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INCIDENCE OF THERMOPHILIC FUNGI FROM DIFFERENT SUBSTRATES IN ANDHRA PRADESH (INDIA)

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ABSTRACT

Total 46 thermophilic fungi were isolated from various substrates such as under ground coalmine soil, bird nest materials, vermicompost, cow dung, poultry litter, decomposing pits, which are prepared with agro waste, municipal waste and zoo dump materials and industrial waste etc. The present paper deals with the isolation of 46 species belonging to 13 genera on different substrates collected from different places of Andhra Pradesh. Among the thermophiles *Humicola lanuginosus* was present nearly in all substrates and *Aspergillus fumigatus* found as a thermotolerant in all the substrates.

KEY WORDS

Thermophilic fungi, ecological substrates, incidence, thermotolerant fungi.

INTRODUCTION

Thermophilic fungi have worldwide distribution. It seems more likely that a generally world wide distribution is a result of the world wide occurrence of self heating masses (Maheshwari *et al.,* 1987). Thermophilic fungi have been reported from various natural habitats such as soils and in habitats where decomposition of plant material takes place. These include compost and wood chip piles, nesting material of birds, animals and municipal refuse. Accumulation of organic matter where in warm, humid and aerobic environment provides the basic physiological condition, in these habitats thermophiles may occur either as resting propagules or as active mycelium depending on the availability of nutrients and favourable environment (Khushaldas, 2009). Cooney defined thermophilic fungi as those which can grow at a temperature with in the range of 20-50 ºC. Thermophilic microorganisms form a diverse group of organisms found in various habitats characterized by different environmental conditions. Among these some fungi possess the ability to grow at higher temperature up to 60 C. Therefore, in the present investigation an attempt has been made to isolate the thermophilic fungi from different substrates like underground coal mine soil, nesting material of birds, animal and municipal refuse, decomposition of plant materials, mushroom compost, vermi compost, poultry litter and tobacco products. In all 450 samples collected from 10 different sources were screened for the presence of thermophilic fungi.

MATERIALS AND METHODS

Isolation of thermophilic fungi from different substrates was carried out using the following techniques.

***DILUTION PLATE TECHNIQUE*:** For the isolation of mycoflora dilution plate method was employed (Apinis, 1963b). 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 serially diluted to obtain 10^{-4} -10^{-5} 0.5 ml of each was transferred to sterile petri plates containing Yeast Extract Starch Agar medium (containing Starch 30 g Yeast extract 5 g $MgSO_4$ 7 H_2O 0.5 g KH_2PO_4 1 g Rose Bengal 0.0001 g and trace amount of streptomycin Agar –agar 20 g and tap water 1000ml). The pH of medium was adjusted to 6.2 with 0.1N HCl or 0.1N NaOH. The isolation of thermophilic fungi from different substrates was also carried out by using the following media, Czapek Dox agar (contain $NaNO_3$ 3g, $MgSO_4$ 7 H_2O 0.05 g KCl 0,5g, $FeSO_4$ 0.01 g, Sucrose 30 g, Agar agar 15 g and tap water 1000ml) and Dextrose Peptone Yeast extract Agar (Dextrose 1 g, Peptone 2 g, Yeast Extract 0.3g, KH_2PO_4 0.2 g $MgSO_4$ 7 H_2O 0.02 g, Agar agar 15 g and tap water 1000ml) and Modified Czapek Dox media (Glucose 2.0g, L-asparagine 10.0g, KH_2PO_4 1.52g, KCl 0.52 g, $MgSO_4$ .7 H_2O 0.52g, $CuNO_3$ .3 H_2O trace, $ZnSO_4$. 7 H_2O trace,$FeSO_4$ 7 H_2O trace , Agar agar 20 g and 1000ml of distilled water, pH was adjusted to 6.2 (Saxena and Sinha, 1981)were employed for isolation.

***PAIRED PETRI PLATE TECHNIQUE (Cooney and Emerson, 1964*):** For isolation of thermophilic fungi we have used this method it provides moisture and suitable environment for the growth of thermophilic fungi. Paired plates are taken and top plate is fixed with sterile filter paper and paired plate is sealed with the cellophane tape to prevent moisture escape. This method gives very good results and maximum thermophiles are isolated by using this method.

