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Production of Enzymes in Predominant Thermophilic Fungi Available From Organic Substrates

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Abstract

Present investigation describes that the study site comes under Aurangabad Division Maharashtra and it falls in Deccan Plateau Zone of India. It was collected different types of organic substrates viz. vermiompost, poultary manure, baggase, farm yard manure (FYM), soil, Ash etc. Isolated thermophilic predominant fungi thermophilic fungi viz.*Aspergillus niger, Mucor mucedo,Humicola insolens,Trichoderma harzianum,T. viride,Penicillium duponti,Fusarium oxysporun* and *Chaetomium thermophilum* were carried out for the production of enzymes. Isolated predominant thermophilic fungi were evaluated on different types of enzymes. Among tested thermophilic fungi, the highest ativity was observed in *C. thermophilium* (20mm) followed by *T. harzianum* (19.50mm) In lipase, *M. mucedo* (15.40mm) was found maximum followed by *F. oxysporun.* Cellulase activity was found highest in *A. nige* (25mm) followed by others. In case of xylanase, catalase, peroxidase and esterase activities were found maximum, minimum and medium even negative in some fungi. Maximum pectinase activity was detected from *H. insolens* (52.26 @ 0 min) and (74.25 @ 10 min) and in case of *M. mucedo, F. oxysporun* and *C. thermophilium* was found most extreme while least in *A. niger* (30.12) and *P. duponti* (33.47) @ 0 minute.

Key words: Organic Substrates, Thermophilic Fungi, Enzymes

I Introduction

Microorganisms play a very important role from the groups according to their temperature ranges i.e.psychrophiles, mesophiles and thermophiles**.** Of the 70,000 formally recorded fungi species only around 30 species have the ability to grow at these elevated temperatures. Temperature is one of the extremely important environmental variables that play a decisive role in the survival, growth, distribution and diversity of microorganisms on the surface of the earth. The response of fungi to temperature varies between the two extremes of obligatorily thermophilic through thermotolerance to psychrophilic species. However, by far the majority of known fungi are mesophiles developing in culture between 5 and 37°C; the psychrophiles extend below that range of temperatures (Dix and Webster, 1995). (Atkinson, 1997) suggested that once temperature goes beyond 40°C, the mesophilic microbes become less aggressive and are replaced by thermophilic microbes, during thermophilic stage high temperatures accelerate up to 65°C, the breakdown of organic and inorganic compounds like cellulose and hemicelluloses, the major structural molecules. Through microbial decomposition of the organic waste matter which can be stabilized, matured and deodorized in to a product rich in humic substances that can be used as organic soil conditioner which is easy to store and distribute (Sahu et al., 2015).

Thermophilic aerobic micro-organisms are physiologically very active and are capable of producing several thermostable enzymes responsible for decomposition of cellulose and to a great extent lignin into simpler compounds. Thermophilic fungi are the chief components of the microflora that develops in heaped masses of plant material, piles of agricultural and forestry products and other accumulations of organic matter wherein the warm, humid, and aerobic environment provides the basic conditions for their development (Allen et al., 1949). Furthermore, compost has a high nutritional value with high concentrations of especially nitrogen, phosphorus and potassium, while the contamination by heavy metals and other toxic substances are very low (Asghar et al., 2006).

Naik (2017) describes the concepts of compost i.e. organic matter is an important component of soil which includes of plant and animal residues that are made up of complex carbohydrates, starch, cellulose, hemicellulose, lignin, protein, fats, organic acids, oils resins etc. Agricultural and industrial wastes were includes rice bran, rice husk, rice straw, sugarcane trash, bagasse, molasses, press mud etc., have a huge potential for recycling nutrient elements. The animal wastes such as cattle dung, buffalo manure, poultry wastes, rural and urban organic wastes, municipal waste can also be used for bioconversion as organic manure. Degradation is carried out by huge mixture of bacteria, fungi, insects, worms and other organisms that eat materials and recycle them into new forms (Singleton and Sambury, 1998).

