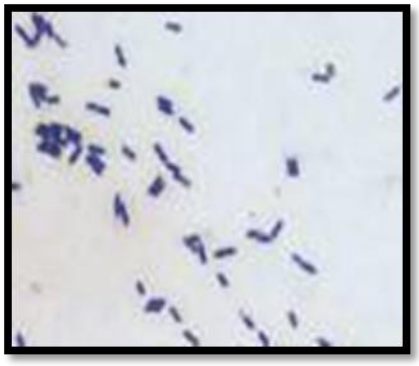

Fig 2 Gram-positive rod shaped bacteria

All the chemical compositions of CMC were weighed with the help of digital balance and dissolved (except agar) in 80ml distilled water. After this, the pH of the media was adjusted and then agar was added. The flask was covered by cotton plug and wrapping was done with wrapping paper. 80ml CMC agar media was prepared and sterilization was done in the autoclave. After sterilization, the flask was then unwrapped inside the laminar and media poured in 8 sterilized test tubes. The test tubes were then kept in a slanting position & media present in them was allowed to solidify. The different colonies that were observed in the CMC plates were streaked with the help of a sterile loop over the slants and the inoculated slants were incubated for 48 hours at 37^0C. These slants were then cotton-plugged, wrapped & preserved inside a refrigerator. After this screening for confirmation of cellulolytic activity on carboxymethylcellulose, agar media was occurred. The pH of media was adjusted and then cellulose was added after warming the broth and then agar was added. Then flask was covered by cotton plug and wrapping was done with wrapping paper. The media was then sterilized in the autoclave. After sterilization, the flask was unwrapped inside the laminar air flow. The media was poured in the petri plates and then the media was allowed to solidify. Bacterial isolates from each of the slants were suspended streaked over the petri plates. The plates were then incubated at 37^0 C for 24 hours. After 24 hours the culture plate was flushed with 1 % congo red solution. Bacterial colonies were surrounded by a zone of clearing were regarded as cellulolytic ones. These bacterial cultures were then preserved over CMC slants for further use.

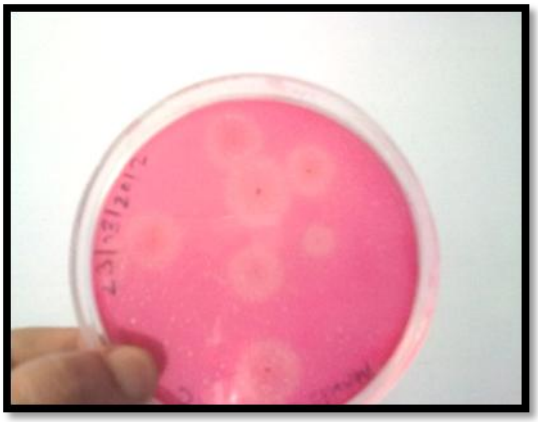

Fig 3 Zone of hydrolysis produced by cellulolytic microorganisms

After the confirmation of cellulolytic microorganism the cellulase assay was performed using 24 hr cellulose broth (containing 1% cellulose) inoculated with cellulolytic bacteria (from the slant) was taken and the supernatant of the broth was transferred into centrifuge tubes inside the laminar. These tubes were then centrifuged at 15000 rpm for 15 minutes. The supernatant was collected in a test-tube & later used as an enzyme source. Test-tubes were taken for making the dilution series of glucose. Then the volume in each test-tube was made up to 2ml by distilled water. 3 ml of DNS reagent was then pipetted out in all the 11 test-tubes. The test tubes are then kept in boiling water bath for 10 to 15 minutes and then the absorbance was taken at 575nm (Table 1).Meanwhile, four separate test-tubes were taken & marked as sample 1, sample 2, sample 3 and sample 4. Each of the test-tube was then filled with 1.8 ml of 0.5 % cellulose in 50 mM phosphate buffer. 0.2 ml of the supernatant from the centrifuge tube was then pipetted out in the test-tube with immediate addition of 3 ml DNS reagent.

Results were then interpreted in terms of enzyme activity in which enzyme activity was defined as the µmols of glucose liberated from 1 ml of the supernatant (Fig 4).

Table 1 Standard plot Preparation of Glucose.

