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Characterization of a thermophilic fungi: *Scytalidium thermophilum* isolated from button mushroom compost

Chetana Jangde, Ananya Rao, Pradeep Badhai, CS Shukla and HK Singh

Abstract
Thermophilic and thermotolerant fungi were isolated from *Agaricus bisporus* compost heap at morning and highest (69.3 °C) temperature observed on 13th day whereas at afternoon it was on 14th day (71.9 °C). In Malt extract agar medium growth of *S. thermophilum* was 67.24 mm as compared to other evaluated media while it was least observed in Czapek dox agar medium. Maximum growth of *S. thermophilum* was recorded at 40 °C and 35 °C respectively. Wheat and sorghum grains gave significantly more 89.33 and 84.97 mm respectively. Wheat grain liquid medium gave more fresh and dry mycelial weight i.e. 8.62 and 2.25 g of *S. thermophilum* respectively. Average fresh weight 10.86 g and dry 1.31 g mycelial weight of *S. thermophilum* was observed on wheat grains liquid medium.

Keywords: Thermophilic fungi, *Scytalidium thermophilum*, temperature

Introduction
The compost is prepared by aerobic process which is brought by fermentation of several microorganisms. The white button mushroom (*Agaricus bisporus*) is cultivated on a substrate consisting of a composted mixture of straw bedded horse manure, wheat straw, chicken manure and gypsum. Among the microorganism thermophilic fungi to contribute significantly play a key role in preparation of compost. The effectiveness of *S. thermophilum* in compost preparation for *A. bisporus* has been shown by Straatsma *et al.* (1994) [9] which obtained a 2 folds increase in the yield of mushrooms on inoculated compost when compared to the pasteurized control. So the aim of the present investigation was to isolate the thermophilic fungi which was present in the compost and prepared by LMC (Mental, 1972).

Materials and Methods
Seventeen fungi were isolated by dilution plate technique while 16 fungi were detected by Waksman inoculation method. In order to isolate thermophilic fungi, compost was prepared by long method and samples were randomly collected at different stages of compost preparation. The fungi were isolated by 2 method (i) dilution plate technique (Apinis, 1963) [1] (ii) Waksman direct inoculation method (Waksman *et al.*, 1939) [10].

Compost preparation
The compost was prepared by long method and following ingredients were used wheat straw 10 Quintals; wheat bran 25 kg; urea 18 kg; gypsum 25 kg. The methodology for compost preparation was followed as per procedure of Mental *et al.* (1972)

Collection of samples
For the isolation of thermophilic fungi samples were taken at different stages of composting i.e. 1st, 2nd, 3rd and 4th turning of compost. For collection of compost 10 g compost was drawn from various spots likewise in zig-zag pattern and collected in a clean sterilized plastic bags, further they were mixed well by hands and a composite sample was made.

Isolation of thermophilic and thermo tolerant fungi
The sterilized plates had solidified media prior to inoculation. For the isolation of fungi 10 g samples was drawn from a composite sample at each turning (1st to 4th turning) from various places of composting from 10 – 45 cm depth. Ten g compost aseptically transferred in 250 ml conical flask which had 90 ml distilled sterilized water. Then flasks were shaken on mechanical shaker for 15 to 20 minutes.

Then content was serially diluted as 10^{-3}, 10^{-4}, and 10^{-5} thereafter, 1.0 ml of each dilution was aseptically transferred on sterilized petri plates with the help of sterile pipette and incubated at 40±1 ℃. Waksman direct inoculation method after solidification of media, a small quantity (0.1g) of compost sample was aseptically sprinkled over on solidify media in dishes.

Morphological Characterization

Colonies were identified with the help of Nikon make microscope at 10X, 40X and 100X and microphotography was with Nikon make digital camera.

Cultural characterization of *S. thermophilum*

To study the growth of *Scytalidium thermophilum* on different media, 4 media (MEA, YpSs, CZA and YGA) were used and each medium was poured in sterilized petri plates and kept for solidification. The growth of *S. thermophilum* was studied on different temperature i.e. 25 ℃, 30 ℃, 35 ℃, 40 ℃ on Emerson's modified Yeast Starch Agar (YpSs) medium. The growth of *S. thermophilum* was studied on 4 different grains i.e. wheat, paddy, kodo and sorghum. Each grain, were separately wetted overnight and next day they were boiled in water for 10-15 minutes. The growth of *S. thermophilum* was studied on liquid medium of different grains. Four types of grains i.e. wheat, paddy, kodo, and sorghum were taken. For mass multiplication of *S. thermophilum* liquid medium was prepared by wheat grains. Two hundred grams of wheat grains were used to make 1 liter liquid medium.

