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Research Article

Cultural Study of Fire-fang Isolates of Mushroom Compost

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ABSTRACT

*Six individual colonies of fire-fang assigned as FI_1, FI_2, FI_3, FI_4, FI_5 and FI_6 were isolated from the four samples of hot compost having 55°C- 60°C temperature. Each one of 1st three samples was drawn at the end of turning 1st (FI_1), turning 2nd (FI_2), turning 3rd (FI_3 and FI_4) of phase I composting. Fourth compost sample represents phase II composting (FI_5 and FI_6). All isolates were found Gram (+) and appeared in white, dry and rough to smooth surface on actinomycetes isolation agar (AIA) medium but varied in shape from circular (FI_1, FI_3 and FI_6) to irregular (FI_2, FI_4 and FI_5) and diffusible pigmentation of pale yellow (FI_1), yellow (FI_5 and FI_6), dark brown (FI_2) and brown (FI_3 and FI_4). The effect of temperature and media on the growth of above isolates was further evaluated with the results that temperature 35°C±1 did not support any growth of above isolates. Temperature 55°C±1has obviously increased average diametric growth of all isolates of fire-fang by 122.61% against temperature 45°C±1 at 10th DAI. Starch ammonium agar (SAA) supported 9.83% more average diametric growth of all isolates of fire-fang at 10 DAI over the actinomycete isolation agar (AIA) medium. Though, the interactions FI_2*55°C, FI_3*55°C, FI_5*55°C and FI_2*KM, FI_3*AIA were appeared best to have highest average growth of thermophilic isolates.*

Key words: *Mushroom compost, Agaricus bisporus, Fire-fang, media, Temperature, Thermophilic actinomycetes.*

INTRODUCTION

Fire-fang is a greyish to whitish speckled presence of thermophilic actinomycetes usually dominated with *Thermomonospora curvata*, *T. alba* and *T. fusca*, *T. chromogena*[12], *Thermoactinomyces thalophilus* and *T. vulgaris*[4&6] in the mushroom compost. That start grows spontaneously from 45°C in phase I compost to 65-70°C in phase II compost[10]. More numbers of colonies forming units of fire-fang are developed at 45°C but decline gradually with increased temperature by 55°C or more[3]. Fire-fang developed in the mushroom compost is found different from that in other composts with alkaliphilic[9], thermophilic, saprophytic and aerobic abilities.

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It normally occurs in the compost prepared from wheat straw/horse manure[11] at high temperature to help in degradation of rather complex and recalcitrant organic molecules, especially cellulose, lignocellulose and lignin[2]. As a result of degradation fire-fang produces variety of antibiotics, enzymes, growth regulators, siderophores and organic acids[14]. Which make compost very selective for button mushroom where it grew better and remained free from any fungal contaminations[16]. Mushroom mycelia also uses the mass of *fire-fang* during spawn run and crop production. Consider the above facts of fire-fang in view; a preliminaire study was done on the cultural behaviour of colony forming units of fire-fang in respect of temperature and media.

MATERIAL AND METHODS

The fire-fang was isolated from the four samples of mushroom compost. The sample 1, 2 and 3 were taken at the end of turning 1st, turning 2nd and turning 3rd of Phase 1st compost material, respectively. Sample 4 was taken from Phase II compost. As per the method of serial dilution technique[1], one gram of compost of each 4 sample was suspended into 10 ml of sterile distilled water followed by 10^{-5} dilution strength. One ml of prepared compost suspension was taken from each of 10^{-5} dilution strength and poured on the actinomycetes isolation agar medium by pour plate method and allowed to solidify. The poured plates were then incubated at 55±1°C. The colonies of fire-fang were appeared within 48 hours (fig.1) Colonies were than selected on the basis of colony characters and transferred to actinomycetes isolation agar plate with the help of four way streaking method to maintain them in form of pure culture. The purified isolates of fire-fang were assigned their name as FI_1, FI_2, FI_3, FI_4, FI_5 and FI_6 after the temperature at which they were isolated, phases from which they belonged to, samples from them isolated and number of isolates as depicted in the table 1.

