RESEARCH ARTICLE

Effect of Temperature, pH and Substrates on CMCase Enzyme Activity of Thermophilic Fungus *Humicola insolens*

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ABSTRACT

Thermophilic fungi are known for their thermostable enzymes. In present work agricultureal wastes like corn cob, groundnut shell, wheat straw, jowar straw and carboxy methyl cellulose were utilized as substrates for cultivation of thermophilic fungus *Humicola insolens*. Culture filtrate was used for analysis of carboxy methyl cellulolase enzyme activity. Effect of different substrates and pH and temperature was studied. On wheat straw, corn cob and jowar straw activity was found to be higher than control on 4th day whereas, groundnut shell and CMC, as substrate inhibited the CMCase activity initially.Temperature 45^{0}Cand pH 5 found to be suitable for better enzyme activity.

Key words: Temperature, pH, substrates, CMCase, thermophilic, *Humicola insolens*.

INTRODUCTION

The thermophilic species are 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, (Cooney & Emerson, 1964). Nowadays, the term thermophilous is used to designate both thermophilic and thermotolerant fungi (Mouchacca 1997). The adaptive mechanisms of thermophilic fungi to withstand temperature/ heat stress may be due to specific and or physiological adaptations such as heavy pigmentation of spores produced by these fungi (Satyanarayana *et al*., 1992), that allow microorganisms to exist in many environments that experience extremes of temperature, pH, chemical content and or pressure. Elevated temperature survival of these fungi is attributed to thermostability and functional permeability of membranes(Redman *et al*., 1999). Plant biomass contains major proportion of cellulose produced continuously by natural photosynthesis. It is the most widespread naturally occurring carbohydrate readily available in every agriculturally developed region on the earth. Cellulose occurs in native fibers in close association with lignin and hemicelluloses, or in relatively pure state as in cotton.

Enzymatic abilities of thermophilic fungi are superior by way of their thermostability and better production under optimum nutritional conditions as compared to mesophiles, (Satyanarayana *et al*., 1988). They reported efficiency of degradation of plant residues by a particular fungus depends

upon its capacity to degrade cellulose, hemicellulose etc. Thermophilic fungi are also reported to secrete other enzymes including xylanase, protease, pectinase that able to degrade cellulose, hemicellulose, pectin and lignin content (Satyanarayana and Johri, 1983). Demirijan *et al*., (2001) stated the reason for number of commercial applications of thermophilic fungi due to their overall inherent stability.

MATERIALS AND METHODS

Cultivation of thermophilic fungi on different substrates:

For the cultivation of fungi on the different substrates 5gm fine powdered Corn cob, groundnut shell, wheat straw, jowar straw and CMC 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. These flasks were sterilized. Mycelial disks of 5mm diameter were inoculated aseptically in flasks from 5 days old culture of *Humicola insolens,* 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.

CMCase enzyme assay:

Temperature and pH plays an important role in enzyme activity. Thus, in the present study temperature and pH optimum of CMCase was determined. To determine the temperature optima, citrate buffer of pH 5.2 was used and the reaction mixture was incubated at 40, 45, 50, 55, 60^{0}C. Similarly to determine the pH optima of CMCase, the reaction mixture was incubated at 45^{0}C and the citrate buffer of pH 4, 5, 6, 7, 8, 9 was used. The reaction mixture was incubated for 30 min.After the incubation, Reducing sugar estimation was done using Nelson-Somogyi (1952) method.

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).

RESULTS & DISCUSSION

Effect of different substrates on CMCase activity

The Corboxy methyl cellulase enzyme activity of *Humicola insolens* was studied in present investigation. Various substrates from agricultural wastes were used as carbon sources. The observations taken during the course of study are as follows.

Overall 4 to 8 days incubation period had shown better enzyme production with all the substrates studied.Theenzyme activity was found to be highest with most of the substrates on 4th day of incubation. On wheat straw, corn cob and jowar straw activity was found to be higher than control on 4th day,however, lateron in case of corn cob was equal to control on 12th day, whereas, groundnut shell and CMC, as substrate inhibited the CMCase activity initially. On Groundnut shell the activity decreased between 8th and 12th day. on the other hand, the activity of CMCase remain almost same throughout incubation period for CMC (Fig. 1). *Humicola insolens* showed CMCase till 8 days on most of the substrates. Thereafter, the activity decreased to a low level (Fig. 1). *Humicola insolens* showed maximum CMCase activity with corn cob, wheat straw and jowar straw, da-Silva *et al*., (2005) reported maximum CMCase activity of *Thermoascusaurantiacus* from corn cob as substrate. Badhan *et al*., (2007) reported high CMCase activity of *Myceliophthora* sp. with rice and wheat straw as substrates. Charles *et al*., (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. Overall weaker enzyme activity was reported after 12-16 days of incubation. Similar observations reported by Joshi (1992), attributed this either to the increasingly resistant cellulose residues left after degradation of

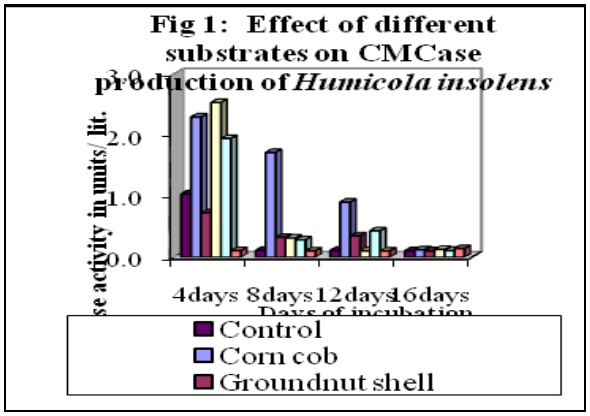
susceptible portion of cellulose or to the repression in soluble hydrolysis products.

