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Qualitative evaluation of some specific media of *Trichoderma* and *Gliocladium* spp.

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Three different media viz., TSM, TME and PDF were screened for selective isolation and qualitative enumeration of *Trichoderma* spp. and *Gliocladium* spp. from soils. Among them TSM appeared better in enumeration and selective isolation of *Trichoderma* and *Gliocladium* from soils. On TSM colonies of *Gliocladium* appeared more compact with restricted growth and normal dirty green in colour. Thus TSM discriminated well *Gliocladium* from *Trichoderma* compared to that obtained in TME and PDF. But TSM failed to restrict the growth of many unwanted microorganisms like *Penicillium, Mucor, Rhizopus* and specially *Fusarium*. Attempts were therefore made to modify TSM to reduce contaminations. Captan 50% W.P. @ 10 mg/litre effectively reduced the contaminations specially for soils rich in fusaria without adversely affecting the recovery percentage of *Gliocladium* from soils.

Key words : *Gliocladium* spp., *Trichoderma* spp., enumeration, selective isolation

INTRODUCTION

The earlier methods using Martin's medium (Elad *et al.*, 1981) and soil extract agar supplemented with rose-bengal (Mouba-Shar, 1965; Mughogho, 1968) failed to eleminate undesirable contaminants. In these media both *Trichoderma* and *Gliocladium* were also very fast growing posing unsuitability for selective and qualitative enumeration of these organisms. Danielson and Davey (1973) used Dextrose-yeast extract agar for isolation and enumeration of *Trichoderma*. The medium has its own limitations.

Davet (1979) deviced an improved technique bringing out the specificity to the medium but had low efficiency of recovery (Maity, 1982) and was subjected to interference with other contaminants. Liu and Baker (1980) proposed a modification of Martin's Rose bengal-Streptomycin agar medium with addition of 100 mg PCNB. Elad *et al.* (1981) developed an improved medium (TSM) fortified with Dexon and PCNB along with other additives to impart specificity to the medium. This medium recovered *Trichoderma* well from soil. Papavizas (1981) and Papavizas and Lumsden (1982) formulated a selective medium (TME) on V-8 juice agar. Maiti (1986) used TSM originally developed by Elad *et al.* (1981) replacing Dexon with methyl orange and claiming precission in results. TSM supplimented with captan @ 20 ppm was yet another modification for *Trichoderma* even in the presence of fusaria in soil (Elad and Chet, 1983).

The present modified TSM medium containing methyl orange (300 ppm) and captan (10 ppm) was found even more suitable and appeared superior among three tested media for selective isolation of *Gliocladium* and *Trichoderma* from soils (Saha, 1995).

MATERIALS AND METHODS

For devicing a medium suitable for specific isolation and with least number of contaminants, a few media were screened for their efficacies in supporting better growth with restricted colony size and for efficient recovery of *Gliocladium* and *Trichoderma* from soil. The media were : (1) TSM (Elad *et al.*, 1981; (2) TME (Papavizas and Lumsden, 1982); and (3) PDF (Mac Faddan and Sutton, 1975).

Among these three media TSM was finally selected for further work. But the modification of TSM was felt essential to avoid contaminants of other soil fungi. The qualitative merits of the media were evaluated by dilution plate technique. Colony morphology of antagonists, their growth habits, quality and quantity of contaminants and the per cent recovery of antagonists from soil were considered the criteria for evaluation. To reduce and /or eliminate contaminants particularly for *Fusarium* and *Rhizopus* modification was made by adding Captan 50% W.P. at different dosages and some other fungicides like Alliette 75% W.P., Blitox 50% W.P. and their combination were tried with TSM. The pH of the medium was found to play a significant role in the recovery and isolation of *Trichoderma* and *Gliocladium*. Acid pH reduced contaminants, particularly fusaria and other microbes. The pH of the medium was maintained with N/10 HCl and N/10 NaOH solution after sterilization. Finally TSM amended with captan 50% W.P. @ 10 ppm at pH 4.5 of the medium was maintained for isolation and enumeration of *Trichoderma* and *Gliocladium* from soils.

