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Full Length Research Paper

Influence of culture media on mycelial growth and sporulation of some soil dermatophytes compared to their clinical isolates

Mukesh Sharma[1]* and Meenakshi Sharma[2]

[1]25, Shri Ram Nagar, Near A.V.M. School, Kalwar Road, Jhotwara, Jaipur-302012, India.
[2]Microbiology Laboratory, Department of Botany, University of Rajasthan, Jaipur-302004, India.

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Culture media significantly affected the growth, sporulation and conidial discharge of any microorganisms. The present investigation was conducted to examine the effect of broth and agar media on the mycelial growth and fungal sporulation of six species belonging to five genera of fungi. Soil fungal dermatophytes (*Trichophyton mentagrophytes*, *Microsporum gypseum* and *Microsporum fulvum*) were isolated from soil using hair-baiting technique and clinical isolates (*Epidermophyton floccosum, Trichophyton schoenleinii* and *Scopulariopsis* sp.) were procured from superficial skin of dermatomycoses patients under the supervision of dermatologists. Variation in mycelial growth and fungal sporulation was observed with media tested. Colony radial growth and sporulation of soil fungi were optimal on Sabouraud's dextrose medium (SDM) followed by Potato dextrose medium (PDM) and Richard medium (RM). For clinical isolates, the suitable broth media were Potato dextrose medium (PDM) and Yeast extract medium (YEM). Among agar media studied, Sabouraud's dextrose agar (SDA) and Potato dextrose agar (PDA) media were found most favored for both soil and clinical isolates.

Key words: Clinical isolates, culture media, mycelial growth, soil dermatophytes, sporulation.

INTRODUCTION

The past two decades has witnessed considerable evolution both of keratinophilic fungi and of the processes by which they degrade keratin and become associated with human and animal diseases. Keratinophilic fungi are generally considered as soil saprophytes (Ajello, 1953, 1956). The keratinophilic fungi include true fungi that vigorously degrade keratin as well as some more important human pathogenic dermatophytes. Soil that is rich in keratinous material is most conducive for the growth and occurrence of keratinophilic fungi. Most of the keratinophilic fungi are not dermatophytes but soil inhabitants. The ubiquity of keratinophilic fungi in soil and various other environments is well recognized. Keratinophilic fungi are ecologically important and recently have attracted the attention throughout the world. Keratinophilic fungi occur on cornfield debris in soil and degrade hard keratin or keratinous materials. Some species of keratinophilic fungi, like *Nattrassia mangiferae* (Syn. *Hendersonula toruloidea*) and *Phoma* species are primarily plant pathogens (Kane et al., 1997; Punithalingam and Western, 1971). Dermatophytes are mycelial and keratinophilic fungi of the mold group, originally saprobial, but have adapted themselves to animal and human parasitism through evolution (Lacaz et al., 1991). The dermatophytes have the capacity to invade keratinized tissue (skin, hair and nails) of humans and other animals to produce an infection, dermatophy-tosis, commonly referred to as ringworm. Dermatophytes are the most important microorganisms, which cause superficial mycosis, and the lesions are characterized by circular disposition, desquamation, alopecia and erythema of the edges (Lacaz et al., 1991). The pre-valence of dermatophytes varies according to geographical

*Corresponding author. E-mail: mukeshsharma_uniraj@yahoo.co.in.

location, season or living conditions and the manipulation to which the susceptible animal or human is exposed (Boyanowski et al., 2000) however, in general, they occur more commonly in countries with a hot and humid climate (Cavalcanti et al., 2003). Nigam et al. (1987) investigated seven keratinophilic fungi from India soil.

The prevalence of keratinophilic species in a region depends upon a large number of factors like climate of a region, living conditions (including nutrition, hygiene and socio-economic conditions etc. of the inhabitants), susceptibilities and edaphic factors. The survival period of keratinophilic fungi in relation to different temperature conditions, media and natural habitats differs from species to species.

