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INCIDENCE OF THERMOPHILIC FUNGI IN DIFFERENT DUNG SAMPLES OF WARANGAL DISTRICT OF AP

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ABSTRACT

The incidence of thermophilic fungi in dung samples of different animals viz., cow, sheep, turkey, pigeon, duck, poultry, bear, rabbit, monkey and zoo dump collected from various places of Warangal were used for the isolation and characterization of several fungi. Samples collected from cow and sheep were reported to associate with more number of coprophilic fungi, whereas, turkey poults were noticed to least. A. flavus and A. fumigatus were observed with all samples of different animals, they proved to be thermotolerant. A positive correlation observed between percentage of incidence, frequency and abundance could be of different.

KEY WORDS

A. flavus, A. fumigatus, Coprophilic fungi, Thermophilic, Thermotolerant.

INTRODUCTION

Thermophilic fungi which grow on animal dung are called coprophiles and are reported to be important in the decomposition and recycling of animal feces, especially of herbivorous mammals. Passage of the spores through the gut of an animal is often necessary to facilitate spore germination of coprophilous fungi [1]. Dung which consists of the remains of plant material plus the microbiota associated with its digestion is a rich substratum for the fungal growth. The material is complex, and includes fatty acids, vitamins, amino acids and the pH is close to neutral. Varieties of thermophilic fungi are reported from different parts of the world [2, 3]. Apart from fungi of the gastro intestinal tract [GIT], dung also includes fungi accidentally consumed and fungal spores settling from air. Coprophilous fungi survive passage through the GIT, germinate and grow in the freshly deposited dung. This produce spores with form adhesive capacity and are actively and violently discharged. The spores thus discharged get deposited on vegetation, and then consumed by herbivores to complete the cycle. Richardson [1] has observed definite succession of coprophilous fungi on freshly laid dung with passage of time. Generally members of the zygomycota followed by ascomycota and then basidiomycota succeeded with time. Deacon [4]; Mouchacca[5]; Wareing[6]; Fulleringer *et al.*[7]; Kushaldas [8]; Ranjith *et al.* [9] have studied the thermophilic fungi from different substrates but only limited information available on the thermophiles inhabiting dung of herbivores [10, 11, 12, 13 and 14].

MATERIALS AND METHODS

Chemicals

Yeast extract, peptone, starch and all other chemicals were purchased from HiMedia Laboratories Pvt Ltd., Mumbai, India.

Isolation of fungi

Thermophilic fungi were isolated from dung of different animals viz., cow, sheep, turkey, pigeon, poultry, bear, Duck, rabbit, monkey and zoo dump collected from Zoo Park, Warangal, Andhra Pradesh, India. Dung samples were collected in sterilized polythene bags and brought to the laboratory and analyzed for the presence of thermophilic fungi [9]. Isolation of thermophilic fungi from different

substrates was carried out using the following techniques.

Dilution plate technique

For the detection and estimation of Thermophilic fungi dilution plate method was employed [15, 16]. Ten g of sample was transferred to 250 ml Erlenmeyer conical flask containing 100 ml sterile water. The contents were shaken on a mechanical shaker for 30 min and then serially diluted to obtain 10^{-4} -10^{-5} dilutions. 0.5 ml of each sample was transferred to sterile Petri plate containing Yeast Extract Starch Agar medium [Starch 15 g, Yeast extract 5 g, $MgSO_4$ 7 H_2O 0.5 g, KH_2PO4 1 g, Rose Bengal 0.0001 g and trace amount of streptomycin Agar –agar 20 g and tap water 1000 ml] by gentle rotational movement of Petri plate to ensure uniform mixing of the sample. Rose Bengal and streptomycin were added to the medium to restrict fungal colonies and suppress the growth of bacteria and actinomycetes. The pH of medium was adjusted to 6.0.

