

Incidence of Thermophilic Fungal Consortium from Different Temperature Gradation Level on Different Organic Substrates

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ARTICLE INFO

Article history:

Received: 8 March 2018;

Received in revised form:

02 April 2018;

Accepted: 12 April 2018;

Keywords

Organic substrates,
Thermophilic fungi,
Different Temperatures,
% of incidence.

ABSTRACT

Present study revealed that thermophile fungal species richness on different substrate in different temperature gradients like room, 35,45,55,65°C. Total 23 fungal species were found from six different organic substrate. Among six substrates, vermiompost (57) and Farm Yard Manure (FYM) (52) were recorded highest species richness and % incidence while less in ash (14). Among isolated thermophilic fungi, *Aspergillus* species found abundant in all the substrate and temperature ranges while poor incidence in case of *Trichoderma harzianum*, *Rhizoctonia*, *Geotrichum*, *Alternaria* species, *Aspergillus tamari* and *Penicillium cinnamon*. Among the thermophiles *Aspergillus niger* and *A. flavus* were found on all the substrates itself act as thermotolerant. Every temperature gradient showed the growth incidence in all substrate but room temp and 35°C showed richness of fungal incidence. Even some species showed the growth on some substrate at higher temp (65°C) in some extent.

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1. Introduction

Thermophilic fungi are the chief components of microflora, they are mainly developed decomposed masses of plant material pile of agricultural and forestry product and other accumulation of organic matter where in the warm humid and aerobic environment provide the basic condition for their development. Thermophilic fungi are occurs in all places of world. They are self-heating masses. (Cooney and Emerson 1964). Accumulation of organic matter where in warm, humid and aerobic environment provides the basic physiological condition. In these habitats thermophilic may occur either as resting propagules as active mycelium depending on the availability of nutrient and favorable environment (Khushaldas 2009). Cooney defined thermophilic fungi as those fungi which can grow at temperature range 20-50°C. Therefore the present study describes the capacity it can grow higher temperature up to 65°C. There are larger organisms present in compost known as the physical decomposers that chew and grind their way through compost heap and are higher up in the food chain (Stainer et al. 1998). Substantial changes occur in microbial populations and species abundance during the various temperature stages (Gupta et al. 1987).

2. Materials and Methods

2.1 Substrates Collection

The organic substrates like Farm Yard Manure (FYM), Soil, Ash, Vermicompost, poultry manure, bagasse were collected from different localities of Osmanabad district of Maharashtra during 2016-2017. The samples were cooled and kept for fungal analysis.

2.2 Isolation of thermophilic fungi

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 on a mechanical shaker for 15 min and then diluted 10^{-3} of 0.5 ml was transferred to sterile petri plates containing Potato Dextrose Agar (PDA) and Martins Rose Bengal (MRB) agar medium 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 °C. Pure cultures of isolates were maintained on respective media slants at 4°C for further study.

The percentage of incidence was calculated by employing the following formula (Girisham et al. 1986)

$$\% \text{ of incidence} = \frac{\text{No of colonies of species in all plates}}{\text{Total no of colony of the all the species in all plates}} \times 100$$

2.3 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 and Mukadam 1997; Kumar et al. 2010).

Result and Discussions

Thermophile fungal species richness on different substrate in different temperature gradients like room, 35, 45, 55,65°C. Total 23 fungal species were found from six different organic substrates. Among six substrates, vermiompost (57) and Farm Yard Manure (FYM) (52) were recorded highest species richness and % incidence while less in ash (14). Among isolated thermophilic fungi, *Aspergillus niger* (23), *A. flavus* (23), *Rhizoctonia solani* (15) and *A. fumigatus* (13) found abundant in all the substrate and temperature ranges

Table 1. Incidence of thermophilic fungi from different temperatures on different organic substrates.