The nature of a particular medium has great role to play in the growth and sporulation of fungi. Zhao and Shamoun (2006) suggested that culture media significantly affected the growth, sporulation, and conidial discharge of *V. heterodoxa*. Kaul and Sumbali (1998) reported that keratinophilic fungi grow well in media rich in nitrogen and carbon contents. The suitability of a growth medium depends upon the specificity of a fungus under study and the purpose of the experiment (Lilly and Barnett, 1951). Vega et al. (2003) investigated the impact of nutrition on spore yields for various fungal entomo-pathogens in liquid culture. The present study was conducted to evaluate the influence of different broth and agar media on mycelial growth and sporulation of *Scopulariopsis* sp. compared with some soil and clinical isolates of dermatophytes.

MATERIALS AND METHODS

Isolation and purification of keratinophiles

Soil samples were collected from various region of Jaipur during 2005 and 2006. All the soil samples were collected from the superficial layers of 2 to 3 cm in sterile polythene bags and tightly closed with rubber bands, to maintain the initial moisture of the soil. The samples were maintained in room temperature until processing. To. Ka.Va. hair baiting technique (Vanbreuseghem, 1952) was followed for isolation of fungi. For purification of fungi, the Dilution Plate Technique/Single Spore Method (Sharma, 1983) with slight modification was followed.

Collection and maintenance of dermatophytes

The test dermatophytes were isolated and collected from superficial layer of human skin, infected with pathogenic fungi, in Out Patient Department (OPD), S.M.S. Hospital, Jaipur under supervision and guidance of dermatologists. All the isolated dermatophytes were maintained on Sabouraud's dextrose agar medium.

Test microorganisms

Soil isolates

Trichophyton mentagrophytes, *Microsporum gypseum* and *Microsporum fulvum*.

Clinical isolates

Epidermophyton floccosum, *Trichophyton schoenleinii* and *Scopulariopsis* sp.

Effect of culture media on test fungi

Eight broth media (Hi Media, Bombay) namely: Sabouraud dextrose medium (SDM), Yeast extract medium (YEM), Richard medium (RM), Czapek medium (CzM), glucose phosphate medium (GPM), potato dextrose medium (PDM), potato carrot medium (PCM), Mannitol Broth medium (MBM) and eight Agar media (Hi Media, Bombay) namely: Sabouraud dextrose agar (SDA), potato dextrose agar (PDA), corn meal agar (CMA), brain heart infusion agar (BHIA), potato carrot agar (PCA), mannitol salt agar (MSA), czapex dox agar (CDA) and chocolate agar (CA) were prepared separately and used to evaluate the mycelial growth and sporulation of test fungi. The isolated pathogenic fungi were inoculated separately in the test media (four replicates) and incubated in incubator at the optimum growth temperature of the microorganisms or between 28 to 33±2°C temperatures for 15 days. After that, the growing mycelial mats were harvested and dried to a constant weight and means were calculated. Radial growth (colony diameter) was estimated on agar plates. Hydrogen ion concentrations of the culture filtrates were measured at the end of each sampling and compared with the initial pH value of the medium.

The degree of sporulation of fungi was determined using standard methods as recommended by Wilson and Knight (1952) and Tuite (1969).

Data analysis

Results are given as mean ± standard error (S.E.) of N observations taken in four replicates (n = 4). Data sets were examined by one-way analysis of variance (ANOVA). P-value of less than 0.05 was considered significant.

RESULTS

The effect of different growth media on mycelial dry weight and sporulation (spore count) of isolated fungi was determined. Colony diameter of keratinophilic fungi was estimated on agar plates. A good medium should contain all requirements, in which fungi can grow and sporulate well and fast. Among all broth media studied, Sabouraud's Dextrose Medium (SDM) was found to be more suitable for maximum growth and sporulation of soil isolates of fungi (Table 1). *T*. *mentagrophytes* (0.452 gm) and *M*. *gypseum* (0.230 gm) showed maximum growth and sporulation on Sabouraud's Dextrose medium followed by Potato Dextrose medium, however Richard's medium was suitable also for latter. In case of *Scopulariopsis* sp., the maximum growth was reported in Richard's medium (0.364 gm) but excellent sporulation was observed in Sabouraud's Dextrose and Potato Dextrose Medium. Czapek's medium was found to be less suitable for growth and sporulation of the afore-mentioned fungi test. In case of clinical isolates of fungi, Potato Dextrose medium was found to be best for growth of *E*. *floccosum* (0.189 g) and *M*. *fulvum* (0.308 g) and

Table 1. Average dry weight and sporulation of soil fungal isolates on different broth media (initial pH 7.5).

