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ISOLATION AND INCIDENCE OF THERMOPHILIC FUNGI FROM VARIOUS THERMOGENIC HABITATS IN ANDHRA PRADESH

S.SANTOSH KUMAR AND EDLA SUJATHA*

Department of Microbiology, Kakatiya University, Warangal - 506609, Andhra Pradesh, India.

ABSTRACT

A total of 400 samples were collected from different thermogenic habitats such as nests of birds, decomposing litter, soils from furnace area, cattle dung, zoo dump, industrial waste, vegetable market compost, mushroom compost, horse dung, municipal waste, chicken manure and coal mine soils of Godavari Khani (Karimnagar) and Bhoopalpally (Warangal) group of mines in Andhra Pradesh. From which 40 isolates including 10species of 10genera were isolated. Among the thermophilic fungi *Rhizomucor pusillus* and *Humicola insolens* present nearly in all substrates. A novel strain *Sordariales sp.A.P-2012 strain* was isolated from nest material and an uncultured compost fungus was isolated from the cattle dung. *Scytalidium thermophilum strain B1* was successfully isolated from mushroom compost and *Sporotrichum thermophile* from decomposed litter are adapted to grow at a temperature maximum of 65°C and 60°C respectively.

KEYWORDS: **Thermophilic fungi, thermogenic ecological substrates, temperature and incidence.**

EDLA SUJATHA
Department of Microbiology, Kakatiya University,
Warangal - 506609, Andhra Pradesh, India.

INTRODUCTION

Thermophilic fungi are a small assemblage in eukaryota that have a unique mechanism of growing at elevated temperature extending up to 60°C to 65°C. During the last four decades many species of thermophilic fungi sporulating at 45°C have been reported. The species included in this account are only those which are thermophilic in the sense of Cooney and Emerson (1964).Therefore, in dealing with this account we have adopted Cooney and Emerson's definition of thermophilic fungi as those that have a maximum temperature for growth at or above 50°C and a minimum temperature for growth at or above 20°C[13].The paper presents a comprehensive account of incidence ,frequency and temperature maxima of the organisms at different temperature ranges and methods for isolation from different thermogenic substrates like nests of birds, decomposing litter, soils from furnace area, cattle dung, zoo dump, industrial waste, vegetable market compost, mushroom compost, horse dung, municipal waste, chicken manure and coal mine soils of Godavari Khani (Karimnagar) and Bhoopalpally (Warangal) group of mines in Andhra Pradesh and other accumulations of organic matter where the warm, humid, and aerobic environment provides the basic physiological conditions for their development and culture of this unique group of fungi[9]. In all samples collected from 12 different sources were screened for the presence of thermophilic fungi.

MATERIALS AND METHODS

The following methods were adopted to isolate thermophilic fungi from thermogenic substrates.

DILUTION PLATE TECHNIQUE[2]

The sample from different thermogenic sources were brought to laboratory in polythene bags and made in to dilution (10 fold), the sample of 1 ml of $10^{-4,}$ 10^{-5} dilutions are placed in the Yeast extract starch agar medium[4] (Yeast extract difco powdered 4.0 gm, K2HPO4 1.0 gm, MgSO4.7H2O 0.5 gm Soluble Starch 15.0 gm, Agar 20.0 gm, Distilled Water 1000.0 ml, The pH of medium was adjusted to 5.5 with 0.1N HCl or 0.1N NaOH). Antibiotics rose bengal, streptomycin are added to the medium to avoid the contamination of bacteria. Yeast Glucose Agar (Yeast extract difco powdered 5.0 gm, Glucose 10.0 gm, Agar 20.0 gm, Distilled Water 1000.0 ml) and potato dextrose agar media were employed for isolation of thermophilic fungi.

PAIRED PETRI PLATE TECHNIQUE[4]

For isolation of thermophilic fungi, paired petriplate technique was employed. 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.