SN	Temp (°C) ↓ Fungi	ORGANIC SUBSTRATES																																
		FYM					VERMICOMPOST					POULRTY MANURE					MOLASES					ASH						SOIL						
		RT	35	45	55	65	RT	35	45	55	65	RT	35	45	55	65	RT	35	45	55	65	RT	35	45	55	65	RT	35	45	55	65	TS	% IN	
1	<i>Af</i>	+	+	+	+	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	13	5.7	
2	<i>An</i>	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+	23	10.2
3	<i>Afl</i>	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	-	23	10.2
4	<i>Ar</i>	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	09	3.9	
5	<i>At</i>	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	-	-	-	07	3.1	
6	<i>Mm</i>	+	+	-	-	-	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	11	4.9	
7	<i>Mmi</i>	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	06	2.7	
8	<i>Hi</i>	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	08	3.6	
9	<i>HL</i>	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	-	-	-	11	4.9	
10	<i>P</i>	-	-	-	-	-	-	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	10	4.5	
11	<i>Rs</i>	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	15	6.7	
12	<i>R</i>	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	04	1.8	
13	<i>Mt</i>	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	09	3.9	
14	<i>Pc</i>	-	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	06	2.7	
15	<i>Pd</i>	+	+	-	-	-	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	08	3.6	
16	<i>Pb</i>	-	-	-	-	-	+	+	-	-	-	+	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	10	4.5	
17	<i>Aa</i>	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	06	2.7	
18	<i>Fo</i>	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	+	+	+	-	-	11	4.9	
19	<i>Tv</i>	+	+	+	-	-	+	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	09	3.9	
20	<i>Th</i>	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	04	1.8	
21	<i>Ct</i>	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	4.5	
22	<i>C</i>	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	08	3.6	
23	<i>G</i>	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	06	2.7	
Total (TS) Species		15	15	10	08	04	16	19	13	07	02	11	08	08	06	01	07	07	07	03	01	03	03	05	02	01	14	14	09	04	04	227		

Legands: *RT*-Room temperature, *Af*-*Aspergillus fumigatus*, *An*-*Aspergillus niger*, *Afl*-*Aspergillus flavus*, *Ar*-*Aspergillus rapens*, *At*- *Aspergillus tamari*, *Mm*-*Mucor muudo*, *Mmi*-*Mucor miehei*, *Hi*-*Humicola insolens*, *Hi*- *Humicola langinos*, *P*-*Pythium* sp, , *Rs*-*Rhizotonia solani*, *R*- *Rhizoctonia* sp, *Mt*- *Myceliophthora thermophila*, *Ct*-*Cladospora thermophila*, *Pc*-*Pinicillium cinnmoni*, *Pd*- *Pinicillium duponti*, *Pb*- *Pinicillium befeldianum*, *Aa*-*Alternaria alternata*, *Fo*-*Fusarium oxysporun*, *Tv*-*Trichoderma viride*, *Th*-*Trichoderma harzianum*, , *C*-*Cladosporum* sp, *G*-*Geotrichum* sp, %IN-Incidence

while poor incidence in case of *Trichoderma harzianum*, *Rhizoctonia*, *Geotrichum*, *Alternaria* species, *Aspergillus tamari* and *Penicillium cinnamoni*. Every temperature gradient showed the growth incidence in all substrate but room temp and 35°C showed richness of fungal incidence. In FYM at higher temp (65°C), *Aspergillus niger*, *Mucor miehei*, *Myceliophthora thermophila* and *Cladospora thermophila*, in vermicompost *Aspergillus niger* and *C.thermophila*, in poultry manure only *A. niger*, in bagasse *Rhizoctonia solani*, in ash *Aspergillus niger* and in soil *A. niger*, *Rhizoctonia*, *Cladospora* and *Geotrichum* species were found dominant (Table 1; Figure 1 & 2).