HUMID CHAMBER TECHNIQUE (Buxton and Mellanby, 1964): This method is employed especially for isolation of thermophiles from the Bird nest materials. Collected bird nest materials are taken and directly placed in a glass chamber which is previously arranged with sterile wet filter paper and sterile glass slide on it. The nest materials directly placed on sterile glass slides.

The internal temperature of the chamber is regularly maintained 40-45˚C Growth of fungus appeared on nest material are taken and they were transferred into sterile yeast extract starch agar slants and checked for thermophilic character.

***WARCUPS SOIL PLATE METHOD*:** 2 ml of the sample was placed in a sterile petridish and 20 ml of sterile cooled (40 C) sterile Czapek Dox medium was added. The contents were thoroughly mixed and the plates were incubated at 47 ±2 ˚C

***WAKS MANS DIRECT INOCULATION METHOD*:** 20 ml of modified Emerson's (YPSS) medium was poured into a sterile petri dish and was allowed to solidify. Small quantities of the samples were sprinkled over the medium in the dishes such plates were incubated in inverted position at 47 ±2 ˚C under high humidity observation on the fungal growth if any were made every day and the fungi were isolated.

The different topographical characters of the colonies were recorded at regular time intervals. The semi permanent slides of the isolated fungi were prepared using lactophenol. Identification of thermophiles were made by referring relevant literature and monographs (Apinis, 1963a, Cooney *et al* 1964 Barnett *et al* 1972). The distribution of thermophilic fungi isolated from 450 sample collected from 11 different substrates is present in table.1 All the substrates showed presence of thermophilic fungi.

Aspergillus fumigatus, *Humicola lanuginosa* were present nearly all of the sources. Underground coal mine soil sample Cattle dung, Municipal waste and Vermicompost sources were rich in thermophilic fungi. The technique used for isolation of thermophilic fungi was compared for their efficiency to obtain different species of fungi. The dilution plate technique using antibiotics, YPSS medium and Cooney Emerson method were found to be suitable to obtain different types of fungi from various sources.

The percentage of incidence, frequency and abundance were calculated by employing the following formulae (Girisham *et al.,* 1986)

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

$$\% \text{ of frequency} = \frac{\text{No of observation in which species appeared}}{\text{Total no of observations}} \times 100$$

$$\% \text{ of abundance} = \frac{\text{No of colonies of species in all observations}}{\text{Total no of colonies in all observations}} \times 100$$

RESULTS AND DISCUSSION

Aspergillus fumigatus abundant in most of the sources and higher in the decomposing litter material. *Mucor species* were also widely distributed and highest in the underground coal mine soil (Table 1). The general *Cheatomium* and *Torula* were restricted to vermicompost (Table 2). *Myricoccum albomyces* and *Penicillium duponti* were recovered from poultry litter and *Humicola lanuginosus* were highly distributed in all sources. *H. insolens* and *H. grisea* were isolated from cattle dung. *Mucor miehei* was recovered from zoo dump and *Cheatomium dissitum (v)* were recovered from municipal waste. The genera

Aspergillus were observed in all substrate. *M. pusillus* and *M. miehei* showed rapid growth at 45 ±2˚C. *H. lanuginosus* showed good growth at 52 ±2˚C which was the highest optimum temperature range among all the fungi. *Torula thermophila* had the optima between 47 ˚C to 50˚C. *Humicola insolens* showed rapid growth at 47 ˚C. *Cheatomium thermophile* had their optimum between 38-42 ˚C. *Mucor* sp.occured with highest percentage of incidence in industrial waste (Table 3).