S.No.	Concentration of Glucose (µg)	Absorbance
1.	100	0.124
2.	200	0.172
3.	300	0.276
4.	400	0.379
5.	500	0.480
6.	600	0.599
7.	700	0.715
8.	800	0.780
9.	900	0.841
10.	1000	0.909

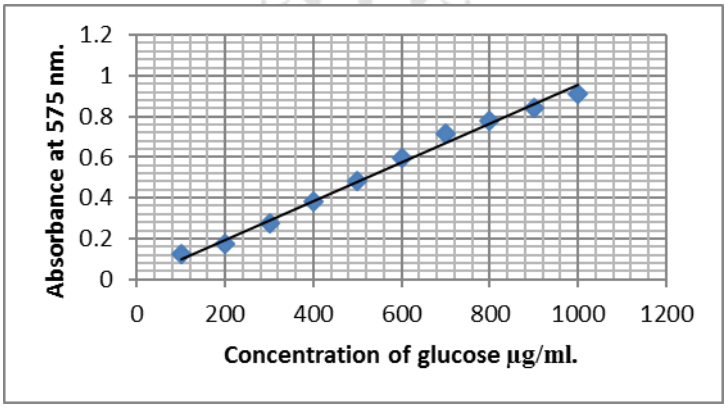

Fig 4 Standard plot of Glucose

Identification of bacterial colony was done with the help of primary stain (crystal violet) applied on the smear slide for 1 minute. Then mordant stain i.e. grams iodine was added on the slide (CVI complex formation) for 1 minute. The stains were washed with tap water. Then decolouriser (95% alcohol) was applied to the slide dropwise until the alcohol excess stain gets washed out. Then the slide was washed with tap water. After this, the counterstain was applied on the slide for 1 minute and after 1min the slide was washed with tap water. Then the slide was air dried and was observed under the microscope.

Effort was also made to optimize the parameters for maximum cellulase production by isolated microorganism. The various parameters studied with CSM Media were effect of initial pH, effect of temp, effect of different carbon source, effect of different cellulose concentration and effect of different nitrogen source.

To Study the effect of initial pH on cellulase production, the pH of Basal CSM media was varied from 4.0 to 7.5 and the other culture conditions remained same. To Study, the effect of temperature on cellulase production, the temperature for incubation was varied from 30^0C to 80 ^{0}C and the other culture conditions was same. While the basal media with different carbon source in the concentration 2 % was taken and sterilized and the remaining culture conditions were same. The media with different carbon source was inoculated with the cellulolytic bacteria and incubated at 37^0C for 24 hours.

