The Cellulolytic Activity of *Myceliophthora Thermophila* and *Talaromyces duponti* two Thermophilic Fungi

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Abstract:

The experiment was conducted with the aim to understand activity of cellulase by thermophilic fungi isolated during decomposition of leafy biomass from agricultural waste leaves. Some of the dominant thermophilic isolates were selected to study their comparative role in decomposition of organic substrate by evaluating their capacity to produce cellulase enzyme, especially endo-□□ -1,4glucanase (CM cellulase or Cx) and □-Glucosidase (Celloobiase) activity.

It was determined by measuring the amount of reducing sugar formed due to cellulolytic activity of crude enzyme extract on carboxymethyle cellulose (CMC) and cellobiose respectively by Nelson-Somogyi, method. The leaves of banana and sugarcane were selected as complex source of cellulose whereas CMC was taken as soluble derivative of cellulose. *Myceliophthora thermophila (*syn. *Sporotrichum therophile)* and *Talaromyces duponti* were selected as thermophilic fungi inoculated separately and were tested for their cellulolytic activity. Both fungi showed Cx and cellobiase activities and it were varied on different cellulosic substrates according to the nutritional requirement of the test organisms *M. thermophila* showed better activity than *T. dupontio*n all the selected substrates.

Keywords:
*Myceliophthora thermophila (*syn.*Sporotrichum therophile), Talaromyces duponti,* endo-□-1,4glucanase, □-Glucosidase.

Introduction:

Several mesophillic and thermophili cmoulds have been known to degrade agricultural lignocellulotic plant residues. In the present study attempt have been made to evaluate the ability to produce extracellular enzyme by thermophilic fungi.Exo-□-1,4 glucanase,endo-□-1,4 glucanase, and b-Glucosidase which are extracelluar cellulolytic enzyme complex formed in these moulds and are very essential for saccharification of cellulose, Reese *et al.*, (1950). Both the selected fungi are known for their enzyme production, *Talaromyces duponti* is good in enzyme production, (Marie-Pierre Bousque, 1998), Maheshwari, *et al. (*2000*), studied physiology of fungal enzymes,* Ismail *et al.*(2013) *M. thermophila in* biodegradation and Rodríguez Couto, *et al* (2006), studied industrial and biotechnological applications of laccases from fungi.

Since rich thermophilic mycoflora was isolated from the leafy biomass during biodegradation of leafy biomass. *Myceliophthora thermophila (*syn. *Sporotrichum therophile), Talaromyces duponti were e*valuated for their role production of cellulase by using complex cellulosic substrates like sugarcane

and banana leaves and carboxymethyle cellulose was use as soluble derivative of cellulose.

Material and methods:

The utilization of highly complex cellulosic substrate by fungi was studied by using the method of Garret, (1962) and Fergus, (1969). The leaves of banana (*Musa paradisiaca*) and sugarcane (*Saccharum officinarum*) were selected as complex source of cellulose, while carboxymethyle cellulose (CMC) was taken as soluble derivative of cellulose.

Leaves were dried at 60^{0}C till constant weight 1 gm of each type of dry leaves and CMC was poured in 150 ml Erlenmeyer flask separately in triplicates. These flasks were poured with 50 ml of nutrient medium YpSs Emerson broth. All flask contain cellulosic substrate and nutrients broth were sterilized in autoclaved at 15 lbs pressure for 20 min. Streptomycin sulphate was added (25 mg/lit) to each flask to avoid bacterial contamination during incubation. Initial pH of the broth containing different cellulosic substrate was recorded in the range of 6-7.

The thermophilic fungi which were taken as test fungi were *Myceliophthora thermophila* (syn. *Sporotrichum therophile)* and *Talaromyces duponti.* Pure culture of fungi were grown on YpSs agar plates at the optimum temperature (45^{0}C), from six days old fungal culture, three discs of 5 mm in diameter was inoculated to each flask, and these were incubated at 45^{0}C $\pm$ 1^{0}C.

At the intervals of 7, 14, and 21 days three flasks each fungus grown on single cellulosic substrates were taken from the incubator. The contents were filtered in Whatman No. 1 filter paper. The filtrate was stored at 3^{0}C for enzyme assay.

Enzyme assay: The extracellular enzyme assay of the culture filtrate was determined for Cx (Carboxymethyl cellulase) and cellobiase by amount of reducing sugar formed due to cellulolytic activity on carboxymetnyle cellulose and cellobiose respectively.

