RESEARCH ARTICLE

Caboxymethyl Cellulase Activity of Thermophilic Fungi from Different Substrates

Borkar KM

Department of Botany, M. B. Patel College of Arts, Commerce & Science, Sakoli-441802 Dist-Bhandara (MS).
Email: khushalborkar@gmail.com

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ABSTRACT

Thermophilic fungi are important organisms for degradation of plant material in nature. These fungi secrete thermostable enzymes that are stable even under harsh environmental conditions. Thermostable properties make the fungal enzymes suitable for industrial use also. In agricultural fields much of the waste material is generated. Different substrates like cron cob, groundnut shell, wheat staw, jowar straw and carboxy methyl cellulose were tried for cultivation of *Myriococcum albomyces* and *Humicola insolens* and their corboxy methyle cellulase activity was assayed. In the fungus *Humicola insolens*, the activity of CMCase was found to be highest with most of the substrates on 4th day of incubation. In Myriococcum albomyces the activity of CMCase was highest on corn cob as substrate.

Key words: Groundnut shell, Wheat staw, Jowar staw, CMCase, Thermophilic fungi.

INTRODUCTION

Thermophilic fungi have economic value as they are able to breakdown dead plant materials and build humus. They are involved in preparation of farm yard manure and garden compost throughout the world and therefore have tremendous significance in human economy as farm yard manure play a important role in sustainable development.

Thermostable enzymes secreted by thermophilic fungi viz., amylases, proteases, lipases, cellulases, xylanases etc. are used in food, fermentation, textile, paper industry, dairy industry, detergent, leather processing, pharmaceutical industry, in pretreatment of biomass that contains cellulose to improve nutrional quality of forage, biobleaching of pulp and many other industries attracting attention towards the enzymes of thermophilic fungi (Satyanarayana et al 1999).

Cooney & Emerson (1964) gave definition as the thermophilic species can conveniently be defined as those with minima for growth at or above 20^{0}C and maxima for growth at 50^{0}C or above, whereas thermotolerant fungi are ones that have a thermal maximum near 50^{0}C and a minimum below 20^{0}C. Nowadays, the term thermophilous is used to designate both thermophilic and thermotolerant fungi (Mouchacca 1997).

In the various industrial processes, cellulolytic enzymes are employed in the color extractions of juices, in detergents causing color brightening and softening, in the biostoning of jeans, in the pretreatment of biomass that contains cellulose to improve nutritional quality of forage and in the pretreatment of industrial wastes (Buchert *et al*., 1997; Niehaus *et al*., 1999; Bhat, 2000; Nakamura *et al*., 2001; Van wyk *et al*., 2001; Zhou *et al*., 2001) Duo et al(2011) attributed faster degradation of biomass to swelling of cellulose during heating.

Thermophilic fungi are known for their faster degradation of agricultural wastes. In the present study author has tried to grow these fungi on agricultural wastes and accounted CMCase activity.

MATERIAL AND METHODS

Thermophilic fungi Myriococcum albomyces and Humicola insolense were isolated from coal mine waste soil dumping area and identified from available literature.

Agricultural waste as substrates for the growth of Thermophilic fungi:

For the cultivation of fungi on the different substrates 5gm fine powder of the Corn cob, groundnut shell, wheat straw, jowar straw was taken in the conical flask of 150ml capacity. To these flask 5ml basal medium containing L-aspargine- medium and microelement solution 1ml/liter was poured (approximately 80% moisture). These flasks were sterilized. Mycelial disks of 5mm diameter were inoculated aseptically in flasks from 5 days old culture of *Humicola insolense* and *Myriococcum albomyces* and these flasks were incubated at 45^{0}C. After the incubation period of 3, 6, 9, 12, 15 days these flasks were removed from the incubator and mycelium was harvested by adding 25 ml water and stirred well for half an hour on magnetic stirrer. Later-on the mycelium was filtered through the Whatman no. 1 filter paper. The filtrate was used as crude enzyme extract to assay the CMCase enzyme activity.

The CMCase enzyme assay:

After the incubation, in a clean and oven dried test tubes 0.5ml of aliquot (prepared by method mentioned as above) was taken, 1ml of alkaline copper tartarate reagent was added to the tube and volume made to 2ml. 2ml distilled water was taken separately instead of enzyme extract as blank. Reducing sugar estimation was done using Nelson-Somogyi (1952) method. Absorbance was measured after 10 minutes of incubation at 560nm. The reducing sugar released was quantified on the basis of standard curve plotted using glucose as standard.

$$\text{Cellulase activity} = \frac{S}{m} \times \frac{1}{t} \times \frac{1}{v}$$

Where S= Reducing sugar liberated in µg,
M= Mol. wt. of glucose,
T= Reaction time in minutes and
V= Volume of enzyme extract in ml.

