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EFFECT OF VARIOUS CULTURE MEDIA ON MYCELIUM GROWTH AND SPORULATION OF DERMATOPHYTES ISOLATED FROM THE PATIENTS OF SMS HOSPITAL, JAIPUR, RAJASTHAN

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ABSTRACT

Earth has been documented as a natural area for fungi which found as individual kingdom through innovation. In their growth, different culture media significantly affected mycelium and sporulation. The present investigation was conducted to examine the effect of different broth media on the mycelial growth and fungal sporulation of *Trichophyton rubrum* and *Microsporum gypseum.* In the present study, Mannitol Salt Broth was found suitable for the growth of *T. rubrum* and *M. gypseum* followed by others broths for each one with a flotation towards the alkaline range.

KEYWORDS: **fungal taxonomy, dermatophytes, culture media, mycelial growth, sporulation**

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INTRODUCTION

The occurrence of dermatophyte infection varies with different geographic area and climatic conditions.[1] Dermatophytes are mycelial and keratinophilic fungi of the mold group, originally saprobial, but have adapted themselves to animal and human parasitism through evolution.[2] Dermatophytoses constitutes an important public health problem, not only in developing countries, but also in immuno-compromised patients' worldwide.[3-5] Dermatophytoses is superficial infection of keratinized tissue caused by an organism of three genera of fungi such as *Epidermophyton, Microsporum* and *Trichophyton i.e.* known as Dermato-Phyton.[4,6] Culture medium can be defined as a substrate which can support the growth of microorganisms outside its normal hosts.[7] The environmental factors and growth medium play an important role in growth of microorganisms and their reproduction of fungi[8,9]. Kaul and Sumbali suggested that dermatophytic fungi grow well in media rich in nitrogen and carbon contents.[10] The present study was conducted to evaluate the influence of different broth media on mycelial growth and sporulation of *Trichophyton rubrum and Microsporum gypseum* isolated from clinical specimens in Jaipur, India.

MATERIALS AND METHODS

Sample collection

A total of one hundred clinical diagnosed patients were randomly selected. Skin and nail scrapings were collected from tinea patients of all age groups, from outpatient department of dermatology, SMS hospital and medical college, Jaipur. The following additional points were also recorded as: name, gender, age of patient, body part involved, the presence of inflammatory margin, symptoms, duration of illness.

KOH mount

The skin scrapings were treated with an aqueous solution of 10-20% potassium hydroxide (as per specimen) and gently heat, examined after 5 min under the microscope for the presence of fungal hyphae.[11]

Fungal Isolation

The specimens were cultured on Sabouraud's dextrose agar with chloramphenicol and cycloheximide (Hi-media) by slant method. The inoculated slants were placed in a mycological incubator at 26 ± 2^{o}C for 14 to 21 days. The dermatophyte isolates were examined by macroscopic, microscopic examination and 18S rRNA sequencing. Sequences were submitted to NCBI Gene bank.

Influence of different broth media on growth and sporulation of fungi

To evaluate the effect of growth media, different liquid media were used as Richard's Synthetic Broth (RSB), Yeast Extract Broth (YEB), Malt Extract Broth (MEB), Modified Sabouraud Dextrose Broth (SDB), Mannitol Salt Broth (MSB) and Czapek Dox Broth (CDB) The medium were prepared separately with a control 6.5 initial pH. In process 100 ml of liquid media was dispensed in 250 ml conical flask and sterilized in the autoclave at l21°C at 15 lbs pressure for 15 min. The pure isolated pathogenic fungi were inoculated separately in the test media and incubated at 26 ± 2°C for 21 days. On 22nd day, the mycelium mats were filtered through a Whatman No.1 filter paper and dried at 40-50°C for 24 hours and dry weight of the mycelium was recorded. The pH of the culture filtrates was also recorded for each medium.

RESULTS

The effects of different growth media on mycelial dry weight and sporulation on the selected fungal growth were analyzed from the dry mycelium weight and spore count in triplicates. Almost all media were found to suitable for growth of selected fungal species but maximum growth and sporulation were found in Mannitol Salt Broth and Malt Extract Broth. In experimentation, *M. gypseum* showed the highest growth on Malt Extract Broth (0.407±0.01gm) followed by Mannitol Salt Broth (0.280±0.04gm), Czapek Dox Broth (0.217±0.03gm), and Richard's Synthetic Broth (0.180±0.02gm) and modified Sabouraud Dextrose Broth (0.159±0.05gm). This fungus less preferred the Yeast Extract Broth (0.131±0.04gm) (Table 1).

Table 1
Effect of culture broth on mycelium growth of dermatophytes

Sr. No.	Name of Culture Broth	*Microsporum gypseum*		*Trichophyton rubrum*	
		Dry weight of mycelium (gm)	Sporulation	Dry weight of mycelium (gm)	Sporulation
1	Richard's Synthetic Broth (RSB)	0.180±0.02	++	0.148±0.04	+
2	Yeast Extract Broth (YEB)	0.131±0.04	+	0.193±0.01	++
3	Malt Extract Broth (MEB)	0.407±0.01	++++	0.267±0.05	+++
4	Sabouraud Dextrose Broth (SDB)	0.159±0.05	++	0.141±0.02	-
5	Mannitol Salt Broth (MSB)	0.280±0.04	+++	0.257±0.06	+++
6	Czapek Dox Broth (CDB)	0.217±0.03	+++	0.203±0.01	+++