HUMID CHAMBER TECHNIQUE[3]

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 (a specially designed humid chamber of glass and steel was employed) 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 45-50°C.Fungi which appeared on nest material are transferred into sterile yeast extract starch agar slants and checked for thermophilic character (fig-2)

FIGURE 2
SHOWING HUMID CULTURE TECHNIQUE FOR ISOLATION OF THERMOPHILIC FUNGI FROM BIRDS NESTS MATERIAL

WARCUPS SOIL PLATE METHOD[15]
1 ml of the sample was placed in a sterile petridish containing 20 ml of sterile cooled (40°C) Yeast extract starch agar medium, contents were thoroughly mixed and the plates were incubated at 45 ± 2 °C.

WAKS MANS DIRECT INOCULATION METHOD[15]
Small quantities of the samples were sprinkled over the medium (YpSs) and allowed to solidify the dishes, such plates were incubated in an inverted position at 45°C ± 2°C and observed for the growth of thermophilic fungi. The fungal species were identified and characterized based on their morphological studies and microscopic analysis by using taxonomic guides, referring relevant literature and standard procedures. The different morphological characteristics of thermophilic fungi were evaluated such as colony growth (length and width), presence or absence of aerial mycelium, colony colour, presence of wrinkles and furrows, pigment production etc[1,4,5,7,11,8,14]. The distribution of thermophilic fungi isolated from 400 samples collected from 12 different thermogenic substrates is presented in tabular form. All the substrates showed presence of thermophilic fungi. *Rhizomucor pusillus, Humicola insolens* were present nearly in all the sources. The technique used for isolation of thermophilic fungi was compared for their efficiency to obtain different species of thermophilic fungi. The percentage of incidence and frequency were calculated by employing the following formulae[6,12].

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

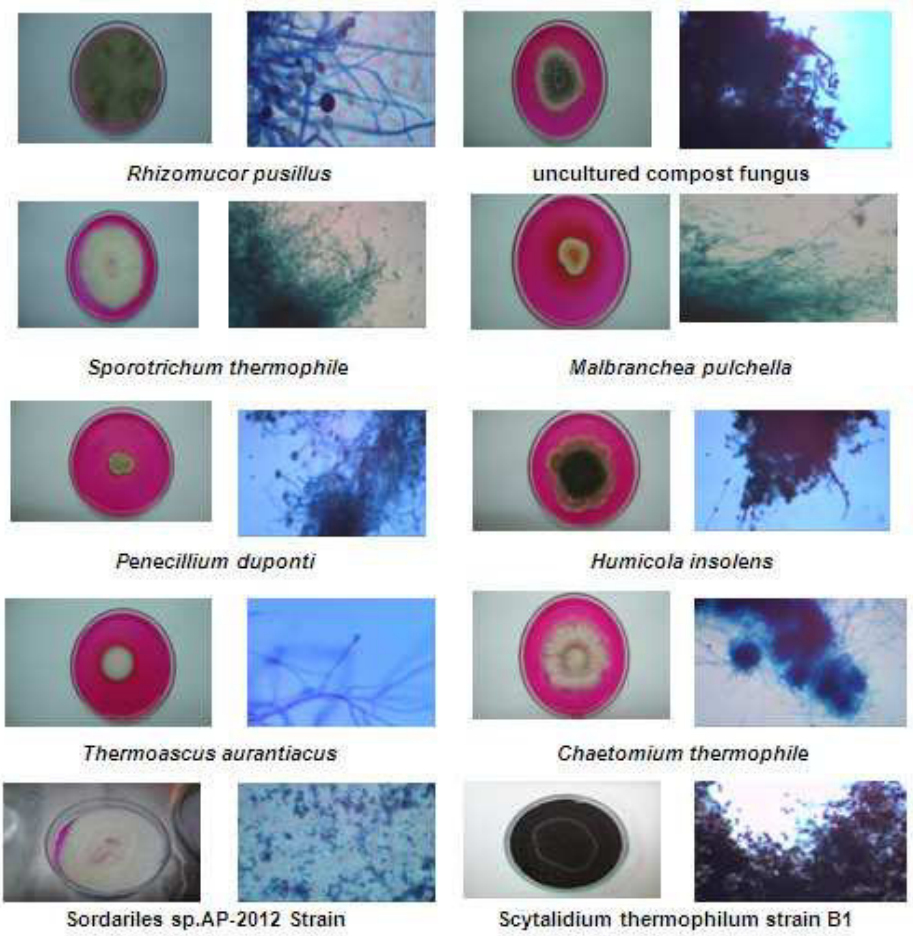
$$\% \text{ of frequency} = \frac{\text{No of observations in which species appeared}}{\text{Total no of observations}} \times 100$$

