J. Indian bot. Soc. 63 : 165—170, 1984

THERMOPHILIC FUNGI OF PADDY STRAW COMPOST: THEIR GROWTH, NUTRITION AND TEMPERATURE RELATIONSHIPS[1]

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ABSTRACT

The thermophilic fungi of paddy straw compost were able to grow in several media; asparagine supported better growth than others although utilization of nitrate by several isolates was rapid. On the basis of temperature relationships, these strains were classified into, (i) Strong Thermophiles (min 25°C or above, opt. 45-50°C): *Acremonium alabamensis*, *Chaetomium thermophile*, *Humicola lanuginosa*, *Rhizopus microsporus*, *Sporotrichum thermophile*, *Thermoascus aurantiacus* and *Torula thermophila*, (ii) Weak Thermophiles (min 25°C or lower, maximum 50-55°C): *Absidia corymbifera*, *Achaetomium macrosporum*, *Mucor pusillus* and *Theilavia minor*, and (iii) Thermotolerants (min. 20-28°C, max. 50°C) : *Aspergilus fumigatus*.

The spores of *A. fumigatus*, *H. lanuginosa*, *S. thermophile* and *T. thermophila* were able to germinate both, on water agar and glucose agar discs; the rate of germination of spores increased with the rise of temperature from 25 to 45°C. A majority of thermophiles could survive short exposure to 60 to 64°C; only *H. lanuginosa* and *T. aurantiacus* were able to survive exposure at 72°C (2 and 10 min, respectively).

INTRODUCTION

Thermophilic fungi of late have been isolated from a variety of substrates (Satyanarayana *et al.*, 1977; Satyanarayana, 1978; Tansey and Brock, 1978; Johri, 1980). Only limited attempts have so far been made to study their nutritional characteristics and temperature relationships (Thakre, 1975; Deploey, 1975; Pandey, 1977; Johri and Pandey, 1980). Sumner *et al.*, (1969) and Dart (1976) have reported lower degree of unsaturation in fatty acids of thermophilic fungi grown at high temperatures. Chapman and Ostrovsky (1975) on the other hand have reported loss of protein bands when fungal extracts of *Paputsapora thermophila* were exposed to high temperatures. It was felt necessary to understand the basic requirements of the thermophiles and the present report is a results of these investigations.

MATERIALS AND METHODS

The thermophilic fungi were isolated, at various intervals, from paddy straw ompost by (i) direct observation (ii) plating out of washed particles and (iii) dilution plate techniques as described by Chang and Hudson (1967).

Growth characteristics were studied in several agar/liquid media. For dry mycelial weight determination, fungi were grown in 100 ml Erlenmeyer fla-

1. Accepted for publication on November 11, 1982.

sks containing 25 ml medium for 6 days at 45°C in duplicate.

Nitrogen nutrition was evaluated in glucose-asparagine medium. Asparagine was replaced in this basal medium with nitrogen equimolar proportions of urea, $NaNo_3$; NH_4NO_3, $(NH_4)_2$ HP SO_4 and $(NH_4)_2$ HPO_4 ; dry weight increment was recorded on these nitrogen sources.

The effect of temperature on growth was studied on YpS³ agar plates which were incubated in the range, 18 to 60°C. The germination of spores of *Aspergillus fumigatus, Humicola lanugionsa, Sporotrichum thermophile* and *Torula thermophila* was studied on water agar and 2% glucose agar at different temperatures. Germination counts for atleast 200 spores were made after 6 hr (*T. thermophila*), 8 hr (*A. fumigatus* and *S. thermophile*) or 10hr (*H. lanuginosa*).

The survival period of thermophiles was evaluated in glass test tubes (18 × 150 mm) containing sterile paddy straw compost upto a depth of 6 cm according to Fergus and Amelung (1971).

RESULTS

The thermophilic strains of paddy straw compost grew well in YpSs broth and agar medium (Table I) suggesting their ability to utilize starch as a source of carbon and amylolytic nature. The growth was moderate on peptone-glucose, glucose-asparagine and Sabour and's liquid/ogar media. Oat meal was a poor substrate expect for *S. thermophile*, and *T. aurantiacus; T. minor* grew in oat meal agar but failed to do so in broth. *Acremonium alabamensis, C. thermophile, S. thermophile, T. thermophila* and and *T. aurantiacus* showed better growth on Abram's cellusose agar suggesting cellulolytic ability of these isolates.

Nitrogen Nutrition (Table II):

The utilization of asparagine was good for all strains. Nitrates ($NaNO_3$, KNO_3) supported moderate growth excepting in *T. minor*. The utilization of ammonium nitrate and ammonium sulphate was poor; *T. minor, C. thermophile* and *T. aurantiacus* did not utilize these sources at all.