Note: Values are means ± standard errors (SE) of measurements taken in triplicates (n=3) and P<0.05 (- = No sporulation, + = Poor sporulation, ++ = Fair sporulation, +++ = Good sporulation, ++++ = Excellent sporulation)

Subsequently, Malt Extract Broth also supported the maximum mycelium growth (0.267±0.05gm) of *T. rubrum*. Mannitol Salt Broth (0.257±0.06gm) was also found suitable for the growth of this fungus followed by Czapek Dox Broth (0.203±0.01gm) and Yeast Extract Broth (0.193±0.01gm) (Figure 1). The fungus showed a less development of mycelium on Richard's Synthetic Broth (0.148±0.04gm) and modified Sabouraud Dextrose Broth (0.141±0.02gm). It was also recorded that the pH of the medium changed at the end of the incubation period where it floated towards the neutrality or an alkaline range from control range (Table 2).

Table 2
Final pH of the Culture Medium after Mycelium Growth of Dermatophytes

Sr. No.	Name of Culture Broth	*Microsporum gypseum*	*Trichophyton rubrum*
		Final pH of the Culture Media	
1	Richard's Synthetic Broth (RSB)	7.45±0.07	6.75±0.15
2	Yeast Extract Broth (YEB)	7.30±0.06	6.58±0.13
3	Malt Extract Broth (MEB)	8.20±0.04	8.95±0.15
4	Sabouraud Dextrose Broth (SDB)	7.20±0.09	6.98±0.18
5	Mannitol Salt Broth (MSB)	8.30±0.08	8.43±0.03
6	Czapek Dox Broth (CDB)	7.46±0.06	7.50±0.10

Note: Values are means ± standard errors (SE) of measurements taken in triplicates (n=3) and P<0.05

DISCUSSION

The nutritional requirement of the test fungi varies from species to species. Each individual medium illustrates great role to play in the growth and sporulation of fungi.[12] In the present investigation, it was observed that different types of culture media affected the mycelial growth rate of dermatophytic fungi. Therefore the certain results showed that MEB was the most suitable liquid culture media followed by MSB. In the present study, the malt extract broth was found as the mycelium growth stimulator for dermatophytic fungi because the richness of concentration of carbon and nitrogen sources due malt extract, dextrose as carbon and peptone as a nitrogen source.Sharma and Sharma[12] also done a similar work and reported the influence of culture media on mycelial growth and sporulation of some dermatophytes on Sabouraud's Dextrose Medium (SDM) followed by Potato Dextrose Medium (PDM) and Richard Medium (RM). Mishra and Khan[13] also reported that the best growth of *Trichoderma viride* was observed on Sabouraud Malt Yeast extract Agar (SYMA) medium with colony diameter of 2 cm after 5 days of incubation. Kadhim[14] reported the effect of culture media (SDA, PDA, CMA, YEA) in growth rate of eight isolates of *Trichophyton rubrum* during different periods of incubation and 30°C.

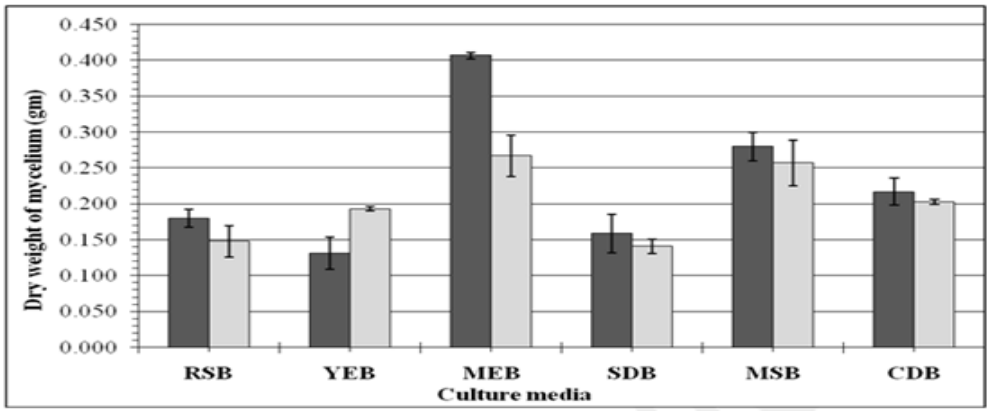

Figure 1
Influence of different liquid culture media on the fungal biomass production of Dermatophytic fungi

Gupta [15] studied the growth of keratinophilic fungi on five different media. Out of all media, SDA, found the best for all *Chrysosporium carmichaelii, Chrysosporium georgii, Chrysosporium indicum, Chrysosporium keratinophilum, Chrysosporium merdarium, Chrysosporium pannicola, Chrysosporium pannorum, Chrysosporium pruinosum, Chrysosporium queenslandicum* and *Chrysosporium tropicum* followed by PDA. Ingle[16] reported that SDB yielded second highest fungal biomass (0.609 g) followed by BCY broth (0.590 g) of Nomuraea rileyi. Al-Musallam[17] reported that growth of mycelial biomass of Chrysosporium zonatum on Sabouraud's dextrose broth (SDB) and found that mycelial biomass increase exponentially to the termination of the experiment when the recorded dry weight of floating mycelial mat was 620 mg.From the above data, it can be concluded that the nature of a particular medium has great role to play in the growth and sporulation of dermatophytic fungi. The carbon and nitrogen contain region on the host support in the increase of dermatophytic infections.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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