TABLE 1
INCIDENCES OF DIFFERENT THERMOPHILIC FUNGI IN COALMINE SOIL, CATTLE DUNG, MUNICIPAL WASTE & POULTRY

NAME OF THE FUNGUS	COALMINE SOIL			CATTLE DUMP			MUNICIPAL WASTE			POULTRY LITTER		
	P I	PF	PA	P I	PF	PA	P I	PF	PA	P I	PF	PA
Chaetomium thermophile	-	-	-	20.7	25	25,0	-	-	-	-	-	-
C.thermophile dissitum v	-	-	-	-	-	-	38.5	48.0	35.0	-	-	-
Humicola grisea	-	-	-	20.0	15.2	15.0	-	-	-	-	-	-
H. insolens	-	-	-	20.3	10.2	10.0	-	-	-	25.0	22.0	23.0
H. lanuginosus	-	-	-	5.0	20.0	3.0	20.5	30.0	38.0	20.0	5.5	18
Humicola spp.	-	-	-	-	-	-	10	15.5	6.0	-	-	-
Humicola spp.	-	-	-	13.5	15.8	19.0	20.0	0.5	16.0	9	20.5	9.5
Mucor miehei	25.5	5.2	20.5	-	-	-	5.0	1.0	1.0	2.0	8.0	6.0
M.pusillus	50.5	60.4	35.5	8.1	0.8	4.0	-	-	-	1.0	15.0	3.0
Mucor spp.	23	31.9	46.0	6.0	6.0	6.0	11.0	5.0	5.0	5.0	15.0	10.0
Myriococcum albomyces	-	-	-	-	-	-	-	-	-	15.0	1.0	12.0
Penicillium duponti	-	-	-	-	-	-	-	-	-	20.0	2.0	14.0
Torula thermophila	-	-	-	4.0	2.0	10.0	-	-	-	-	-	-
Torula spp.	-	-	-	1.0	1.0	8.0	-	-	-	-	-	-

TABLE 2 INCIDENCE OF DIFFERENT THERMOPHILIC FUNGI IN BIRD NESTS, ZOO DUMP, VERMI COMPOST AND DECOMPOSING LITTER

NAME OF THE FUNGUS	Industrial waste			Furnase soil			Tobacco products		
	P I	PF	PA	P I	PF	PA	P I	PF	PA
Chaetomium thermophile	10.0	5.0	5.0	-	-	-	-	-	-
Humicola lanuginosus	40.0	20.0	50.0	-	-	-	50.0	20.0	70.0
Humicola spp.	-	-	-	75.0	10.0	50.0	-	-	-
Mucor miehei	-	5.0	5.0	-	-	-	-	-	-
M. pusillus	-	-	20.0	-	-	-	-	-	-
Mucor spp.	50.0	70.0	20.0	15.0	30.0	50.0	50.0	80.0	30.0

TABLE 3. INCIDENCE OF DIFFERENT THERMOPHILIC FUNGI IN INDUSTRIALEFFLUENT,FURANSE SOIL TOBACCO PRODUCTS

NAME OF THE FUNGUS	BIRD NESTS			ZOO DUMP			VERMI COM0POST			DECOMPOSING LITTER		
	P I	PF	PA	P I	PF	PA	P I	PF	PA	P I	PF	PA
Chaetomium thermophile	-	-	-	7.0	10.0	15.0	10.0	5.0	5.0	-	-	-
Chrysosporium spp.	-	-	-	20.0	20.0	20.0	-	-	-	-	-	-
Humicola grisea	40.0	8.0	30.0	-	-	-	5.0	15.0	10.0	-	-	-
H.insolens	-	-	-	-	-	-	10.00	3.0	5.0	-	-	-
H.lanuginosus	-	-	-	-	-	-	15.0	20.0	10.0	-	-	-
Humicola spp.	44.0	82.0	40.0	28.0	40.0	47.0	7.0	4.5	19.0	100.0	100.0	100.0
Mucor miehei	-	-	-	10.0	5.0	5.0	-	-	-	-	-	-
M. pusillus	-	-	-	10.0	3.0	5.0	-	-	-	-	-	-
Mucor spp.	16	8.0	30.0	25.0	22.0	8.0	8	12.5	3	-	-	-
Torula thermophila	-	-	-	-	-	-	20.0	23.0	40.0	-	-	-
Torula spp.	-	-	-	-	-	-	15.0	20.0	7.0	-	-	-

TABLE3.

PI = Percentage of Incidence, PF = Percentage of Frequency, PA=Percentage of Abundance

CONCLUSION

Abundance of thermophilic fungi in the cattle dung obtained 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 lanaginosus* 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. Crisan (1964) has described methods for collecting and isolating thermophilic fungi.

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