Paired Petri plate technique

Paired Petri plate technique [16] which provides moisture and suitable environment for the growth of thermophilic fungi was employed. This method gave good results and many thermophilic fungi would be isolated by using this method. Identification of thermophiles was made by referring relevant literature and monographs [17, 18, and 19]. The number of colonies of each species appearing in Petri plate was counted from which percentage of incidence, percentage of frequency and percentage of abundance were calculated by the formula:

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

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

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

RESULTS AND DISCUSSION

Fungi isolated from dung are précised in **Table I**, in all 15 species representing 10 genera in the dung of different herbivore animals. *Aspergillus flavus* followed by *A.fumigatus* and *Mucor miehei* were with highest incidence, while *R. arrhizus was* with lowest percentage of incidence. *M. pusillus* and *H. grsea* differed significantly in their percentage of incidence but was same in percentage of frequency. Similarly *H. grisea* and *P. duponti* incidence was almost same in zoo dump but they differed significantly in their percentage of frequency. *Aspergillus fumigatus*, *A. flavus* and *M.miehei were* present in nearly all of the sources.

Among 15 species of fungi isolated, 12 were identified as thermophilic, while others were thermotolerant (table II). Association of some fungi with thermogenic substrates may be an ecological adaptation. Similar results were also reported by Antonella Anastasi *et al.* [20] who also isolated fungi from different thermogenic places.

Dung of sheep followed by pigeon and poultry feces supported many thermophilic members of fungi, while turkey poults were least favorable for supporting the growth of fungi. Dung of cow and monkey supported intermediate number of fungal species. Feces of duck and bear were also poor substrate for fungal growth. Dung of rabbit, duck, and turkey failed to support the growth of species of *Humicola*. Dung of cow and sheep supported three species of *Humicola*, while monkey and hens dung favored the growth of only two species of *Humicola* [*H. grisea and H. lanuginosus*]. Excreta of pigeon supported growth of *H. lanuginosus* and *H.stellata. A.*

flavus could be isolated in excreta of all animals except those of rabbit and cow. *A.fumigatus* could not be isolated from dung of turkey poults and poultry birds. Interestingly cellulolytic thermophilic *Chaetomium thermophilus* could be isolated from dung of sheep and rabbit only. *Chrysosporium* sp could be detected from excreta of pigeon only. *Mucor pusillus* could be isolated from dung of variety of animals such as cow, bear, turkey and zoo wastes. *Myriococcum albomyces* a typical thermophile was associated with the dung of cow and poultry birds. *Penicillium duponti* was detected only in decomposing waste of Zoo Park, while *Rhizopus arrhizus* was detected in the Droplets of pigeon and zoo wastes. *Torula thermophila* could be recorded on the dung of sheep, cow and monkey. *Aspergillus flavus, A.fumigatus* and *Mucor miehei* occurred with highest percentage of frequency, while *P. duponti* with least percentage of frequency. Rest of the fungi occurred with intermediate percentage of frequency. Almost same trend was observed with percentage of abundance.

Table II reveals that *A. flavus,* A. *fumigatus* and *R. arrhizus* were thermotolerant, while rest of the fungi were thermophiles as they failed to grow below 20 ^{0}C.

TABLE I: Percentage of Incidence, frequency and abundance of thermophilic Coprophilous fungi in different dung of animals