S/N	Broth media	Soil fungal isolates					
		Average dry weight of mycelium (g)	Sporulation	Average dry weight of mycelium (g)	Sporulation	Average dry weight of mycelium (g)	Sporulation
		Trichophyton mentagrophytes		***Microsporum gypseum***		***Scopulariopsis* sp.**	
1.	SDM	0.452±0.12	++++	0.230±0.01	++++	0.328±0.31	++++
2.	YEM	0.277±0.16	+++	0.213±0.83	+++	0.117±0.41	++
3.	RM	0.258±0.03	++	0.221±0.12	++	0.364±0.04	+++
4.	GPM	0.265±0.06	+++	0.203±0.51	++	0.321±0.17	+++
5.	PDM	0.298±0.05	++++	0.221±0.04	+++	0.336±0.62	++++
6.	PCM	0.251±0.14	++	0.191±0.16	++	0.208±0.43	++
7.	MBM	0.273±0.31	+++	0.218±0.13	+++	0.231±0.25	++
8.	CzM	0.123±0.01	+	0.144±0.70	+	0.183±0.03	++
		Clinical fungal isolates					
		Epidermophyton floccosum		***Trichophyton sc h oe n l e i n i i***		***Microsporum fulvum***	
1.	SDM	0.149±0.01	+++	0.339±0.05	+++	0.291±0.06	++++
2.	YEM	0.138±0.09	++	0.558±0.08	++++	0.296±0.08	+++
3.	RM	0.175±0.13	+++	0.442±0.11	++	0.280±0.14	+++
4.	GPM	0.134±0.09	+++	0.551±0.13	++++	0.289±0.09	++++
5.	PDM	0.189±0.12	+++	0.523±0.09	++++	0.308±0.16	++++
6.	PCM	0.117±0.15	+	0.319±0.15	+	0.219±0.08	++
7.	MBM	0.131±0.06	+	0.357±0.02	+++	0.253±0.01	+++
8.	CzM	0.127±0.01	++	0.381±0.06	-	0.154±0.05	++

SDM- Sabouraud dextrose medium; YEM- Yeast extract medium; RM- Richard medium; GPM- glucose phosphate medium; PDM- Potato dextrose medium; PCM- Potato carrot medium; MBM- Mannitol Broth medium; CzM- Czapax medium. (+ = Poor sporulation, ++ = Fair sporulation, +++ = Good sporulation, ++++ = Excellent sporulation). Values are means ± standard errors (SE) of measurements taken in four replicates (n = 4) and P<0.05.

Yeast extract medium for growth of *T. s c h o e n l e i n i i* (0.558 gm) (Table 1) . Among all agar media studied for fungal growth, *T. mentagrophytes* showed maximum growth on Potato dextrose agar medium (6.9 cm) followed by Sabouraud dextrose agar medium (5.2 cm) (Table 2). Chocolate Agar medium showed slowest growth.

The maximum growth of *M. gypseum* was observed on Sabouraud dextrose agar (4.2 cm) while the least growth was on Potato dextrose agar. The two media Potato carrot agar (2.9 cm) and Chocolate agar (2.9 cm) showed similar growth and both are found to be less suitable for fungal growth. *Scopulariopsis* sp. also showed maximum growth on Sabouraud dextrose agar (6.5 cm) medium followed by Mannitol salt agar (5.5 cm). Chocolate agar showed poor growth of the fungus. All the clinical isolates of fungi showed their maximum growth on Sabouraud dextrose agar medium (Table 2).