Temperature maxima of the thermophilic fungi was studied by incubating the plates at different temperatures (50°C, 55°C, 60°C, 65°C,) on Yeast extract starch agar medium for 4 days. Incubation of plates at elevated temperature may cause desiccation of the agar in petri plates, this problem can be solved by placing the agar plates in humid chamber.

RESULTS AND DISCUSSION

The 10 species of thermophilic fungi could be isolated from nests of birds, decomposing litter, soils from furnace area, cattle dung, zoo dump, industrial waste, vegetable market compost, mushroom compost, horse dung, municipal waste, chicken manure and coal mine soils of Godavari Khani (Karimnagar) and Bhoopalpally (Warangal) group of mines in Andhra Pradesh (fig1).

FIGURE 1
THERMOPHILIC FUNGI AND MICROSCOPIC IMAGE



Rhizomucor pusillus, Humicola insolens was most abundant in all the thermogenic sources. *Chaetomium thermophile var. dissitum* occurred with high percentage of incidence in birds nests material followed by *Rhizomucor pusillus and Humicola insolens.* The decomposing litter, municipal waste are good substrate for the growth of all the thermophilic fungal species isolated, this states that the decomposing material, municipal waste have a good nutritive value for encouraging the growth of the thermophilic fungi. *Chaetomium thermophile var. dissitum, Rhizomucor pusillus*, and *Humicola insolens* could be isolated with maximum percentage of incidence and frequency in industrial wastes, nests of birds, horse organic manure and furnace soils (table-1, 2, 3)

TABLE 1
INCIDENCE OF DIFFERENT THERMOPHILIC FUNGI IN COALMINE SOIL, CATTLE DUNG, MUNICIPAL WASTE, POULTRY MANNURE

NAME OF THE FUNGUS	COALMINE SOIL		CATTLE DUNG		MUNICIPAL WASTE		POULTRY ANNURE	
	PI	PF	PI	PF	PI	PF	PI	PF
Chaetomium thermophile var. dissitum	-	-	-	-	-	-	-	-
Humicola insolens	10	16.66	15	25	5.71	6	16.66	20
Rhizomucor pusillus	25	33.33	12.5		4.76	10	25	35
Uncultured compost fungus	-	-	2.85	10	-	-	-	-
Penecillium duponti	-	-	-	-	2.38	6	-	-
Thermoascus aurantiacus.	-	-	-	-	1.42	4	-	-
Sporotrichum thermophile	-	-	-	-	-	-	-	-
Malbranchea pulchella var. sulfurea	-	-	-	-	2.38	4	-	-

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

NAME OF THE FUNGUS	DECOMPOSING LITTER		ZOO DUMP		BIRD NESTS MATERIAL		MUSHROOM COMPOST	
	PI	PF	PI	PF	PI	PF	PI	PF
Chaetomium thermophile var. dissitum	-	-	-	-	12.5	30	-	-
Humicola insolens	3.5	20	20	8	7.5	25	10	20
Rhizomucor pusillus	7.01	25	14	10	6.25	15	5	8
Penecillium duponti	7.01	37.5	-	-	-	-	-	-
Thermoascus aurantiacus.	2.45	7.5	-	-	-	-	-	-
Sporotrichum thermophile	2.45	12.5	-	-	-	-	-	-
Malbranchea pulchella var. sulfurea	5.26	22.5	-	-	-	-	-	-
Sordariales sp.AP-2012 strain	-	-	-	-	10	15	-	-
Scytalidium thermophilum strain B1	-	-	-	-	-	-	10	15