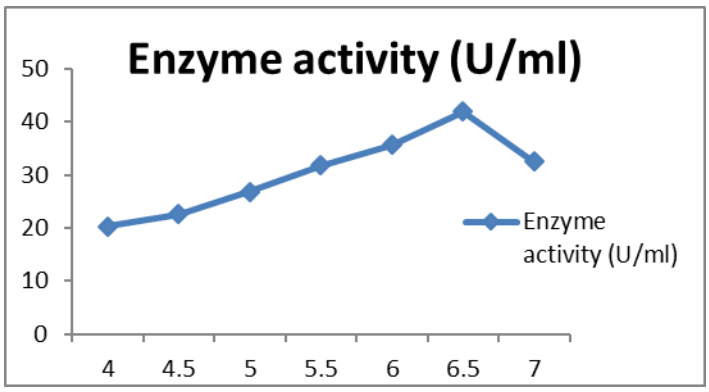

Fig 5 Effect of pH on Cellulase production

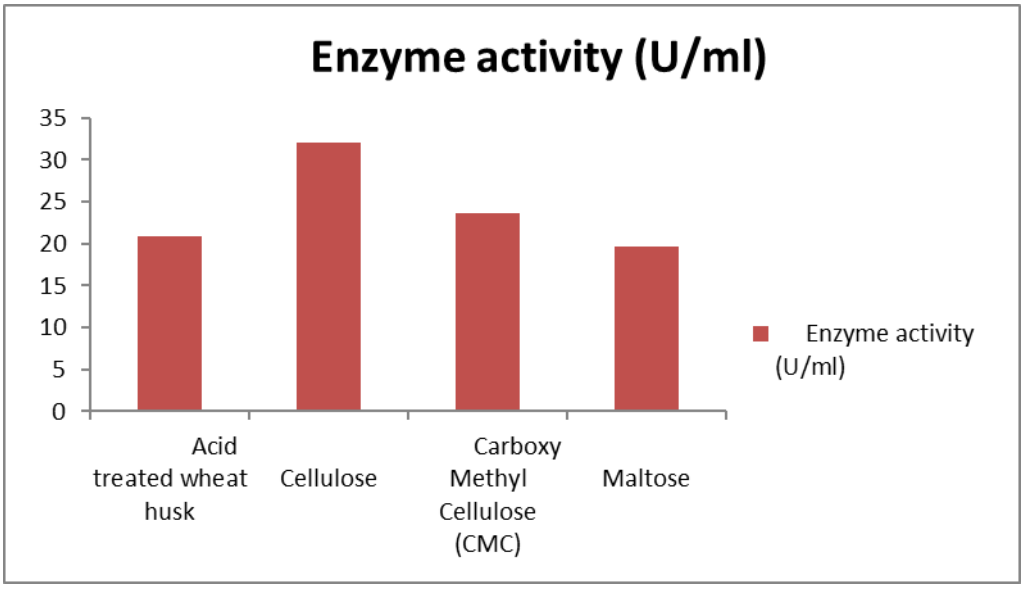

Fig 6 Effect of different carbon source on Cellulase Production

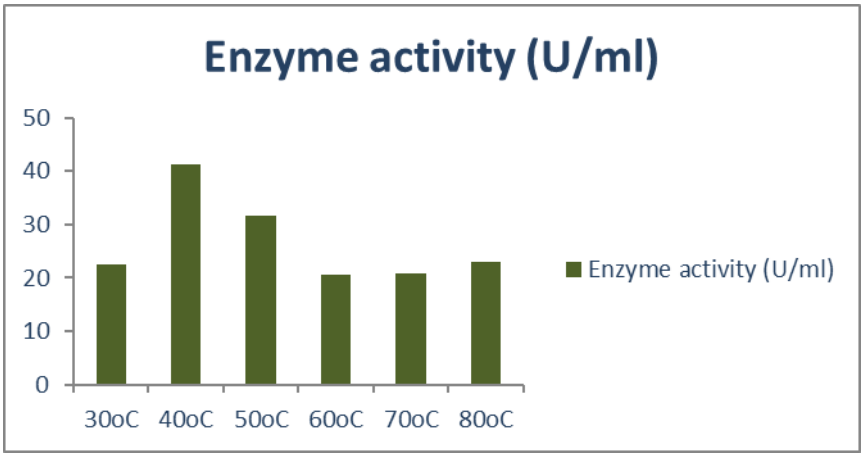

Fig 7 Effect of different temperature on Cellulase Production

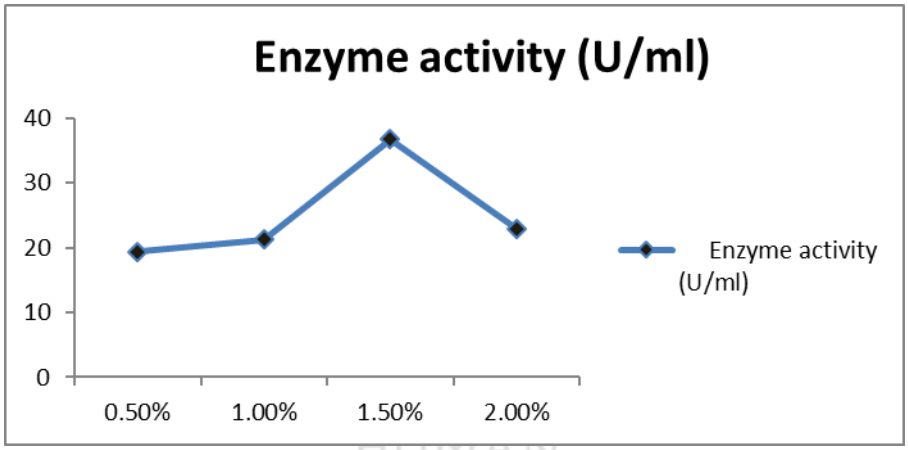

Fig 8 Effect of different cellulose concentration on cellulase production

RESULTS AND DISCUSSION

The present study was aimed at isolating cellulolytic bacteria from soil sample (Fig 1) and was screened for its cellulose degrading activity using Congo red. The bacterial isolates were Gram-positive rods (Fig 2). Screening of bacteria was conducted by using the Congo red test as a preliminary study for identifying cellulose producers. After the 24 hrs incubation, these bacteria showed ring of growth on cellulose agar. When Congo red test was applied, some bacterial isolates showed positive results with clear zone of hydrolysis ranging from 1 to 7 mm (Fig 3). The result showed that the dye formed complexes with unhydrolysed polysaccharides. It was difficult to differentiate colonies of cellulose utilizing organism bacteria from another organism on solid media even with polysaccharide precipitants. To examine the ability of polysaccharide precipitants to enhance visualization of zones of clearing was done by flooding the plates of the bacterial cultures with its 1% solution.

(Crudan *et al.* 1979). The isolated bacteria showed rod shape and were gram-positive bacteria (Fig 2).

The various parameters studied with Cellulase Screening Media were effect of shaking and agitation, effect of initial pH, selection of carbon source and nitrogen source.