Determination of recovery percentage of *Trichoderma* and *Gliocladium* in modified TSM

Efficiency of Captan @ 10 ppm in TSM on recovery percentage of *Trichoderma* and *Gliocladium* by adding known concentration of spore suspension in natural and sterilized soil was studied under laboratory condition by dilution plate technique using a 10^{-2} dilution of the soil samples. The result on per cent recovery of *Trichoderma* and *Gliocladium* in both amended and non-amended TSM media were compared among themselves.

RESULTS AND DISCUSSION

Devicing specific media for isolation

Three different media viz., TSM, TME and PDF were evaluated for their efficacies in isolation and quantative enumeration of *Trichoderma* and *Gliocladium* from soils. Among these three media (Table 1), TSM appeared better in qualitative and quantative enumeration of *Trichoderma* and *Gliocladium* from soils. In TSM the individual colony of *Trichoderma* and *Gliocladium* was compact and restricted in growth (3.0 - 4.0 mm in diameter), dirty green and dark green in colour respectively compared to other two media (TME and PDF). TSM efficiently recovered the organisms from soil. Although TME medium recovered *Trichoderma* and *Gliocladium* well from soil but the colonies developed were sparse and spreading type. Thus found unsatisfactory for discrimination between *Trichoderma* and *Gliocladium* and their enumeration from soil. In TME, contaminatins were far less than TSM and PDF, particularly fusaria. In PDF medium *Trichoderm* and *Gliocladium* formed well defined, coloured and compact colonies but with unrestricted growth, creating difficulties in enumeration either of *Trichoderma* and *Gliocladium* from soils and also failed to prevent the appearence of *Fusarium* in culture plates. So, from comparative analysis of the performance of these media, TSM was selected for enumeration of *Trichoderma* and *Gliocladium* from soil (Table 2).

Modification of TSM

As the medium failed to prevent the growth of many undesired microorganisms from soils especially the fusaria, it was therefore, felt necessary to bring about modifications in the

Table 1. Assay of media on enumeration of *Trichoderma* and *Gliocladium* from soil*

Medium	Population (c.f.n X 10^3)				
	T	+	G	+	TC ***
TSM	5.5	+	0.0	+	53.75**
TME	7.75	+	0.0	+	16.75
PDF	4.25	+	0.0	+	10.5

* Soil Sample - 1B
** Average of four replications
*** *Trichoderma* + *Gliocladium* + Total count

Table 2. Colony morphology of *Trichoderma* and other contaminants in TSM, TME and PDF media.

TSM	TME	PDF
Compact, restricted colony growth	Non-restricted, Non-compact faint colony growth	Compact, Non-restricted vigorous colony grwoth
Early sporulation (days)	Not early as compare to TSM (days)	Early sporulation (days)
Usual dark green colour colony	Faint green colour colony	Usual dark green color colony
Colony discreat	Colony coalessing,	Colony coalessing,
Contaminations of *Fsarium, Penicillium Aspegillus* are present	Not present except others	Contaminations of *Fusarium* are present with vigorous colony growth

Table 3. Effect of Captan in TSM on *Trichoderma, Gliocladium* and *Fsarium.*

Conc.of Captan mg/L	Trichoderma	Gliocladium	Fusarium
5	Well developed restricted compact colony of dark green in colour. Av. dia 3 mm; sporulation appeared within 72 hours	Well developed restricted compactly growing colony light dirty green colour. Av. dia of colony - 3 mm; sporulation appeared within 72 hours	Number of colonies are fewer than *Trichoderma* of *Gliocladium*
10	Av.dia of colony-4mm	-do-	No colony
15	Size of the colony slightly larger than in 10 mg/L treatment	Number of colonies lesser than in 10 mg/L treatment	-do-
20	Av. dia of colony-9mm, Dispersed colony growth	-do-	-do-
25	-do-	-do-	-do-