Several thermophiles were observed to grow on starch, cellulose, hemicellulose, lignocellulose, lignin and pectin, but their ability to degrade lignin is doubtful (Johri et al., 1999). It was reported previously that the thermophilc fungi are known to produce thermostable proteases, lipases, amylases, cellulases, xylanases, lactases, trehalases and other extracellular enzymes (Johri et.al. 1985; Satyanarayana et al., 1992).

According to Bending and Read (1997) the lactase production ability of the microorganisms has not been measured in the selected thermophiles with high polyphenol oxidase activity, but phenol oxidase producing ability of the isolates could accelerate the degradation of lignin. However, cellulose recalcitrance to biodegradation poses several major bottlenecks in the thermophilic digestion of biomass with the major impediment being the lack of availability of robust cellulases that can function efficiently at relatively higher temperatures (Rastogi et al., 2010).

In literature, many thermophilic cellulolytic fungal species such as *Sporotrichum thermophile*, *Thermoascus aurantiacus* and *Thielavia terrestris* have been reported (Maheshwari et. al. 2000). Thermophilic microbes growing at temperature of 50-80°C are the sources of highly active and thermostable enzymes studied (Haki and Rakshit 2003, Viikari et al., 2007,Yeoman et al., 2010, Zambare et al., 2011).

Similarly, during the production of lipase by *Aspergillus* sp. (Cihangir and Sarıkaya, 2004) and *Penicillium restrictum* (De Azeredo et al.,2007) the highest lipolytic activity has been attained in media containing olive oil, *Thermus thermophiles*, whereas the optimum lipase production was attained at 70°C (Dominguez, 2005). Joshi et.al. (2008) reported the alkaline proteases, chitinases, amylases, lipases and caseinases enzymes in a wide range of microorganisms isolated from Soda Lake environments, such as Rift valley Soda lakes. Other lignolytic microorganism reported from *Asperigillus flavus* (Betts and Dart, 1988), *Trichoderma harzianum* (Harper and Lynch,1985; Bhale and Rajkonda, 2012), *Nocardia* sp. (Trojanowski et al., 1977)

II Materials and Methods

Survey of various compost

Study site (Osmanabad district) is comes under Marathwada regions of Maharashtra state of India. The region comes under Aurangabad Division. It was a part of Nizam's domain, which was known as 'The Princely State of Hyderabad'. This region lies between 17^0 35' N & 20^0 40' N Latitude and 70^0 40' E & 78^0 15' E Longitude. It falls in Deccan Plateau Zone of India with geographical area of 6.5 million hectare occupying 21 % of total area of the Maharashtra. This region is situated at an average height of about 300-650 m. above mean sea level, gradually sloping ranges originating from the Sahyadri's in the west and Satpuda ranges in the north.

Isolation of thermophilic fungi by Dilution plate technique

The isolation of thermophilic fungi from different substrates was carried out using dilution plate technique (Apinis 1963; Waksman, 1939). Ten grams of sample were transferred to a flask containing 100 ml sterile water. The contents were shaken with centrifuge machine for 15 min and then diluted 10^{-3} of 0.5 ml was transferred to sterile petri plates containing different media in triplicates. The pH of medium was adjusted to 6.5 with 0.1N HCl or 0.1N NaOH. Petri plates were incubated in an inverted position at room temperature (RT) and adjusted the temperature in hot air oven at 35 to 65 ^{0}C. Pure cultures of isolates were maintained on respective media slants at 4^0C for further study.

Identification of thermophilic fungi

The different topographical characters of the colonies were recorded at regular time intervals. The semi-permanent slides of the isolated fungi were prepared using 1 % cotton blue and lactophenol. Identification of thermophilic fungi was made by referring relevant literature and monographs (Subramanian, 1971; Barnett, 1972; Kumar et al., 2010). Key to the identification of thermophilic fungi was used according to Salar and Aneja (2007).