Pure culture of all six isolates of fire-fang FI_1, FI_2, FI_3, FI_4, FI_5 and FI_6, were further visualized under stereoscopic microscope Olympus, SZH 10 to observe their clearer shape, surface and pigment production. Effect of temperature on the growth of above all six isolates of actinomycetes were studied on actinomycetes isolation agar medium at 35±1°C, 45±1°C, 55±1°C with three replications. Two days old culture of actively growing isolates transferred aseptically in the centre of culture media. Inoculated Petri plates were kept at 35±1°C, 45±1°C, 55±1°C and data were recorded on 10th day after inoculation (DAI).

The vegetative growth of six isolates of fire-fang was also evaluated on actinomycetes isolation agar (AIA), starch ammonium agar (SAA), Kenknight & Munaier's (KM) medium with three replications. Ten ml sterilized melted media of temperature 35°C were poured in sterilized Petri plates. The solidified plates were inoculated with two days old culture of actively growing isolates and incubated at 55±1°C. The data were recorded on10th DAI. All the data were analysed using 2factorial CRD statistical method at 5% level of significance.

RESULTS AND DISCUSSION

Isolation of fire-fang from the mushroom compost

The fire-fang was isolated from the four samples of compost mass that continue to be decomposed at the 55^{o}C- 60^{o}C temperature. Compost sample 1, 2 and 3 were taken at the end of turning 1st, turning 2nd and turning 3rd of Phase I compost, respectively. Compost sample 4 was taken from Phase II compost as shown in the table 1. All isolates of fire-fang were identified on the basis of temperature 55^{o}C at which only fire-fang was appeared, Gram staining and morphological characters. Initially, total 10 colonies were appeared on AIA medium. Out of them, only six colonies as FI_1, FI_2, FI_3, FI_4, FI_5 and FI_6 were found gram positive with white and dry surface (table 2 and Fig 1). However, all isolates were grown into beads like colonies in the broth of AIA. These colonies were joined together and form the circle on the top surface of broth.

Some beads like colonies were also appeared within the circle, though the colonies would not grow deep in the broth. The isolates were differentiated in circular (FI_1, FI_3 and FI_6) and irregular (FI_2, FI_4 and FI_5) shape. They also produced pale yellow (FI_1), dark brown (FI_2), brown (FI_3 and FI_4) and yellow (FI_5 and FI_6) diffusible pigment (table 1). However, sometimes diffusible pigment in the substrate did not noticed[15]. *Thermoactinomyces* were white and yellow in colour with the production of endospores singly on aerial and substrate mycelium, however, *Thermomonospora* were appeared as white mycelial colonies on which single spores were formed on sessile or dichotomously branched sporophores[7]. All isolates of fire-fang produced foul smell in the broth. The above characteristics thus enough to confirm that isolates of fire fang were resembled with the characters of thermophilic actinomycetes.

Diametric growth of fire-fang at different temperature

The results of interaction between isolates (FI_1, FI_2, FI_3, FI_4, FI_5 and FI_6) and temperatures (45°C and 55°C) were recorded in table 3, revealed that FI_2*55°C, FI_3*55°C and FI_5*55°C were *at par* statistically with highest 7.7 cm (0.77 cm growth/day), 7.53 cm (0.75 cm growth/day) and 6.6 cm (0.66 cm growth/day) growth as recorded on 10th DAI. As a result, 327.75 %, 318.3% and 266.64% higher growth was achieved in above treatments against the lowest 1.8 cm growth of the AI_4*55°C. Among the six isolates of fire-fang, AI_3 and AI_2 as depicted in table 3 were statistically *at par* with 5.33 and 5.29 cm mean growth on the 10th DAI with 0.5cm growth/day and resulted 170.55% and 168.52 % more diametric growth, respectively over to the minimum 1.97 cm diametric growth of AI_4. The temperature also influenced the growth of fire-fang (table 3). Among three temperatures 35±1˚C, 45±1˚C and 55±1˚C, the previous one did not show any growth of thermophilic isolates. However, 55±11˚C was found more effective than to that of 45±11˚C to be recorded with maximum 6.08 cm average diametric growth of isolates on 10th DAI with 0.61 cm growth/day. On the other hand, 122.61% more average diametric growth of fire-fang was recorded at temperature 55±11˚C if calculated over to the temperature 45±11˚C. The temperature 45±11˚C was supported only 2.73 cm growth with a 0.27 cm increases in the growth/day. In an earlier study thermophilic population became dominant at gradual increase in temperature, which often includes some actinomycetes[17]. Jin *et al.*[5] actinomycetes called thermophilic for those that grow between 37^{o}C and 65°C but showed optimum growth from 55^{o}C-60°C and moderately thermophilic actinobacteria to thrive at 28°C-60°C with optimum growth in 45°C -55°C[5]. The strains of *Thermoactinomyces candidus* could not grow at 25°C, showed slight growth at 37°C and growth vigour was gradually increased at the 45°C, 50°C, 55°C and 60°C after a week of inoculation[8]. The temperature 55°C -60°C was found optimum for the growth of thermophilic actinomycetes[4].