DISCUSSION

An individual medium shows great role to play in the growth and sporulation of fungi. Jacques et al. (2002) studied the effect of liquid culture media on morphology, growth, propagule production, and pathogenic activity of the Hyphomycete. *Metarhizium*

Table 2. Average colony diameter (cm) of soil fungal isolates of fungi on different agar media.

S/N	Agar media	Soil fungal isolates		
		Average fungal colony diameter	Average fungal colony diameter	Average fungal colony diameter
		Trichophyton mentagrophytes	***Microsporum gypseum***	***Scopulariopsis* sp.**
1.	SDA	5.2±0.31	4.2±0.12	6.5±0.30
2.	PDA	6.9±0.01	1.5±0.21	3.2±0.41
3.	CMA	4.8±0.12	3.1±0.15	3.0±0.17
4.	BHIA	4.6±0.11	3.2±0.09	2.9±0.13
5.	MSA	5.0±0.04	3.0±0.16	5.5±0.24
6.	CDA	4.0±0.15	3.3±0.01	3.1±0.51
7.	PCA	3.1±0.37	2.9±0.17	2.8±0.09
8.	CA	3.0±0.07	2.9±0.08	2.0±0.06
		Clinical fungal isolates		
		Epidermophyton floccosum	***Trichophyton sc h oe n l e i n i i***	***Microsporum fulvum***
1.	SDA	4.2±0.04	5.5±0.16	5.2±0.07
2.	PDA	3.0±0.14	4.9±0.08	3.9±0.09
3.	CMA	4.1±0.09	4.2±0.07	4.4±0.16
4.	BHIA	2.9±0.03	4.3±0.08	4.2±0.05
5.	MSA	3.0±0.12	4.5±0.04	3.5±0.05
6.	CDA	2.8±0.05	4.1±0.09	3.0±0.26
7.	PCA	4.0±0.08	4.3±0.03	2.8±0.06
8.	CA	3.1±0.15	3.0±0.06	2.9±0.01

SDA- Sabouraud dextrose agar; PDA- Potato dextrose agar; CMA- corn meal agar; BHIA- brain heart infusion agar; MSA- Mannitol salt agar; CDA- czapax dox agar; PCA- Potato carrot agar; CA- chocolate agar. Values are means ± standard errors (SE) of measurements taken in four replicates (n = 4) and P<0.05.

flavoviride and found that liquid medium possess excellent response. Singh (1983) used different media and concluded that Sabouraud's Dextrose Agar was the best among natural media and glucose aspergin was the best among synthetic media for the growth of *T. equinum* and strains of *Nanizia fulva*. In present investigations, Sabouraud's dextrose agar was also found to be best for growth and sporulation of test fungi. Ooijkaas et al. (2000) studied the growth and sporulation stoichiometry and kinetics of *Coniothyrium minitans* on agar media and concluded that optimum concentration required for best growth and sporulation by organism. Ibrahim et al. (2002) studied the effect of artificial culture media on germination, growth, virulence and surface properties of the entomopathogenic hyphomycete *Metarhizium anisopliae* and suggested that culture media influence the germination of conidia, appressorial development and mycelial growth of *M*. *anisopliae*. Rombach et al. (1988) investigated the production of *Beauveria bassiana* dry mycelium in different liquid media and subsequent conidiation and concluded that sucrose and maltose yeast extract media produced most conidia. Jain (2001) studied the effect of four liquid media on keratinophilic and dermatophytic fungal growth and found that SDA medium showed maximum growth and sporulation of all fungi. This is in agreement with present investigation and findings.

Sharma (1983) also agrees with present investigation and suggested that the SDA medium used to be as an excellent source of almost all dermatophytic and keratinophilic fungi. The present study will help to maintain the fungus in the laboratory condition for preparation of inocula for different studies concerning control of the human pathogen causing dermatophytic infections. The study also concluded that the culture media are essential growing fator for controling the growth and sporulation of human pathogenic fungi.