	Percentage of Incidence											
Name of the fungus	**S**	**C**	**B**	**M**	**D**	**T**	**P**	**Po**	**R**	**Z**	**F**	**A**
Aspergillus flavus	28.7	-	50.0	33.34	47.65	36.36	13.63	29.16	-	15.38	80	12.9
A. fumigatus	4.76	12.50	25.0	16.67	25.92	-	13.63	-	47.68	-	70	11.3
Chaetomium thermophile	4.76	-	-	-	-	-	-	-	11.56	3.84	30	4.8
Chrysosporium spp	-	-	-	-	-	-	9.09	-	-	-	20	3.2
Humicola grisea	4.76	12.50	-	8.34	-	-	-	4.16	-	7.69	50	8.0
H. insolens	4.76	6.25	-	-	-	-	-	-	-	5.79	20	3.2
H. lanuginosus	14.28	25.00	-	16.67	-	-	18.18	12.50	-	19.21	60	9.6
H. stellata	-	-	-	-	-	-	13.16	4.16	-	-	20	3.2
M.pulchella var.sulfuria	-	-	-	-			9.09	25.00	19.23	7.69	30	4.8
Mucor miehei	24.56	-	-	16.67	22.23	26.46	18.18	12.50	21.53	-	70	11.3
Mucor pusillus	-	31.50	18.75	-	-	37.18	-	-	-	19.23	40	6.4
Myriococcum albomyces	-	6.25	-	-	-	-	-	8.33	-	-	20	3.2
Penicillium duponti	-	-	-	-	-	-	-	-	-	7.69	10	1.6
Rhizopus arrhizus	-	-	-	-	-	-	4.54	-	-	5.79	20	3.2
Torula thermophila	9.52	6.00	-	8.33	-	-	-	-	-	-	30	4.8
Sterile mycelium	4.76	-	6.25	-	4.2	-	-	4.16	-	7.69	50	8.0

S- Sheep; **C-** Cow; **B-**Bear; **M-** Monkey; **D-**Duck; **T-**Turkey; **P-**Pigeon; **Po-** Poultry; **R-**Rabbit; **Z-**Zoo dump; **F-**percentage of Frequency; **A-** Percentage of Abundance

TABLE II: Lists of thermophilic and thermotolerant species of fungi isolated from Dung of different animals

Name of the fungus	Nature of Organism	SD	CD	BD	MD	DuD	TE	PE	RD	PoW	ZW
Aspergillus flavus	TT	+	-	+	+	+	+	+	-	+	+
Aspergillus fumigatus	TT	+	+	+	+	+	-	+	+	-	-
Chaetomium thermophile	TP	+	-	-	-	-	-	-	-	-	+
Chrysosporium spp	TP	-	-	-	-	-	-	+	+	-	-
Humicola grisea	TP	+	+	-	+	-	-	-	-	+	+
H. insolens	TP	+	+	-	-	-	-	-	-	-	+
H. lanuginosus	TP	+	+	-	+	-	-	+	-	+	+
H.stellata	TP	-	-	-	-	-	-	+	-	+	-
M.pulchella var.sulfuria	TP	-	-	-	-	-	-	+	-	+	+
Mucor miehei	TP	+	-	-	+	+	+	+	+	+	-
Mucor pusillus	TP	-	+	+	-	-	+	-	+	-	+
Myriococcum albomyces	TP	-	+	-	-	-	-	-	+	-	-
Penicillium duponti	TP	-	-	-	-	-	-	-	-	-	+
Rhizopus arrhizus	TT	-	-	-	-	-	-	+	-	-	+
Torula thermophila	TP	+	+	-	+	-	-	-	-	-	-
Sterile mycelium	TT	+	-	+	-	+	-	-	+	-	+

S- Sheep; **C**- Cow; **B**-Bear; **M**- Monkey; **Du**-Duck; **D**- Dung; **T**-Turkey; **P**-Pigeon; **Po**- Poultry; **R**-Rabbit; **Z**-Zoo dump; **E**- Excreta; **W**- waste; **TP**- Thermophilic; **TT**- Thermotolerant; **(+)** - present; **(-)** - absent

CONCLUSION

From the present investigations it is clear that thermophiles prefer to colonize dung of different animals. However, colonization of these fungi varied with different animals. In all 15 species representing 10 genera could be isolated. Out of 15 fungal species isolated 12 belonged to thermophilic and 3 were thermotolerant. Dung of cow and sheep supported comparatively more number of thermophilic fungi. Thermotolerant fungi of *Aspergillus flavus* and *A. fumigatus* were constantly associated with the dung of different animals. A positive correlation observed between percentage of incidence, frequency and abundance could be of different.

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