Diametric growth of fire-fang on different media

The results of the interactions of isolates and media were depicted in table 4. FI_2*KM and FI_3*AIA were recorded with 84.7% and 82% more growth, respectively against FI_2*AIA on 10 DAI and consequently showed highest growth rate of 0.48 and 0.47 cm/day. The treatment FI_6*KMM was statistically *at par* with the treatment FI_2*AIA. However, remaining interactions were showed moderate increase in the growth of isolates ranged from 19.25-63.91% over to that of FI_2*AIA. Among the six isolates, FI_3 and FI_5 were recorded with 4.18 cm (25.9%) and 4.02 cm (21.08%) higher average growth on 10th DAI in comparison to 3.32 cm mean growth of FI_1. The strains FI_6 and FI_4 were found statistically *at par* with the strain FI_1. The effects of media were also comparatively tested with the mean growth of isolates and revealed that the starch ammonium agar (SAA) was found one of the best-supported media in which 3.24 cm (0.32 cm radial growth/day) highest average growth of isolates was achieved with 9.83% increases on 10 DAI over to the actinomycete isolation

agar (AIA) medium. The effect of Kenknight & Munaier's medium (KMM) was statistically identical with the AIA medium in order to support the growth of isolates. AIA and SAA (ISP 4) were the best medium for supporting abundant growth of actinomycetes isolates[13].

Table 1: Nomenclature of isolates of fire-fang (thermophilic actinomycetes)

Name of isolate	Temperature	Name of Phase	Sample No.	Isolate No.	Name
Fire-fang (F)	Temperature 55˚C (T_{55})	Phase I	Turning 1(S_1)	Isolate **2(I_2)**	$FTP_IS_1I_2$**(FI_1)**
			Turning 2(S_2)	Isolate **2(I_2)**	$FTP_IS_2I_2$**(FI_2)**
			Turning 3(S_3)	Isolate **1(I_1)**	$FTP_IS_3I_1$**(FI_3)**
			Turning 3(S_3)	Isolate **2(I_2)**	$FTP_IS_3I_2$**(FI_4)**
		Phase II	-	Isolate **3(I_3)**	$FTP_{II}I_3$ **(FI_5)**
				Isolate **4(I_4)**	$FTP_{II}I_4$ **(FI_6)**

F=Fire-fang, T=Temperature P=Phase, S=Sample, I=Isolate and FI= Fire-fang Isolate. An each cfu of fire-fang from turning 1st and 2nd and 2 no. of cfu_s of fire-fang from turning 3rd and 2 no. of cfu_s from phase 2 were finally assigned with names FI_1, FI_2, FI_3, FI_4, FI_5 and FI_6, respectively.

Table 2: Morphogenic characters of fire-fang isolates

S. No.	Isolates	Gram reaction	Colony Morphology			
			Shape	Chromogenesis	Diffusible Pigment	Surface
1.	FI_1	+	Circular	White	Pale yellow	Dry
2.	FI_2	+	Irregular	White	Dark brown	Dry
3.	FI_3	+	Circular	White	Brown	Dry
4.	FI_4	+	Irregular	White	Brown	Dry
5.	FI_5	+	Irregular	White	Yellow	Dry
6.	FI_6	+	Circular	White	Yellow	Dry

"+" = gram positive

Table 3: Effect of temperature on fire-fang isolates

Isolates	Diametric growth of fire-fang (cm)					
	45°C		55°C		Mean of strains	
	Growth	Growth rate/day	Growth	Growth rate/day	Growth	Growth rate/day
FI_1	2.53 (40.55)	0.25	6.40 (255.53)	0.64	4.47 (126.75)	0.45
FI_2	2.87 (59.44)	0.29	7.70 (327.75)	0.77	5.29 (168.32)	0.53
FI_3	3.13 (73.88)	0.31	7.53 (318.30)	0.75	5.33 (170.35)	0.53
FI_4	2.13 (18.33)	0.21	1.80 (0.00)	0.18	1.97 (0.00)	0.20
FI_5	2.96 (64.44)	0.30	6.60 (266.64)	0.66	4.78 (142.47)	0.48
FI_6	2.80 (55.55)	0.28	6.43 (257.19)	0.64	4.62 (134.36)	0.46
Mean of temperatures	2.73 (0.00)	0.27	6.08 (122.61)	0.61	-	-