Enzymes production of predominant thermophilic Fungi

Detection of enzyme activities of isolated thermophilic fungi from various composts was tested. Hydrolytic enzymes, cellulase, amylase, lipase and pectinase, which provide the fungi chemical means of entrance into the host and a process whereby nutrients, can be digested. The extracellular production of hydrolytic enzymes was important activity with respect to mycoparasitism and antibiosis of the fungal species. Commercial production of extracellular hydrolytic enzymes was employed commercially and the work about the enzyme activities is still going progressively. Cellulose & Lipase production activity was determined by Cup plate method (Dingle et al., 1953). The amylase activity was determined with the help of cup method (Singh and Saxena, 1982; Hankin and Anagnostakis, 1975). The enzyme assay for pectinolytic enzyme was tested by viscometer method (Papdiwal, 1982). Determination of xylanase activity was performed by Nakamura et al. (1993) Determination of Catalase and Peroxidase activity was performed by (Balasundaran, 2008). Determination of esterase activity was described by Sierra (1957).

III Results and Discussion

Isolation and Identification of thermophilic fungi

Study sites shows different types of available compost viz. vermiompost, poultary manure, baggase, farm yard manure (FYM), soil, Ash etc. Isolation of fungi were separated from substrate and among six substrates and isolated predominant thermophilic fungi viz.*Aspergillus niger*,*Mucor mucedo*,*Humicola insolens*,*Trichoderma harzianum*,*Trichoderma viride*,*Penicillium duponti*,*Fusarium oxysporun* and *Chaetomium thermophilum*

Enzyme Production

Isolated predominant thermophilic fungi were evaluated on different types of enzymes. In case of amylase, the fungi i.e. *Aspergillus niger, Mucor mucedo, Humicola insolens* and *Chaetomium thermophilium* were found most extreme activity while *Penicillium duponti* and *Fusarium oxysporun* was least activity. In lipase, *A. niger, H. insolens, F. oxysporun* and *C. thermophilium* was found least activity while there was negative activity found in *Trichoderma harzianum* and *Penicillium duponti*. In cellulase, *A. niger*, *T. harzianum* and *P. duponti*, were found most extreme activity while *F. oxysporun* was least. In *cellulase* there was negative activity in case of *M. mucedo*, *H.insolens* and *Chaetomium thermophilium*. In xylanase, *A. niger, H. insolens*, *P. duponti* and *C. thermophilium* found least while *T. harzianum* was most extreme activity. In addition there was negative activity in *M.mucedo* and *F. oxysporun.* In catalase, *A. niger, F. oxysporun* and *Chaetomium* was least while there was negative activity in *H. insolens* and *T. harzianum.* In peroxidase, *A. niger*, *T. harzianum* and *P. duponti* were found medium activity and there was negative activity in case of *M. mucedo, F.* oxysporun and *C. thermophilium* species. In esterase, there was negative activity in *A. niger, M.mucedo* and *T. harzianum* while *P. duponti* was found least(Tab.1,Fig.1).

Table 1 . Detection of different enzymes of predominant thermophilic fungi.

Predominant thermophilic Fungi	Enzyme Activity						
	Amylase (mm)	**Lipase (mm)**	**Cellulase (mm)**	**Xylanase**	**Catalase**	**Peroxidase**	**Esterase**
Aspergillus niger	17.00	12.00	25.00	+	+	++	-
Mucor mucedo	15.60	15.40	00.00	-	++	-	-
Humicola insolens	0.00	11.00	00.00	+	-	+	++
Trichoderma harzianum	19.50	00.00	19.00	+++	-	++	-
Trichoderma viride	00.00	12.06	15.32	+++	++	-	+
Penicillium duponti	13.90	00.00	20.00	+	++	++	+
Fusarium oxysporun	14.50	14.50	15.00	-	+	-	-
Chaetomium thermophilum	20.00	13.00	00.00	+	+	-	++