Result and Discussion

Temperature of compost during composting

Temperature was noted twice a day (Morning and evening) during its composting period. In morning highest (69.3 ℃) temperature was noted on 13th day whereas it was on 14th day (71.9 ℃) and mean highest temperature was 70.5 ℃.

Morphological characterization

***Scytalidium thermophilum* (Cooney and Emerson) Austwick**

Hyphae was septate and colorless; colonies were greyish to jet black in color; conidia were dark brown, smooth walled, globose and had chains of conidia having 5-10 µm in diameter.

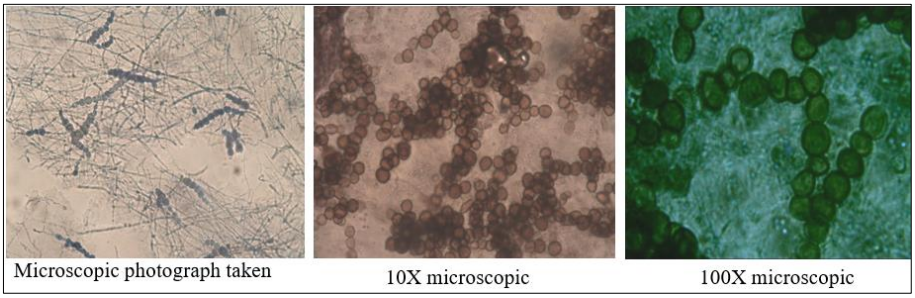

Fig 1: After 6 days of incubation

Cultural characterization

Effect of different solid media on mycelial growth of *S. thermophilum*

An experiment was conducted to know the impact of different media on growth of *S. thermophilum.* The results have been obtained and depicted in table 3.

The data presented in table 3 revealed that malt extract agar medium showed significant more (67.24 mm) growth of *S. thermophilum* as compared to other evaluated media however it was least observed in czapek doz agar medium (32.67 mm). The growth of *S. thermophilum* in other media was significantly less with malt extract agar medium but more czapek dox agar medium. The growth of *S. thermophilum* was dense and white on malt extract agar medium, sparse growth was observed on YGA, dense white mycelium was seen in YpSs and CZA gave white asymmetric mycelia.

The present findings are in accordance with the investigation made by Goh *et al.* (2015) [4] and they suggested malt extract agar peptone medium to be better for growth of *S. parasiticum.* Malt extract agar medium has been used by Cooney and Emerson (1964) [2] for growing and maintaining culture of *S. thermophilum.*

Effect of different temperature on growth of *S. thermophilum*

In vitro, growth of *S. thermophilum* was studied on various temperature and data are presented on table 3.

Data presented in table 4 clearly indicates that the growth of *S. thermophilum* was significant higher (63.57 and 62.77 mm) observed at 40 °C and 35°C respectively with other studied temperature. However, other temperature did not perform well but growth was significantly superior at 30°C (41.63 mm) then 25 °C (37.33 mm).

At 40 °C, mycelial growth of *S. thermophilum* was creamy white on 16th day of incubation and sporulation was noticed on 18th day. However other temperature did not show sporulation till incubated period

Present investigation are tally with the findings of Saha (2003) [8] and found 45-50 ℃ as best temperature for growth of *S. thermophilum*. Optimum temperature 35-45 ℃ for ideal growth of *S. indonesicum* have been reported by Hedger *et al.* (1982) [5]. On the other hand, Cooney and Emerson, 1964; Craveri and Colla, 1966 [3] have reported 35-45 ℃ as optimum for growth of *S. thermophilum.*

Effect on different grains on growth of *S. thermophilim*

A study was undertaken to know the best suited grains for growth of *S. thermophilium* and 4 types of grains (wheat, sorghum, paddy, kodo) were taken. The results have been obtained and are given in table 4.

From the table 5 it is evident that wheat and sorghum grains gave significantly more (89.33 and 84.97 mm) growth of *S. thermophilium* respectively and both were at par with each other. In paddy, growth of *S. thermophilium* was significantly superior (73.56) with kodo but it was significantly less with wheat and sorghum. In paddy, growth of *S. thermophilium*

were significantly superior with kodo but inferior to wheat and sorghum grains. However, significantly lower (46.70 mm) growth was observed in kodo grains with other grains.

The present work are in line with the study made by Saha (2003) [8] who reported earlier and maximum growth of *S. thermophilum* on bajra and wheat grains. Wheat grains are used by Pathak (2014) [7] for the multiplication of *S. thermophilum* and *Humicola* spp.