Enzyme production / liter = Cellulase activity/ ml/ min. x 1000.

One unit of cellulolytic activity is defined as the amount of sugar liberates in one micromole of reducing sugar (as glucose) per min. per ml of enzyme samples under conditions defined (Joshi 1992).

RESULT AND DISCUSSION

Effect of different substrates on CMCase activity of thermophilic fungi

The Corboxymethyl cellulase enzyme activity of *Myriococcum albomyces* and *Humicola insolens* was studied in present investigation. Four different substrates from agricultural wastes were used as carbon sources. *Humicola insolens* showed the highest activity of CMCase with most of the substrates on 4th day of incubation. Wheat straw, corn cob and jowar straw induced the CMCase activity rapidly and the activity was found to be higher than control on 4th day. However, lateron the activity in case of corn cob was equal to control on 12th day, whereas, groundnut shell and CMC, when used as substrate inhibited the CMCase activity initially. However, in case of Groundnut shell the activity decreased between 8th and 12th day. CMC, on the other hand, the activity of CMCase remain almost same throughout the incubation period (Fig. 1).

In Myriococcum albomyces the activity of CMCase was highest on corn cob as substrate. However, it then declined gradually till 16th day. Corn cob induced the CMCase activity most and CMC had induced the activity least. Moreover, corn cob along with groundnut shell and wheat straw induced the highest activity on 4th day, which declined subsequently till 16th day. In contrast to this, jowar straw induced the highest activity on 8th day which rapidly declined on 12th day and then remain constant till 16th day.

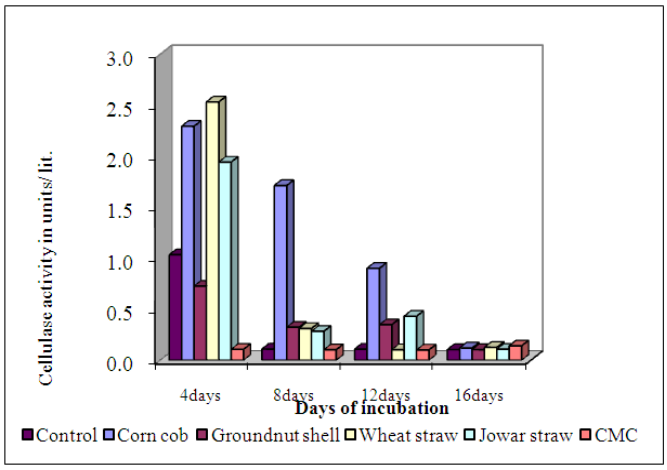

Fig 1: Effect of different substrates on CMCase production of *Humicola insolens*

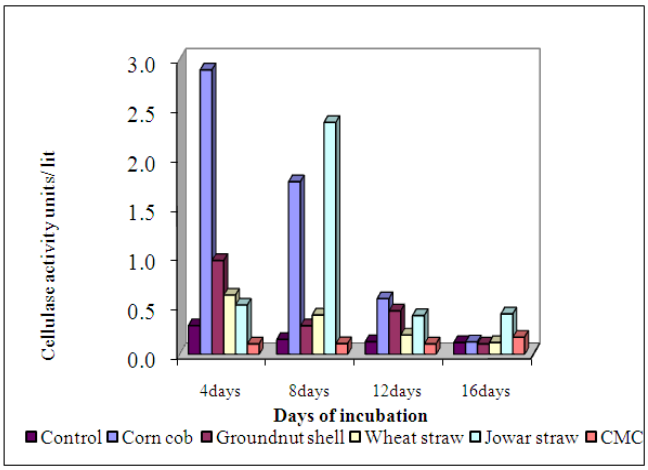

Fig 2: Effect of different substrates on CMCase enzyme production of *Myriococcum albomyces*

Similarly, CMC also induced more or less equal activity till 12th day of incubation. The marginal increase in activity of CMCase was observed on 16th day in case of CMC (Fig. 2).