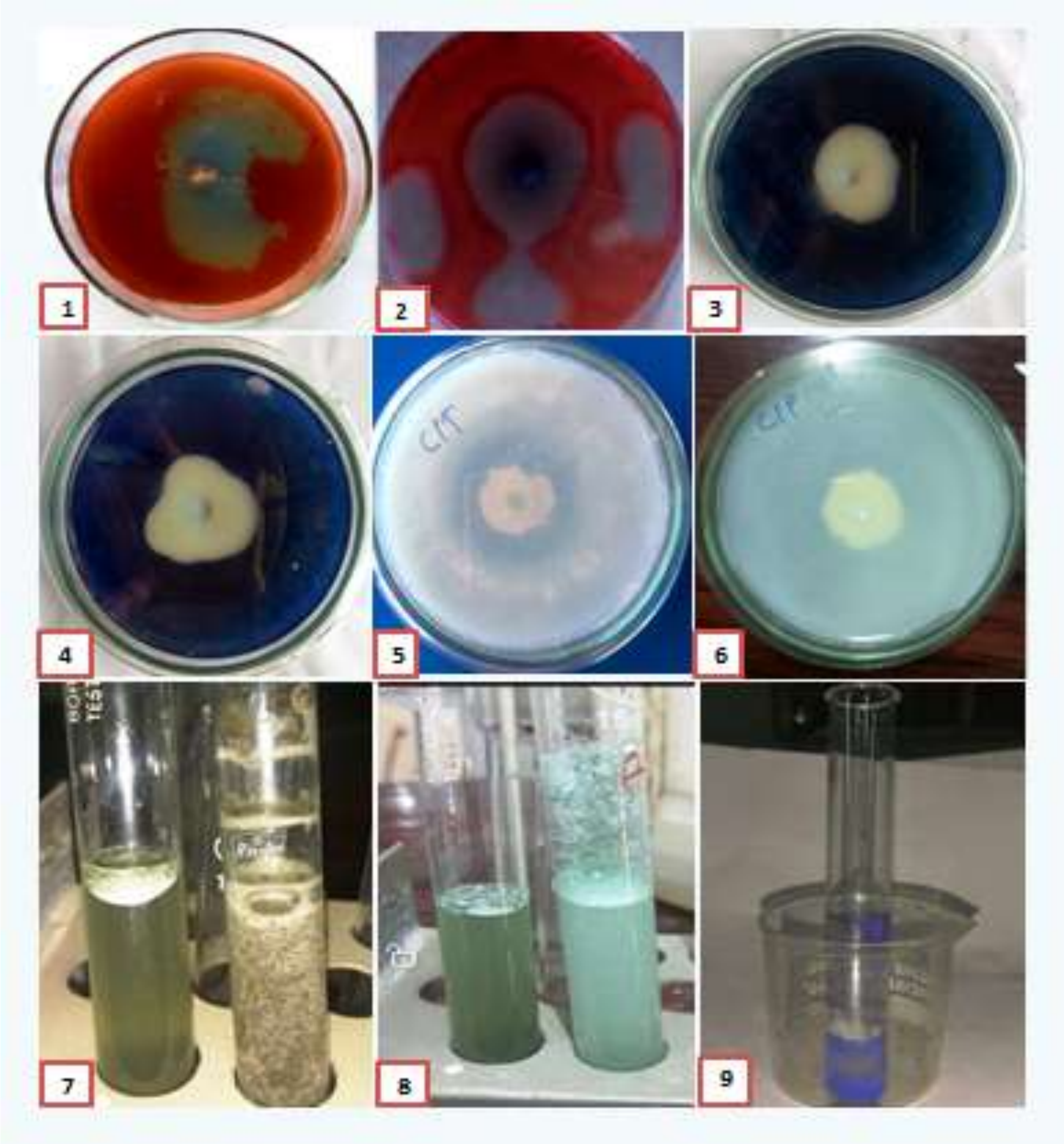

Fig.1.Enzyme Production Of Predominant Fungi (**1**-Xylanase activity (*A.niger*), **2**- Xylanase Activity (*T.harzianum*), **3**-Amylase Activity (*P.dupanti*), **4**-Amylase Activity (*C.thermophylium*), **5**-Cellulase Activity(*T.viride*), **6**- Cellulase Activity (*P.dupanti*), **7**-Catalase activity (*A.niger*), 8- Catalase activity (*T.viride*), **9**-Peroxidase activity(*A.niger*)