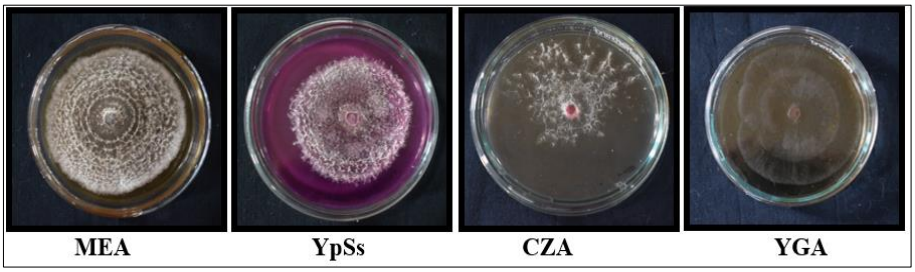

Fig 2: Effect of different solid media on mycelial growth of *S. thermophilum*

Fig 3: Effect of different grains on growth of *S. thermophilum*

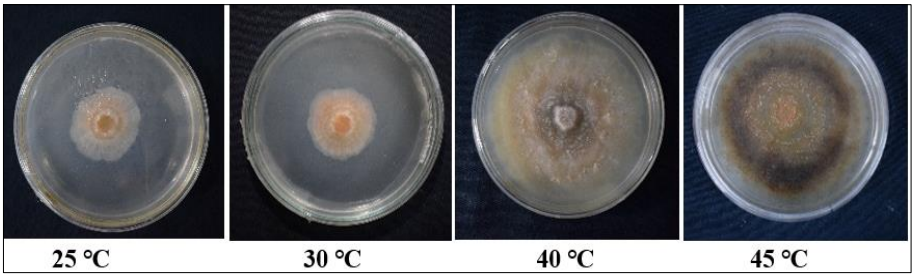

Fig 4: Effect of different temperature on growth of *S. thermophilum*

Fig 5: Growth of *S. thermophilum* in different grains liquid medium

Effect of different grains broth on biomass of *S. thermophilum*

It is clear from the table that fresh mycelial weight of *S. thermophilum* in different grains liquid medium was significantly differ with each other and it was significantly more (8.62 g) observed on wheat grains liquid medium as compared to other liquid medium. However, it was least (2.25 g) noticed on kodo grains and next were by sorghum (3.73 g) and paddy (6.21 g) grains prepared medium.

It clear from table 6 that wheat (1.31 g) grains liquid medium gave significantly more dry mycelial of *S. thermophilum* as compared to other liquid medium. However significantly less (0.21 g) growth was observed in kodo, sorghum (0.28 g) prepared medium and both were at par with each other. Dry mycelial weight of *S. thermophilium* on paddy liquid medium was significantly inferior with wheat medium but it was superior to kodo medium. The growth was at par in sorghum and kodo grains medium.

In present investigation malt extract agar medium was found superior for the growth of *S. thermophilum.* The findings supported by Goh *et al.* (2015) [4] and Cooney and Emerson (1964) [2] who obtained maximum growth and biomass on malt extract agar medium.

Mass multiplication of *S. thermophilum*: This study was under taken for mass multiplication of *S. thermophilum*. the results are obtained and given in table 7.

It is clear from table that fresh mycelial weight of *S. thermophilum* was 10.86 g and dry weight (1.31 g) was obtained with average of 25 replications.

Table 1: Temperature during composting at different days

Days of Composting (Days)	Morning temperature (°C)	Afternoon temperature (°C)	Mean temperature (°C)
0	0	22.3	22.3
1	37.5	39.1	38.3
2	46.6	47.2	46.9
3	45.7	46.5	46.1
4	46.1	47.6	46.5
5	47.3	49.7	48.5
6	49.4	50.8	50.1
7	50.2	52.9	51.5
8	55.3	56.1	55.7
9	61.6	62.9	62.2
10	62.4	65.8	64.1
11	63.5	65.9	64.7
12	65.9	68.6	67.5
13	69.3	70.1	69.7
14	69.1	71.9	**70.5**
15	65.9	69.5	67.7
16	65.8	68.4	67.1
17	63.9	68.3	66.1
18	62.3	59.2	60.7
19	63.5	68.1	65.8
20	59.8	61.0	60.4
21	55.1	60.2	57.6
22	51.3	58.3	54.8
23	53.2	55.5	54.3
24	46.1	47.8	46.9
25	45.0	47.7	46.3
26	45.1	48.7	46.9
27	43.2	45.0	44.1
28	43.3	44.1	43.7
29	46.5	44.0	45.2