The present investigation revealed cellulase production by both the tested thermophilic fungal isolates viz., *Humicola insolens* and *Myriococcum albomyces* on different agricultural wastes as substrates. These fungi were grown on powdered agricultural wastes and incubated for 4, 8, 12, 16 days, and CMCase enzyme activity was measured. The enzyme activity was highest on 4th day of incubation in present work. Similar results were observed by Coutts and Smith (1976) and Da- Silva *et al.,* (2005). Both the isolates viz., *Humicola insolens* and *Myriococcum albomyces* showed CMCase till 8 days on most of the substrates. Thereafter, the activity decreased to a low level (Fig. 11 and 12). *Humicola insolens* showed maximum CMCase activity with corn cob, wheat straw and jowar straw; whereas *Myriococcum albomyces* showed maximum activity of CMCase with corn cob and jowar straw (Fig. 1 and 2). da-Silva *et al*., (2005) reported maximum CMCase activity of *Thermoascus aurantiacus* from corn cob as substrate. Badhan *et al*., (2007) reported high CMCase activity of *Myceliophthora* sp. with rice and wheat straw as substrates. Charles and Ling-Chang Chiang (1980) described in detail the cellulase enzyme complex and the process of cellulose degradation. They have illustrated that cellulose degradation process of different cellulosic substrates depends on various factors such as moisture content, degree of cellulose crystallinity and degree of polymerization of cellulose molecules as well as its association with hemicellulose and lignin.

CONCLUSION

Among the substrates studied, highest activity was recorded from corn cob, wheat straw and jowar straw from *Humicola insolens* after 4 days of incubation. Availability of carbon source in these substrates for rapid breakdown may be the reason for occurrence of highest cellulase activity. After 8 days of incubation still higher activity on jowar straw was seen from *Myriococcum albomyces* and from corn cob by *Humicola insolens*. *Myriococcum albomyces* may be producing CMCase enzyme in quite later and comparative slower rate but remain functional for slightly longer time. However, same may be the case for corn cob and *Humicola insolens*. These fungi are able to degrade agricultural wastes at faster rate by degrading cellulose.

REFERENCES

Badhan AK, Chadha BS, Kaur J, Saini HS and Bhat MK (2007) Production of multiple xylanolytic and cellulytic enzymes by thermophilic fungus *Myceliophthora* sp. IMI 387099. *Biores. Technol*. 98: 504-510.

Bhat MK (2000) Cellulases and related enzymes in biotechnology. *Biotechnol. Adv*. 18, 355–383.

Buchert J, Pere J, Oijusluoma L, Rahkamo L and Viikari L (1997) Cellulases––tools for modification of cellulosic materials. In: Niches in the World of Textiles World

Conference of the Textile Institute, Manchester, England. 284–290.

Charles ED and Ling-Chang Chiang (1980) Cellulose degradation- A common link in: Utilization and recycle of agricultural waste and residues ed. M. L. Shuler, Pub. by CRC, CRC press Inc. Boca Raton Florida USA. 19-65.

Cooney DG and Emerson R (1964) Thermophilic fungi. An Account of Their Biology, Activities and Classification. W. H. Freeman & Co., San Francisco, Calif.

Coutts AD and Smith RE (1976) Factors influencing the production of celluloses by *Sporotrichum thermophile*. *Appl. Enviro. Microbiol., American Soc. for Microbilogy*. 31(6): 819-825.

Da- Silva, R Lago ES, Merbheb CW, Machione MM, Park YK and Gomes E (2005) Production of xylanase and CMCase on solid state fermentation in different residues by *Thermoascus aurantiacus* Miehe. *Brazilian J. Microbiol*. 36: 235-241.

Duo-Chuan- Li, An- Na-Li and Anastassios CP (2011) Cellulase from Thermophilic fungi: Recent insight and Botechnological Potential, Enzyme Research, pp. 1-9.

Joshi P (1992) Studies on Decomposition of organic materials with special reference to the role of thermophilic fungi. Ph. D. Thesis. Nagpur University, Nagpur, M.S. India.

Mouchacca J(1997) Thermophilous fungi: biodiversity and taxonomic status. *Cryptogamie, Mycologie*. 18: 19-69.

Nakamura H, Kubota H, Kono T, Isogai A and Onabe F (2001) Modification of pulp properties by cellulase treatment and application of cellulase to wastepaper deinking and mechanical pulp refining. In: 68th Pulp and Paper Research Conference. Proceedings of the Pulp and Paper Research Conference, 18–19/06, Japan, pp. 2–5.

Nelson-Somogyi (1952) *J. Biol. Chem*. 200: 245.

Niehaus F, Bertoldo C, Kahler M and Antranikian G (1999) Extremophiles as a source of novel enzymes for industrial applications. *Appl. Microbiol. Biotechnol*. 51: 711–729.

Satyanarayana T, Johri BN and Olsen J (1999) Thermophilic Moulds in Biotecnology. (eds.) Kluwer Academic Publishers.

Van wyk J, Mogale A, Seseng T (2001) Bioconversion of wastepaper to sugars by cellulase from *Aspergillus niger*, *Trichoderma viride* and *Penicillium funiculosum. J. Solid Waste Technol. Manage*. 27: 82–86.

Zhou L, Yeung K, Yuen C (2001) Combined cellulase and wrinklefree treatment on cotton fabric. *J. Dong Hua University* 18, 11–15.