Values in parenthesis represent per cent increase growth against the lowest growth of isolates
CD at 5%
Isolate :0.78
Temperature :0.46
Isolate x Interaction :1.11

Table 4: Effect of media on fire-fang isolates

Isolates	Diametric growth of fire-fang (cm) on different media							
	AIA		SAA		KM		Mean of strains	
	Growth	Growth rate/day	Growth	Growth rate/day	Growth	Growth rate/day	Growth	Growth rate/day
FI_1	3.20 (23.1)	0.32	3.46 (33.11)	0.35	3.30 (26.95)	0.33	3.32 (0.00)	0.33
FI_2	2.60 (0.00)	0.26	3.96 (52.36)	0.40	4.80 (84.70)	0.48	3.79 (14.16)	0.38
FI_3	4.73 (82)	0.47	4.26 (63.91)	0.43	3.56 (36.96)	0.36	4.18 (25.90)	0.42
FI_4	3.83 (47.36)	0.38	3.46 (3.11)	0.35	3.10 (19.25)	0.31	3.46 (4.22)	0.35
FI_5	3.76 (44.66)	0.38	4.16 (60.06)	0.42	4.13 (58.91)	0.41	4.02 (21.08)	0.40
FI_6	3.23 (24.26)	0.32	3.86 (48.51)	0.39	2.93 (12.71)	0.29	3.34 (0.60)	0.33
Mean of media	2.95 (0.00)	0.30	3.24 (9.83)	0.32 (2.37)	3.02	0.30		

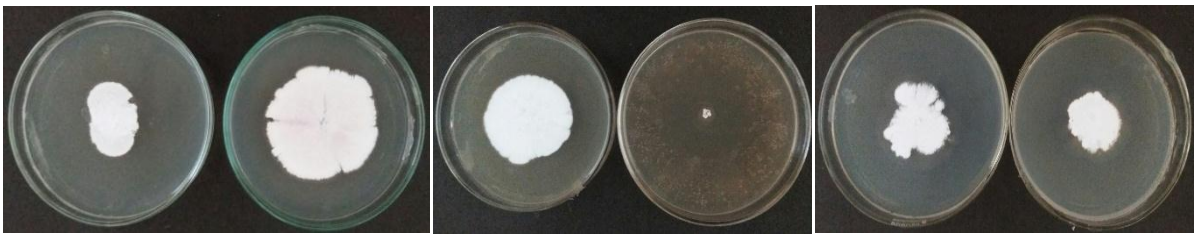
Values in parenthesis represent per cent growth against the lowest growth of isolates

CD at 5%

Isolate : 0.25

Temperature : 0.18

Isolate x Interaction : 0.45



$FI_{1,}$ $FI_{2,}$ $FI_{3,}$ FI_4 FI_5 FI_6

Fig. 1: Isolates of Fire-fang

CONCLUSION

Six isolates of fire-fang $FI_{1,}$ $FI_{2,}$ $FI_{3,}$ $FI_{4,}$ FI_5 and FI_6 were characterized and further evaluated on different temperatures and media. It was found that all of them characterized with white, dry, compact powdery surface with ray type wrinkles and showed aerial mycelium composed of more or less erect filaments. Some isolates were also formed concentric rings with rhythmic growth on actinomycetes isolation agar medium at 55^{o}C media. All isolates of fire-fang were Gram (+) and diffused pale yellow to dark brown pigments in the growing medium of AIA. They were also produced foul smell. Among three temperatures 35±1^{o}C, 45±1^{o}C and 55±1^{o}C, the previous one did not show any growth of thermophilic isolates. However, 55˚C±1was found more effective than to that of 45˚C±1on which FI_2, FI_3 and FI_5 were grown very fast. The growth performance of FIs was also varied with different growing medium. The interactions FI_2*KM and FI_3*AIA, media SAA and isolate FI_3 and FI_5 were found best in order to support fast growth. The present work may support further to the study on the effect of fire-fang on the quality and productivity of compost of button mushroom.

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