Temperature Relationships:

The pattern of mycelial growth of these strains was studied on YpSs agar medium in the temperature range, 18 to 60°C. On the basis of growth, minimum, optimum and maximum temperatures for each thermophile were obtained (Table III) and this was then used in categorising the strains into the following :

Group I-Strong Thermophiles : This class eomprised of thermophiles depicting high minimum temperature (25°C or above) but grew best at 45 to50°C viz., *Acremonium alabamensis, C. thermophile, H. lanuginosa, R. microsporus, S. thermophile, T. aurantiacus* and *T. thermophila;* of these only *H. lanuginosa* and *T. aurantiacus* were able to grow at 60°C. Among these, *C. thermophile, H. lanuginosa* and *T. aurantiacus* failed to grow at temp. below 30°C suggesting their strong thermophilic nature. Mature perithecia of *T. aurantiaus* were produced in the temp. range, 40 to 50°C, below 40°C and above 50°C it produced only vegetative mycelium. *Chaetomium thermophile* was able to produce perithecia at only 40 to 45°C.

Group II-Weak Thermophiles : Strains which grew at 25°C or below but which did not show any noticeable growth at 50 to 55°C. The optimum growth of these strains usually occurred at 40°C, viz., *A. corymbifera, A. macrosporum, Mu-*

TABLE I

GROWTH OF GTHERMOPHILIC FUNGI OF PADDY STRAW COMPOST IN LIQUID AND AGAR MEDIA

Organism	Dry mycelial wt (mg/day)							Area covered in plates (sq cm/day)							
	YpSs	PG	OM	SAB	GA	PDA	RDS	YpSs	PG	OM	SAB	GA	PDA	RDS	ABC
Absidia corymbifera	10.3	12.6	5.6	20.0	12.4	8.4	15.4	3.0	4.7	3.4	1.6	10.6	3.1	10.6	0.62
Acremonium alabamensis	10.2	10.0	6.4	12.4	17.4	12.4	11.3	3.8	3.0	5.7	5.2	3.4	1.4	4.0	3.0
Achaetomium macrosporum	10.4	9.0	1.5	2.8	12.4	4.0	5.6	2.4	2.4	2.5	3.82	2.9	6.8	3.4	0.28
Humicola lanugnosa	12.0	9.2	3.8	6.7	8.6	10.0	12.6	4.9	5.7	4.0	9.2	5.2	3.8	8.3	0.36
Chaetomium thermophile	14.4	6.6	8.4	7.4	5.6	7.8	15.0	10.6	3.0	10.6	4.21	5.0	15.9	10.6	2.7
Mucor pussillus	18.4	10.4	4.3	12.0	8.7	3.0	7.1	6.8	3.0	4.50	3.3	6.8	7.2	4.1	0.4
Rhizopus microsporus	10.1	10.3	4.2	17.4	11.8	8.4	13.0	15.9	15.9	10.6	15.9	12.8	15.9	10.6	0.6
Sporotrichum thermophile	21.0	19.0	10.0	32.4	15.0	20.2	18.6	15.9	10.6	10.6	10.6	8.3		10.6	9.24
Thermoascus aurantiacus	16.0	26.4	14.4	28.9	19.0	23.0	21.0	15.9	15.9	15.9	15.9	15.9	15.9	15.9	15.9
Thielavia minor	8.0	9.4	0.0	4.0	12.4	4.2	3.6	3.01	2.9	2.96	3.8	3.8	3.96	3.4	0.0
Torula thermophila	14.2	5.6	9.0	6.4	8.0	8.0	3.0	2.8	3.2	3.8	2.5	3.8	3.8	0.6	2.31

YpSS—Emerson *YpSs*—medium *PG*—peptone glucose, *OM*—Oat meal, *SAB*—Sabouraud's dextrose, *GA*—Glucose asparagine, *PDA*—Potato doxtrose, *RDS*—Richard's medium, *ABC*—Abram's cellulose medium

TABLE II

GROWTH OF PADDY STRAW STRAINS ON VARIOUS NITROGEN SOURCES IN LIQUID MEDIUM AT 45°C

Organism	Dry mycelial wt in broth (mg) on 7th day							
	Asparagine	Urea	$NaNo_3$	KNO_3	NH_4NO_3	$(NH_4)_2SO_4$	$(NH_4)_2HPO_4$	Control*
Absidia corymbifera	81.5	0.0	56.0	33.0	15.0	26.0	28.0	12.0
Achaetomium macrosporum	76.0	0.0	75.0	76.5	32.0	27.0	20.0	5.0
Acremonium alabamensis	86.0	36.0	85.0	73.0	28.0	33.0	60.0	0.0
Chaetomium thermophile	91.0	7.0	12.0	6.0	10.5	0.0	5.0	0.0
Humicola lanuginosa	53.0	30.5	18.0	28.0	23.0	24.6	27.0	13.0
Rhizopus microsporus	70.8	15.0	12.0	10.0	18.0	21.0	68.0	11.5
Sporotrichum thermophile	78.0	0.0	42.0	101.0	42.0	28.0	33.0	21.0
Thielavia minor	72.5	0.0	0.0	0.0	0.0	22.0	0.0	10.0
Thermoascus aurantiacus	91.0	0.0	40.0	82.0	48.5	0.0	50.0	0.0
Torula thermophila	56.0	7.0	8.0	0.0	22.0	12.0	14.0	10.5