Table 2: Colonies of thermophilic and thermotolerant fungi at different dilution

	Number of colonies			
Mycoflora	**10^{-3}/ml**	**10^{-4}/ml**	**10^{-5}/ml**	**Total**
Ascomycetes				
Aspergillus equatis	1.00	0.67	1.00	2.67
A. flavus	0.33	1.00	1.00	2.33
A. fumigatus	5.67	1.67	1.67	9.01
A. granulosus	1.67	-	-	1.67
A. niger	2.67	4.67	0.33	7.67
A. nidulans	3.67	2.67	1.67	8.01
A. sydowii	0.33	1.00	0.67	2.00
A. terreus	1.33	1.33	0.33	2.99
A. versicolor	1.33	1.33	-	2.66
C. sengalensis	15.67	8.00	6.67	30.34
Deuteromycetes				
H. insolens	9.00	6.00	5.00	20.00
H. fuscoatra	2.67	5.67	7.00	15.34
M. fergusii	0.67	1.33	0.33	2.33
S. thermophilum	31.67	14.33	12.33	58.33
T. lanuginosis	1.33	0.33	1.67	3.33
Zygomycetes				
A. corymbifera	5.67	-	0.33	6.00
Mucor sp.	3.00	2.67	0.33	6.00
Total	87.68	52.67	40.33	180.68

Table 3: Effect of different media on mycelial growth of *S. thermophilum*

S. No.	Medium	After 6 days*	After 8 days*	After 12 days*	Characteristics of mycelium
	Mean colony diameter(mm)				
1	Malt Extract agar	33.25	45.30	67.24	White, dense mycelial growth was observed
2	Yeast Glucose agar	27.80	39.29	64.74	White sparse growth of mycelium was noticed
3	Emerson's modified Yeast Starch agar	26.12	36.33	62.07	White, dense mycelia
4	Czapek Dox agar	11.01	28.06	32.67	White, mycelial mat is more asymmetric
	C.D.	3.303			
	SE(m)	0.997			
	(*)-Average of three replication				

Table 4: Effect of different temperature on growth of *S. thermophilum*

S. No.	Temperature (°C)	Growth (mm)	Characteristics of mycelium
1	25	37.33	Very slow growth, was observed till 2nd day, no sporulation was observed
2	35	41.63	Mycelial growth was observed on 3rd day and no sporulation observed
3	35	62.77	Mycelium was creamy white and no sporulation was observed
4	40	63.57	Mycelium was creamy white till 16th day and sporulation was observed on 18th day and dark in appearance
	C.D.	3.075	
	SE(m)	0.929	
	(*)-Average of three replication		

Table 5: Effect of different grains on growth of *S. thermophilum*

S. No.	Grains	6th day*	10th day*	16th day*	Mycelial characteristics
			Growth (mm)		
1	Wheat	41.77	53.02	86.33	Growth was good and all grains were covered by the mycelium of *S. thermophilium*
2	Sorghum	38.22	48.85	84.97	Growth was good, all grains were completely covered by cottony growth of *S. thermophilium*
3	Paddy	28.06	52.69	73.56	Growth was poor and grains were not fully covered by the mycelium of *S. thermophilium* and they were in scattered manner.
4	Kodo	12.56	22.93	46.70	Growth was limited and in scattered manner
	C.D.	2.232			
	SE(m)	0.674			
	(*)-Average of three replication				

Table 6: Effect of different grains broth on biomass of *S. thermophilum*

S. No.	Grain broth	Fresh mycelial weight (g)*	Dry mycelial weight (g)*	Mycelial characteristics
1	Wheat	8.62	1.31	Mycelial growth was good
2	Sorghum	3.73	0.028	Mycelial growth was scattered and limited
3	Paddy	6.21	0.039	Mycelial growth was limited
4	Kodo	2.25	0.021	Mycelial growth was good but dense in limited area
	C.D.	0.017	0.16	
	SE(m)	0.005	0.05	

Table 7: Mass multiplication of *S. thermophilum*

Liquid medium	Fresh weight (g)*	Dry weight (g)*
Wheat broth	10.86	1.31
(*)-Average of 25 replication		