Each insertion is based on the observation of five replications, concentration of spore used was equal for each organism.
Av. : Average; dia. : diameter

Table 4. Effect of Blitox, Alliette and their combination on antagonist in soil with TSM

Medium	*Trichoderma* setrilised soil	Percent recovery	*Trichoderma* Natural soil	Percent recovery
	(TC+T)*		(TC+T)*	
TSM+Blitox 100 ppm	20.33+19.33**	55.12	19.0+14.0	50.84
TSM + Alleitte 100 ppm	22.01+21.66	83.33	23.66+22.0	84.61
TSM+Alleitte + Blitox(each at 100 ppm)	19.66+19.0	80.76	22.0+20.0	76.91
TSM	26.33+24.33	93.58	26.66+25.33	97.43

* Total count + *Trichoderma*
** Population (c.f.u/ml soil suspension) - average of three replications in 10^{-2} dilution
*** Concentration of added spores in sterilized and natural soil-26 spores/ml soil suspension in 10^{-2} dilution.

Table 5. Enumeration of *Trichoderma* and *Gliocladium* of IB soil sample with TSM+Captan in different dosage at different pH

Conc. of Captan mg/L	pH 4.5	pH 7.0	Non corrected pH
TSM + 00	(T + G + TC)* 8.8 + 0.0 + 68.8**	(T + G + TC) 2.8 + 0.0 + 32.0	(T + G + TC) 46.2+ 0.0 + 219.9
TSM +7.5	1.2 + 0.0 + 3.4	2.0 + 0.0 + 16.4	26.0 + 0.0 + 87.0
TSM +10	1.6 + 0.0 + 4.0	1.2 + 0.0 + 29.0	30.2 + 0.0 + 100.0

* *Trichoderma* + *Gliocladium* + Total count
** Average population (c.f.u/ml soil suspension in 10^{-2} dilution) of five replications

composition of medium. Accordingly, TSM was modified with different dosages of Captan 50 W.P.

TSM fortified with Captan @ 10 ppm showed substantial improvement over other dosage of Captan like 5,15,20 and 25 ppm respectively in respect of colony morphology and elimination of contaminants (Table 3). Other modifications also enumerated *Trichoderma* and *Gliocladium* well and contaminations of fusaria was eliminated but the recovery percentage of antagonists was less than TSM fortified with Captan (Table 4).

Table 6. Effect of Captans[a] on recovery[b] of *Gliocladium* sp. from natural and sterilized soil samples in TSM

Medium	Soil condition							
	Natural[c]				Sterilized[d]			
	Gliocladium sp.	Other[e] contaminants	Total[f] count	Percentage recovery	Gliocladium sp.	Other contaminants	Total count	Percentage revovery
	Population/ml suspension				Population/ml suspension			
TSM	9	34	43		17	0	17	
	6	73	79		12	0	12	
	7	35	42		5	1	6	
	12	45	57		15	0	15	
	10	74	84		13	1	14	
	8	38	46	92	9	0	9	98
	9	38	47		9	0	9	
	11	61	72		8	0	8	
	10	90	100		10	0	10	
	10	52	62					
TSM + Captan	8	27	35		9	0	9	
	10	34	44		2	1	3	
	10	40	50		13	0	13	
	12	48	60		14	0	14	
	7	45	52		12	0	12	
	15	38	53	91	14	0	14	96
	12	43	65		12	0	12	
	4	44	48		8	0	8	
	10	60	70		4	0	4	
	3	60	63		8	0	8	

a) Captan was used at 10 ppm
b) Recovery of *Gliocladium* sp. was done by mixing spore with 1 g narural and sterilized soil samples, so that 10^{-2} dilution of 10 ml contained 100 spores.
c) Natural soil samples were collected from field
d) Soil samples were sterilized in autoclave for one hr. at 15 psi for three consecutive days.
e) Number of colonies of different organisms other than *Gliocladium* sp. were also counted.
f) Summation of colony numbers of *Gliocladium* sp. and other.