In the present investigation supports earlier findings and Blom et al. (1962) made no mention of bagasse as a source of thermophilic fungi. The baled or heaped bagasse made development of thermophilic microorganisms (Cooney et al 1964). The occurrence of *Humicola lanuginosus* in the manure was also reported (Crisan 1964 and Fergus 1964). Cooney and Emerson 1964 reported occurrence of *Cheatomium thermophile var coprophile* on goat dung. Recently, Ch. Ramesh and Anil Kumar (2005) isolated 22 thermophilic fungi belonging to 8 genera on different substrates from different region of Dharwad (Karnataka State). Similar results were also reported by Anastasi et al. (2005) who also isolated fungi from different thermogenic places. The thermophile *T. lanuginosus* is one of the most common fungi occurring in soil (Maheshwari et al. 1987). Fungal consortium of *Aspergillus* and *Humicola* sp., and actinomycetes, especially *Streptomyces* had been earlier used for the conversion of nutrient rich compost (Cowan 1999). Ashraf et al. (2007) reported microorganisms isolated and characterized from the above composts include the species of fungi viz., *Aspergillus*, *Trichoderma*, *Mucor*, *Penicillium*, *Alternaria*, *Cladosporium*, *Monilia*, *Helminthosporium*, *Coccidioides*, *Scedosporium*, actinomycete viz., *Nocardia* and bacteria viz., *Bacillus*, *Lactobacilli*, *Micrococcus*, *Pseudomonas*, *Clostridium*. Of these isolates, members of the genus *Aspergillus* were most prevalent (38%) followed by *Bacillus* comprising of 20% of the total microbial isolates. Bhale et al. (2008) reported that the isolation of 20 species belonging to 7 genera on different substrates collected from different places of Osmanabad district and *Aspergillus fumigatus* was present nearly in all of the sources.

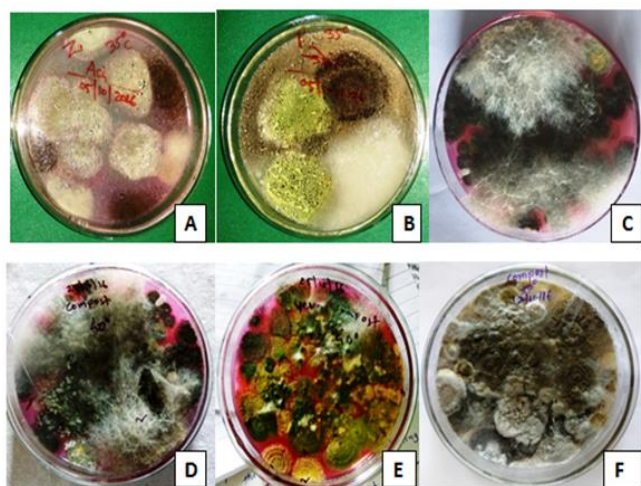


Figure 1. Thermophilic Fungal Colonies on and MRB medium. (A-Ash, B-Poultry Manure, C-Bagasse, D-FYM, E-Vermicompost F-Soil).

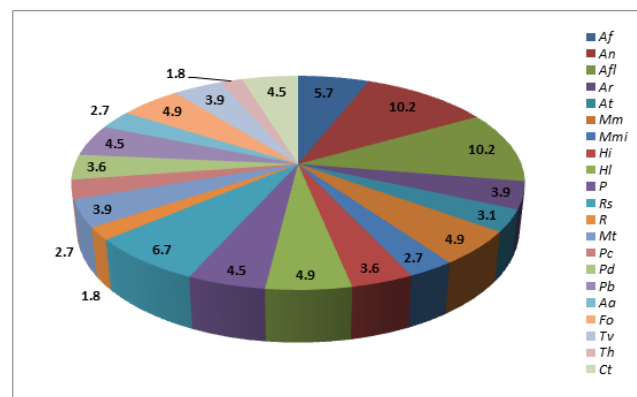


Figure 2. Incidence (%) of different groups of thermophilic fungi in different organic substrates.

Conclusions

Abundance of thermophilic fungi in FYM, Vermicompost and Poultry manure substrate were the main sources of thermophilic fungi. Common thermophilic fungi are *A.niger* and *A.flavus*. Among six substrates, vermicompost and FYM was recorded highest species richness and % incidence while less in ash.

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