The aliquot was prepared by mixing 1 ml of CMC solution (0.5 %), 1 ml of sodium acetate buffer (0.2 M) 5.2 pH and 1ml of enzyme extract, to calculate carboxymethyle cellulase activity and for cellobiase activity aliquot was prepared by mixing 1 ml of 15 nMcellobiose solution, citric acid buffer 1 ml (1 M, 4.8 pH) and 1 ml of enzyme extract. The reaction mixture was kept at 45^{0}C $\pm$ 1^{0}C in a water bath for rapid enzyme activity for 60 min. and 30 min respectively, reaction was stopped by Deeping test tube in boiling water. Amount of reducing sugar was determined by Nelson –Somogyi, (1952) method. Enzyme activity was expressed in terms of reducing sugar ug/ml of fungal filtrate from CMC and cellobiose in 1 hr at 45^{0}C.

Result and discussion:

Cellulase activity by thermophilic fungi:

Estimation of reducing sugar after incubation for enzymatic activities by thermophilic fungi *M. thermophila* and *T. duponti* were found to be good decomposers of cellulose and were able to produce carboxymethylecellulase (Table No. 1) as well as cellobiase (Table No. 2) on media containing leafy biomass or CMC as sole source of cellulose.

It was observed that both the fungi produced more enzymes in the medium containing CMC as compared to its production on leafy materials as cellulosic substrate. As compared to banana leaves enzyme activity was more on sugarcane leaves. *M. thermophila* showed more cellulolytic activity on all three selected substrates. It was also observed that cellulose activity on all the substrate was increased upto 14 days and then declined Hajny and Reese (1969), recorded similar observation and attributes. This may be due to increasingly resistant cellulose residues left after degradation of susceptible portion of cellulose or it may be due to repression in soluble hydrolysis product. *M. thermophila* showed higher enzymatic activity as compared to *T. duponti*, (Fig. 1 and 2).

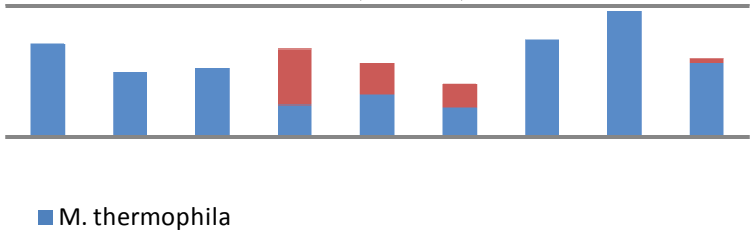
Table 1: Carboxymethyl cellulase (Cx) activity of thermophilic fungi *M. thermophila*and *T. duponti* in terms of reducing sugar released (ug/ml)

Fungi	Days --→	Reducing sugar released								
		Sugarcane leaves			Banana leaves			CMC		
		7	15	21	7	15	21	7	15	21
M. thermophila		71	49	52	24	32	22	74	96	56
T. duponti		34	38	15	67	56	40	62	82	60

Table 2: Cellobiase activity of thermophilic fungi *M. thermophila* and *T. duponti* in terms of reducing sugar released (ug/ml)

Fungi	Days --→	Reducing sugar released								
		Sugarcane leaves			Banana leaves			CMC		
		7	15	21	7	15	21	7	15	21
M. thermophila		49	24	24	19	20	25	27	31	29
T. duponti		45	30	36	22	24	17	20	48	27

Figure 1: Cx activity by *M. thermophila* and *T. Duponti* on various substrates (ug/ml)



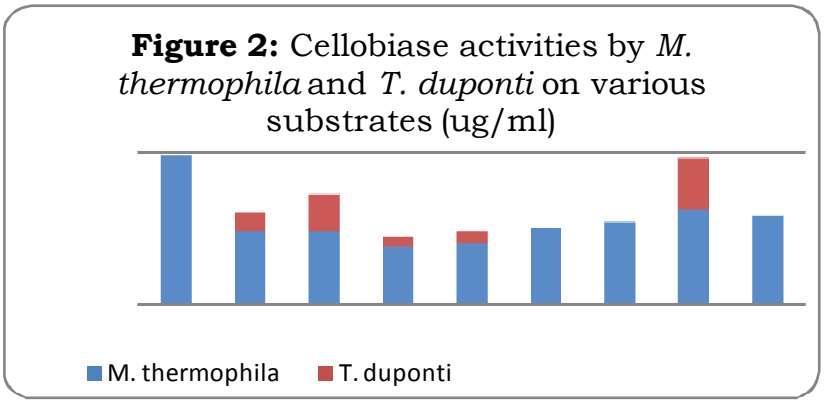


Figure 2: Cellobiase activities by *M. thermophila* and *T. duponti* on various substrates (ug/ml)

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