Cellulase yield appears to depend on pH value. Regarding the effect of pH on enzyme synthesis, it was found that the cellulase production, expressed as enzyme activity, gradually increased as the pH value increased from 5-6.5 and reached its maximum at pH of 6.5. The enzyme activity gradually increased when increasing the pH up to the optimum followed by a gradual full in activity (Fig 5). As the probable phenomenon, it can be stated that at pH 6.5 the configuration of enzyme favored higher enzyme activity. The production of cellulase is significantly influenced by the type of carbon source in the basal salt medium. The different carbon sources were wheat straw, cellulose, CMC and starch. Cellulose was the most effective as a sole carbon source for cellulase enzyme production; results increased in enzyme activity.It may be due to the faster & better release of the glucose (Fig 6). Cellulase yield appears to depend on temperature value. Regarding the effect of pH on enzyme synthesis, it was found that cellulase production, expressed as enzyme activity The enzyme cellulase was found to be stable up to 40^0C where it shows significant activity. After 40^0C there was a sharp decline in enzyme activity. The decline is the enzyme activity may be due to denaturation of the hydrogen bonds (Fig 7).

Cellulase yield also depends on the concentration of substrate, It was observed that cellulose influenced the biosynthesis of enzyme and 1.5 % cellulose was the best among different substrates used. It may be due to the faster & better release of the glucose (Fig 8).

Future Scope

Research has shed light into the mechanisms of microbial cellulose production and has led to the development of technologies for the production and applications of cellulose degrading enzyme. The major goals for future cellulase research would be reduction in the cost of cellulose production and improving the performance of cellulases to make them more effective so that less enzyme is needed. The formal task may include such majors as optimizing growth conditions or processes whereas the latter required directed efforts in protein engineering and microbial genetics to improve the properties of enzyme.

Acknowledgement

The authors acknowledge to the Management Board of Blossom Institute of Pharmacy and biotech for providing all lab facilities to carry out the present study.

REFERENCES

1. Lynch JM, Letcherm F and Lathamm J: Biological interactions. In Microbial Ecology. A Conceptual Approach 1979; 171-187.
2. Lynch JM: Acetic acid in anaerobic soils containing plant residues. Soil Biology and Biochemistry 1978;10, 131- 135
3. Lynch JM: Straw residues as substrates for growth and product formation by soil microorganisms. In Straw Decay and its Effect on Utilization and Disposal. Chichester Wiley 1979; 47-55.
4. Lynch JM and Gum KB: The use of the chemostat to study the decomposition of wheat straw in soil slurries. Journal of Soil Science 1980; 29.
5. Lynch JM: Effects of organic acids on the germination of seeds and growth of seedlings. Plant, Cell and Environment 1980; 3,255-259.
6. Ryu DD and Mandels M: Cellulases: Biosynthesis and Applications. Enzyme Microbiol. Technol 1980; 2, 92-102.
7. Smiths JP, Rinzema A, Tramper J, Van HM and Knol W: Solid-state fermentation of wheat bran by Trichoderma reesei QM9414: Substrate composition, changes, C-balance, enzyme production, growth and kinetics. Appl. Microbiol. Biotechnol1996; 46,489-496.
8. Fan LT, Gharpuray MM and Lee YN: Cellulose Hydrolysis Berlin, Germany, Springer-Verlag 1987; 3,1-68.
9. Kubicek CP : From cellulose to cellulase inducers: facts and fiction," in Proceedings of the 2nd Symposium Trichoderma Reesei Cellulases and Other Hydrolases (TRICEL '93) Foundation for Biotechnical and Industrial Fermentation Research 1993; 8, 181–188.
10. Sang-Mok L and Koo YM: "Pilot-scale production of cellulase using Trichoderma reesei Rut C-30 in fed-batch mode," Journal of Microbiology and Biotechnology 2001; 11, 229–233.
11. Philippidis GP: Cellulase Production technology. In: Enzymatic Conversion of Biomass for Fuel Production 1994; 566.
12. Montenecourt BS and Eveleigh DE: Semiquantitative Plate Assay for Determination of Cellulase Production by *Trichoderma viride*, Appl. Environ. Microbiol 1977; 33, 178-183.
13. Shimokawa T, Ishida M, Yoshida S and Nojiri M: Effects of growth stage on enzymatic saccharification and simultaneous saccharification and fermentation of bamboo shoots for bioethanol production, Bioresour Technol 2009; 24, 6651- 6654.
14. Dominguez J: State of the art and new perspectives in vermicomposting research. In: Edwards, C.A. (Ed.), Earthworm Ecology CRC Press, Boca Raton 2004; 401-425.
15. Goyal A, Ghosh B and Eveleigh D: Characteristics of fungal cellulases. Microbiol. Bioresour Technol 1991; 46, 728-737.
16. Lee KE: Some trends and opportunities in resources because of either the appearance of microbial earthworm research or: Darwins children- the future species in fresh substrate or the pool of easily assimilable of our discipline, Soil Biol. Biochem 1992; 24: 1765-1771.