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REFERENCES

Ajello L (1953). The dermatophytes *Microsporum gypseum* as a

saprophyte and parasite. J. Invest. Derm., 21: 157-171.
Ajello L (1956). Soil as a natural reservoir for human pathogenic fungi. Science, 123: 876-879.
Boyanowski KJ, Ihrke PJ, Moriello KA, Kass PH (2000). Isolation of fungal flora from hair coats of shelter cats in the Pacific Coastal USA. Vet. Dermatol. 11: 143-150.
Cavalcanti MP, Faustino MAG, Gomes-Filho JB, Alves LC (2003) Frequência de dermatófitos e fungos saprófitas em caninos e felinos com sintomatologia sugestiva de dermatopatia micótica atendidos no Hospital Veterinário da UFRPE. Clín. Vet., 8: 24-28.
Ibrahim L, Butt TM, Jenkinson P (2002). Effect of artificial culture media on germination, growth, virulence and surface properties of the entomopathogenic hyphomycete Metarhizium Anisopliae. Mycological Res., 106: 705-715.
Jacques F, Smits N, Claire V, Alain V, Vega F, Guy M, Paul Q (2002). Effect of liquid culture media on morphology, growth, propagule production, and pathogenic activity of the Hyphomycete, *Metarhizium flavoviride*. Mycopathologia, 154(3): 127-138.
Jain N (2001). Antidermatophytic activity of some plant metabolites. Ph. D. Thesis, Botany Department, University of Rajasthan, Jaipur, India.
Kane J, Summerbell R, Sigler L, Krajden S, Land G (1997). Laboratory Handbook of Dermatophytes. Belmont, Ca (USA), Star Publishers, pp. 213-231.
Kaul S, Sumbali G (1998). Impact of some ecological factors on the occurrence of poultry soil-inhabiting keratinophiles. Mycopathologia, 143(3): 155-159.
Lacaz CS, Porto E, Martins JEC (1991). Micologia médica-fungos, actinomicetos e algas de interesse médico. Sarvier Ltda, São Paulo, p. 695.
Lilly VG, Barnett HL (1951). Physiology of Fungi. McGraw Hill Book Company Inc. New York.
Nigam N, Kushwaha RKS, Nigam N (1987). Seven new keratinophilic fungal records from India. Kavaka, 15(1-2): 29-31.
Ooijkaas LP, Buitelaar RM, Tramper J, Rinzema A (2000). Growth and sporulation stoichiometry and kinetics of *Coniothyrium minitans* on agar media. Biotechnol. Bioengineer., 69(3): 292-300.
Rombach MC, Aguda RM, Roberts DW (1988). Production of *Beauveria bassiana* [Deuteromycotina: Hyphomycetes] in different liquid media and subsequent conidiation of dry mycelium. Biocontrol, 33(3): 315-324.
Sharma M (1983). Taxonomical, Physiological and Para-clinical studies of fungi causing skin and other infections in human beings. Ph. D. Thesis, Department of Botany, University of Rajasthan, Jaipur, India.
Singh KV (1983). Radial growth of soil inhabiting keratinophilic fungi and related dermatophytes on various media and at different temperatures. Geobios, 10(6): 284-286.
Tuite J (1969). Plant Pathological Methods- Fungi and Bacteria. Burgess Publishing Company Minneapolis, Minnesotta, p. 239.
Vanbreuseghem R (1952). Technique biologique pour 1'isolment des dermatophytes du sol (Biological technique for the isolation of dermatophytes from the soil). Ann. Soc. Belg. Med. Tech., 32: 173-178.
Vega FE, Jackson MA, Mercadier G, Poprawski TJ (2003). The impact of nutrition on spore yields for various fungal entomopathogens in liquid culture. World J. Microbiol. Biotechnol., 19(4): 363-368.
Wilson M, Knight D (1952). In: Methods of Plant Pathology. (Ed.) Tuite, John. London: Academic Press, p. 343.
Zhao S, Shamoun SF (2006) The effects of culture media, solid substrates, and relative humidity on growth, sporulation and conidial discharge of *Valdensinia heterodoxa*. Mycol. Res., 110(11): 1340-1346.