*Liquid broth without any added nitrogen source

TABLE III

TEMPERATURE REQUIREMENTS FOR GROWTH OF THERMOPHILIC FUNGI OF PADDY STRAW COMPOST

Organism	Temperature characteristics (°C)		
	Minimum	Optimum	Maximum
Absidia corymbifera	18	40	50
Achaetomium macrosporum	25	40	50
Acremonium alabamensis	25	45	55
Aspergillus fumigatus	18	40	50
Chaetomium thermophile	30	45	55
Humicola grisea	18	35	45
H. lanuginosa	30	40	60
Mucor pusillus	18	40	50
Rhizopus microsporus	18	45	55
Sporotrichum thermophile	25	45	55
Thielavia minor	18	40	45
Thermoascus aurantiacus	30	50	60
Torula thermophila	25	45	55

cor pusillus and *T. minor;* the perithecia production was restricted to 35 to 40°C.

Group III-Thermotolerants : Aspergillus fumigatus was only organism in this class. This mould was able to tolerate high temperatures like a true thermorphile but also grew well at 20 to 28°C, and failed to grow beyond 50°C.

Spore Germination:

The conidia of *A. fumagatus, H. lanuginosa, S. thermophile* and *T. thermophila* were able to germinate well both on water agar and glucose agar. The time required to achieve 100% germination on water and glucose agar respectively was 10 and 8 hr for *A. fumigatus,* 16 and 10 hr for *H. lanuginosa,* 14 and 8 hr for *S. thermophile,* and 8 and 6 hr for *T. thermophila.*

Thermal Survival:

The thermal resistance of the straw strains in terms of survival time at 60, 64, 68 and 72°C is given in Table IV

TABLE IV

SURVIVAL TIME (HOUR—HR, MINUTES—MIN) OF THERMOPHILIC FUNGI EXPOSED TO SELECTED TEMPERATURES IN PADDY STRAW COMPOST

Organism	Temperature °C			
	60	64	68	72
Absidia corymbifera	2 hr	30 min	5 min	0
Achaetomium macrosporum	1 hr	30 min	0	0
Acremonium alabamensis	4 hr	1 hr	10 min	0
Aspergillus fumigatus	4 hr	1 hr	5 min	0
Chaetomium thermophile	24 hr	2 hr	10 min	0
Humicola grisea	1 hr	15 min	0	0
H. lanuginosa	24 hr	5 hr	15 min	2 min
Mucor pusillus	1 hr	30 min	0	0
Rhizopus microsporus	2 hr	30 min	5 min	0
Sporotrichum thermophile	12 hr	2 hr	5 min	0
Thielavia minor	1 hr	30 min	0	0
Thermoascus aurantiacus	48 hr	2 hr	30 min	10 min
Torula thermophila	4 hr	1 hr	10 min	0

0=No survival even at the shortest exposure period.

which amply demonstrates that expecting *H. lanuginosa* and *T. aurantiacus* (survived 10 and 2 min exposure to 72°C all others failed to resist 2 min exposure to the highes. temperature; the peak temperatures during composting in large heaps often reach 70°C.

DISCUSSION

The temperature makes the basis for delineating species into mesophiles and thermophiles and the need to undertake such studies for latter group has been emphasized by Fergus (1964), Emerson(1968), Tansey and Brock (1978) and Johri (1980). There is however some difference of opinion on the definition of a thermophile but according to Crisan (1973) a broad working one includes all organisms growing at or above 40°C into his group. Thakre (1975) has reported that the coal-mine isolate of *R. microsporus* was able to grow at 60°C but the straw isolate of this thermophile was not able to do so ; only *H. lanuginosa* and *T. aruantiacus* were able to grow at 60°C which incidentally approaches the near limits for the eukaryotic organisms (Tansey and Brock, 1972). The over all growth pattern and temperature characteristics of straw strains were quite similar to thermophiles described from other sources (Ofosu-Asiedu and Smith, 1973; Subrahmanyam and Gopala Krishna, 1975; Tansey and Borck ,1978).

The conidia of *A. fumigatus, H. lanuginosa, S. thermophile* and *T. thermophila* showed complete nutritional independence by germinating on water agar devoid of any other nutrients; the enhancement of germination rate by glucose was remarkable but this phenomenon has been reported for *Alternaria solani* and *Trichoderma viride* (mesophiles) and *Rhizopus rhizopodiformis,* a thermophile (Satyanarayana, 1974 ; Thakre and Johri, 1974; Johri and Pande, 1980). The temperature response of compost strains of thermphilic fungi is similar to that described for mesophiles (Cochrane, 1958). The only known report pertaining to survival timing for thermophiles is that of Fergus and Amelung (1971) and with which the behaviour of straw isolates compared favourably; only *H. lanuginosa* and *T. aurantia us* survived exposure to 72°C; detailed investigations of this nature can help in successful selective isolation of slow growing thermophiles from natural substrata which is much needed too

because of the ubiquity of *A. fumigatus* and the problems posed by it in normal isolations.

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