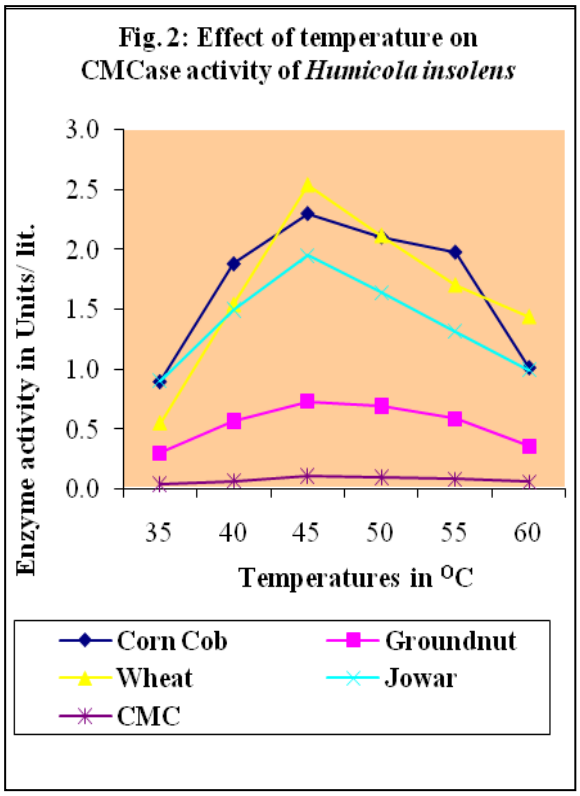
Effect of temperature on CMCase activity:

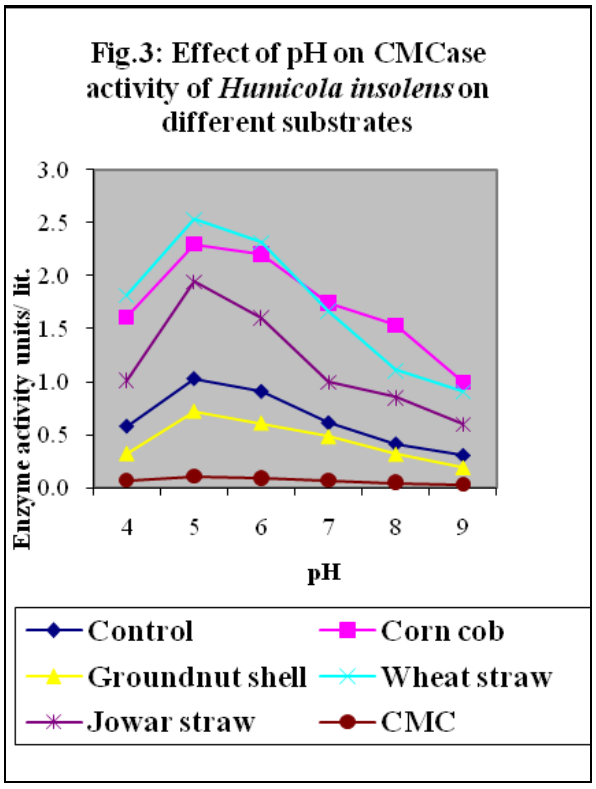
Crude enzyme extract of corn cob as a substrate has shown favourable temperature of 40-55^{0}C for the better enzyme activity. Although this extract has shown slight increase at 45^{0}C. The crude enzyme extract from Jowar straw as a substrate has shown better enzyme activity at 45^{0}C and further gradual decrease in enzyme activity at higher temperatures was observed. In case of crude enzyme extract from groundnut and CMC did not show significant difference in enzyme activity with respect to temperature (Fig. 2) Almost all the extracts tested under various temperature regimes showed better enzyme activity at 40-45^{0}C (Fig.2). Moreover, highest enzyme activity was recorded from almost all the extracts except jowar straw and CMC for *Humicola insolens*. For rest of the substrates broader range of temperature was observed. These findings are in agreement with the results Gomes *et al*., (2000) and da-Silva *et al*., (2005).

Effect of pH on CMCase activity:

The pH of the growth medium affect the enzyme activity at various levels. However, pH 5 had seen to be favourable for the production of maximum enzyme activity at all the substrates and pH studied. Highest enzyme activity (2.295 units/ liter) on wheat straw at pH 5 was recorded. Later on, gradual decrease till pH 8 was observed. This trend was followed by the enzyme extract from corn cob as a substrate. However, after pH 5 linear decrease in activity was observed in case of control. Whereas, no change in activity at various pH was observed in case of CMC as a substrate (Fig. 3). CMCase activity in crude enzyme extracts of both the isolates was found to be maximum at pH 5.0 (Fig. 3) Hayashida and Yashioka (1980) and Hayashida *et al*., (1988) found pH 5.0 to be optimum for CMCase activity of *Humicola insolens*. Similarly, da-Silva *et al*., (2005) reported pH 4.5-6 suitable for CMCase enzyme activity for *Thermoascusaurantiacus*.







CONCLUSIONS

Among the substrates studied, highest activity was recorded from corn cob, wheat straw and jowar straw from after 4 days of incubation. Availability of carbon source in these substrates for the rapid breakdown may be the reason for occurrence of highest CMCaseactivity.After 8 days of incubation still higher CMCase activity of jowar straw was seen from corn cob by *Humicola insolens*. The crude enzyme extract was subjected to the temperature and pH treatment for determination of temperature and pH stability and it was observed that, 40-55^{0}C temperature for both the fungi and pH 5.0-6.0 may be suitable for the production of CMCase activity.

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