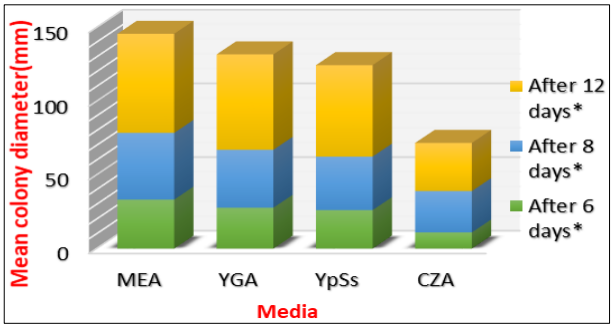

Fig 6: Effect of different solid media on mycelial growth of *S. thermophilum*

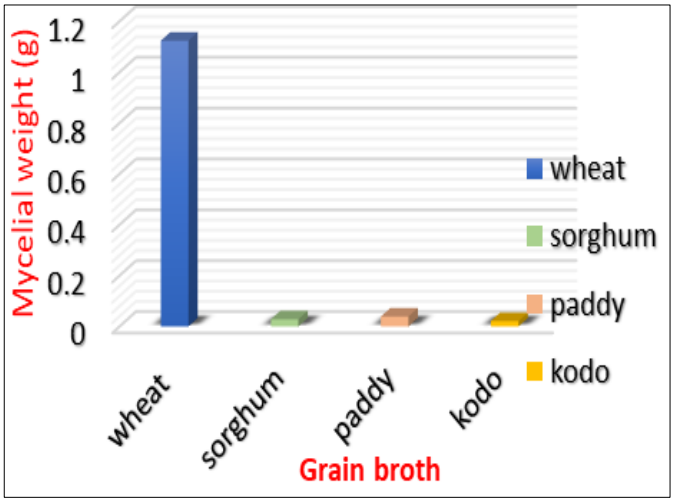

Fig 7: Effect of different grains broth on biomass of *S. thermophilum*

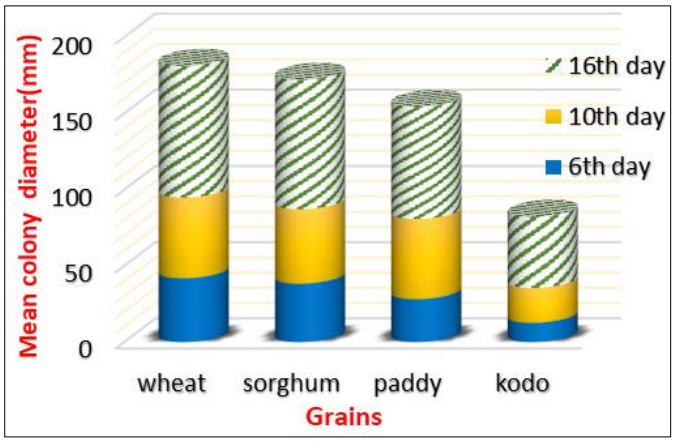

Fig 8: Effect of different grains on *S. thermophilum*

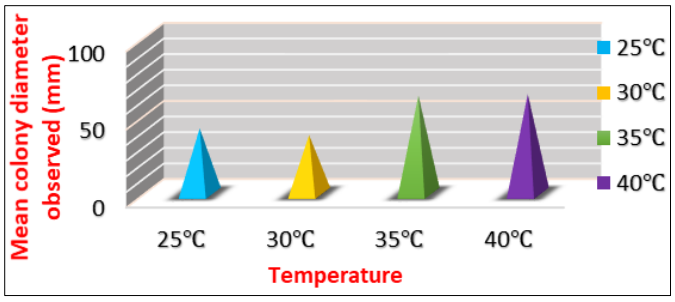

Fig 9: Effect of different temperature on growth of *S. thermophilum*

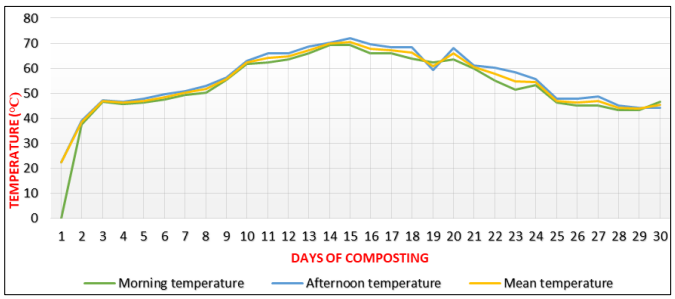

Fig 10: Temperature of compost during composting

Conclusion

Maximum growth of *S. thermophilum* (63.57 and 62.77 mm) was recorded at 40 °C and 35 °C respectively with other studied temperature. Wheat and sorghum grains gave significantly more (89.33 and 84.97 mm) respectively. Wheat grain liquid medium gave more (8.62 and 2.25 g) fresh and dry mycelial weight of *S. thermophilum* respectively. Average fresh (10.86 g) and dry (1.31 g) mycelial weight of *S. thermophilum* was obtained on wheat grains liquid medium.

Acknowledgement

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