Both *Trichoderma* and *Gliocladium* are known to grow well at pH ranging between 4.0 to 5.0 (Kang *et al.*, 1989). The pH 4.5 of the medium improved the percentage recovery of *Trichoderma* and *Gliocladium*. The acid pH also reduced contamination(s) significantly as compared to pH 7.0 of the medium (TAble 5).

Recovery of *Trichoderma* and *Gliocladium in specific medium*

The results (Tables 6 and 7) on per cent recovery of *Trichoderma* and *Gliocladium* and extent of contaminants in Captan amended TSM when compared with non-amended TSM showed

Table 7. Effect of Captans[a] on recovery[b] of *Trichoderma* sp. from natural and sterilized soil samples in TSM meidum

Medium	Soil condition							
	Natural				Sterilized			
	Tricho-derma sp.	Other conta-minants	Total count	Percentage recovery	Tricho-derma sp.	Other conta minants	Total count	Percentage revovery
	Population/ml suspension				Population/ml suspension			
TSM	24	58	82		28	2	30	
	25	53	78	92.0	30	1	31	94.4
	22	45	67		30	1	31	
	25	58	83		20	0	20	
	24	45	69		27	3	30	
	20	67	87		15	2	17	
	14	70	84		24	2	26	
	25	51	76		20	2	22	
	27	75	102		20	2	22	
	20	31	51		22	0	22	
TSM +	18	30	48		27	0	27	
Captan	22	28	50	92.4	22	0	22	93.0
	24	26	50		33	0	33	
	23	8	31		40	0	40	
	22.	12	34		32	0	32	
	25	25	68		22	5	37	
	27	26	53		17	0	17	
	22	26	48		3	0	3	
	23	28	51		11	0	11	
	25	31	56		26	0	26	

a = Captan was used at 10 ppm concentration

b = Recovery of *Trichoderma* sp. was estimated after mixing certai conc. of spore with1 g, natural and sterilized soil and diluted spore mixed such soil to 10^{-2} dilution which contain 250 spore in 10 ml.

only insignificant effect. The percentage of recovery of *Trichoderma*`and *Gliocladium* was 92.07 in natural soil and sterlized soil was 98.0 and 94.0 respectively in non-amended TSM, 91.0 and 92.0 (natural soil); 96.0 and 93.0 (sterilized soil) respectively in Captan amended TSM.

The need for a selective medium for the study of soil ecology and selective isolation of any soil-borne microorganism can not be ruled out. These media have their own merits and limitations and attempts are always being made to improve their qualitative values (Papavizas and Lumsden, 1982). Restricted colony growth with usual colony morphology, early sporulation and colour are a few important attributes for selective media (Elad *et al.*, 1981). The semiselective media of Papavizas (1981) and the selective medium of Davet (1979) and Papavizas and Lumsden (1982) have their own limitations particularly in soils rich with mucorales. (Chet, 1987).

Elad and Chet (1983) used Captan at 20 ppm. The dosage of Captan when reduced to 10 ppm was found not to affect the qualitative value of the medium. Due to non-availability of Dexon, Maiti (1986) used methylorange without any alteration of the qualitative character of medium.

The presently modified TSM medium containing methylorange and Captan @ 300 ppm and 10 ppm respectively was found equally suitable and appeared best among the three tested media for selective isolation of *Trichoderma* and *Gliocladium* from the natural soil (Saha, 1995).

The original TSM when used showed a lot of contaminants, Accordingly, soils harbouring high population of fusaria always create problems in selective isolation of other microorganisms. Modifications have therefore been felt essential. Addition of Benomyl as an antifungal agent improved the efficiency of the medium for isolation of *Fusarium* from soils while Captan makes it selectivity specific for isolation of *Trichoderma* even in the presence of *Fusarium* in the soil (Elad and Chet, 1983).

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