TABLE 3
INCIDENCE OF DIFFERENT THERMOPHILIC FUNGI IN INDUSTRIAL WASTE, HORSE ORGANIC MATTER, FURNACE SOIL, VEGETABLE MARKET COMPOST

NAME OF THE FUNGUS	INDUSTRIAL WASTE		HORSE ORGANIC MATTER		FURNACE SOIL		VEGETABLE MARKET COMPOST	
	PI	PF	PI	PF	PI	PF	PI	PF
Chaetomium thermophile var.dissitum	10	13.33	-	-	-	-	-	-
Humicola insolens	25	20	20	30	16.66	25	10	20
Rhizomucor pusillus	12	20	8.33	7.5	6.66	15	5	8

Novel strain *Sordariales sp.AP-2012* was isolated from birds nest material and uncultured compost fungus was isolated from cattle dung. The temperature maxima for the growth of thermophilic fungi was studied and found that *Scytalidium thermophilum strain B1* was tolerant at a temperature maxima of 65°C where as the *Humicola insolens, Sporotrichum thermophile* was able to grow at 60°C temperature. *Humicola insolens,* uncultured compost fungus showed a good growth at 55°C, where as the *Chaetomium thermophile var. dissitum, Sordariales sp.AP-2012* strain, *Sporotrichum thermophile* showed a moderate growth at temperature of 50°C (table-4).

TABLE 4
GROWTH OF THERMOPHILIC FUNGI AT ELEVATED TEMPERATURE

NAME OF THE FUNGUS	TEMPARATURE IN DEGREE CELSIUS							
	50°C		55°C		60°C		65°C	
	GROWTH	Diameter Of colony (cm)	GROWTH	Diameter Of colony (cm)	GROWTH	Diameter Of colony (cm)	GROWTH	Diameter Of colony (cm)
Chaetomium thermophile var. dissitum	M	2	M	1.7	N	-	N	-
Humicola insolens	G	3.5	G	3.5	M	1	N	-
Rhizomucor pusillus	G	2.5	N	-	N	-	N	-
Penecillium duponti	M	1	N	-	N	-	N	-
Thermoascus aurantiacus.	M	1	N	-	N	-	N	-
Sporotrichum thermophile	M	2.5	N	1	M	1	N	-
Uncultured compost fungus	G	4	G	3.5	N	-	N	-
Malbranchea pulchella var. sulfurea	M	2	N	-	N	-	N	-
Sordariales sp.AP-2012 strain	M	2.5	M	2	N	-	N	-
Scytalidium thermophilum strain B1	M	2.5	M	2	M	1	M	1

CONCLUSION

Isolation of thermophilic fungi from different thermogenic habitats was investigated in the present paper and it supports the earlier findings. The dilution plate technique using antibiotics, Cooney Emerson method were found to be suitable to obtain different types of thermophilic fungi from various thermogenic habitats. Incidence of thermophilic fungi in thermogenic habitats obtained in the present investigation reveals that most frequently isolated organisms from almost all environments was *Humicola insolens* and *Rhizomucor pusillus*. The occurrence of Scytalidium thermophilum strain B1 from mushroom compost was able to grow at 65°C, adaptation of this fungus to grow at elevated temperature may have many applications in industrial fermentation process. Fungi are well known agents of decomposition of organic matter in general[10]. Among all the substrates municipal waste and decomposed litter was able to encourage the growth of most of the thermophilic fungi because, it may contain wide variety of nutrients for luxurious growth of fungi.

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