Pectinase Enzyme

Detection of another extracellular hydrolytic enzyme, pectinase was also undertaken in the present work. The pectinase activity was determined in terms of percent loss of viscosity by viscometry method. The percent loss of viscosity was obtained at a particular time intervals (Tab. 2). It was cleared from the results that loss of viscosity was directly related with the time. As time interval increased more viscosity loss was observed. Pectinase activity was detected from *Aspergillus niger* (30.12@0 min.) and (55.25 @10 min.). Maximum pectinase activity was detected from. *Humicola insolens* (52.26 at 0 min) and (74.25 @ 10 min). In case of *Mucor mucedo*, *Fusarium oxysporun* and *C. thermophilium* was found most extreme while least in *A. niger* (30.12) and *Penicillium duponti* (33.47) @ 0 minute.

Table 2. Detection of pectinase enzymes from predominant thermophilic fungi.

Sr. No .	Predominant thermophilic Fungi	% Viscosity loss after time (Minutes)			
		0	**01**	**05**	**10**
1	*Aspergillus niger*	30.12	42.52	49.12	55.25
2	*Mucor mucedo*	48.21	53.42	58.97	64.32
3	*Humicola insolens*	52.26	59.25	67.24	74.25
4	*Trichoderma harzianum*	44.27	45.54	50.82	52.73
5	*Trichoderma viride*	43.85	46.22	50.42	53.06
5	*Penicillium duponti,*	33.47	46.58	52.12	58.54
6	*Fusarium oxysporun*	40.33	54.69	61.59	67.56
7	*Chaetomium thermophilum*	48.84	57.72	63.84	67.63

Study discussed here the different types of enzymes production by dominant thermophiles fungi involved in composts was detected and observed very promising role by these fungi for decomposition of composts and found passive and negative enzyme activity.

In literature, thermophilic cellulolytic fungal species such as *Sporotrichum thermophile, Thermoascus aurantiacus* and *Thielavia terrestris* have been reported and shows enzyme systems produced by various cellulolytic microorganisms for the degradation of cellulose and xylan (Aro et.al., 2005). In comparison to mesophilic fungi, thermophillic ones have been found to show rapid growth rates and higher rates of cellulose decomposition, making thermophilic fungi an attractive potential source of cellulases (Rajasekaran and Maheshwari, 1990, Bhalla et al., 2013). Lee et al (2014) reported three species of thermophiles were isolated from compost and were identified as *Myriococcum thermophilum, Thermoascus aurantiacus,* and *Thermomyces lanuginosus* and grow at temperatures above 50^{o}C and produce high levels of cellulolytic and xylanolytic enzymes at high temperatures. Msaraha et.al. (2018) reported eight thermophilic bacteria were isolated and determined to have at least three strong enzyme activity including protease, lipase, amylase, cellulase, pectinase and xylanase. Bairagi (2016) reported total 151 fungal isolates were isolated from soil samples and determined the potency of microbes in producing cellulase and xylanase which were indicated by clear zones formation around the cultures and found maximum enzyme production at 30°C and pH of 6.0 in *Trichoderma atroviride* on 5th day of incubation.

Mansfield et al. (1999) reported the *Trichoderma* spp. and *Aspergillus* spp. have most widely been used for production of cellulase and xylanase enzymes. Gautam et al. (2010) observed that the *Trichoderma* sp. is well known among the cellulolytic fungi for their potential to degrade organic municipal solid waste. Doolotkeldieva and Bobusheva (2011) have been reported the soft-rot fungi, *Trichoderma viride* and *Trichoderma reesei* are the most extensively studied cellulolytic fungi.

IV Conclusion

Thermophilic microbes are preparing high quality of compost with useful micro and macro elements in short duration. This observation would help in improvement of plant productivity and promotes the need of introducing methods of farming for better utility of organic amendments. Hence the different types of enzymes production by dominant thermophiles fungi involved in composts was detected and observed very promising role by these fungi for decomposition of composts and found passive and negative enzyme activity. Hence, the fortification of organic wastes and their composts as a source of organic nutrients are imperative for sustainable agriculture.

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