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Original Research Article

Optimization of Environmental Parameters for *Trichophyton rubrum* Growth and its Antigen Production

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ABSTRACT

Trichophyton rubrum is a dermatophyte cause dermatophytosis in humans and animals worldwide. It is the most prevalent form of dermatophyte. In the present study, the effect of different environmental factors on growth and antigen production of *T. rubrum* was studied. The optimum temperature and pH for the growth and antigen production of *T. rubrum* was found to be 32° C and 7 respectively. Among four media used in this study, SDA1 and SDB2 were found to be most suitable for *T. rubrum's* growth and antigen production respectively. The optimal incubation time was found to be 30 days. Light intensity and aeration were induced both growth and antigen production. This study may help in understanding of the pathogenicity and host immune responses against *T. rubrum.*

Keywords: *Trichophyton rubrum*, dermatophyte, colony diameter, anthropophilic and Optimization

1. INTRODUCTION

Trichophyton rubrum is the widely distributed anthropophilic dermatophyte responsible for causing the majority of superficial fungal infections called dermatophytosis (tinea or ringworm infection) in humans and animals worldwide. [1,2] It causes at least 60%-80% of all superficial fungal infections and especially dominant in onychomycosis in humans. [3] *Trichophyton rubrum* infections usually present as superficial, scaling eruptions. [4] These conditions are relatively benign and easy to treat, but therapy is expensive and can fail in a significant proportion of cases. [5] It has increased during the last decades, particularly among high-risk patients. [6] It is the most frequent in clinical cases of tinea pedis, tinea unguium, tinea corporis and tinea cruris. [7, 8]

Environmental factors play an important role in the growth and sporulation of keratinophilic fungi. Usually most of the fungi grow at a temperature ranging from 15°C to 35°C; some of the fungi require a range of higher temperatures for their optimum growth. Anima Sharma investigated the influence of temperature and relative humidity on common dermatophytes. Maximum growth was obtained at 30°C and highest sporulation was obtained from 25°C to 35°C. [9]

The aim of this work is to detect and optimize the different environmental factors such as pH, temperature, culture media, incubation time, light, aeration and fermentation media on *T. rubrum's* growth and its antigen production.

2. MATERIALS AND METHODS

2.1 Effect of Environmental parameters on growth

Temperature:

Temperature is one of the environmental factors which influence the growth of dermatophytes. To study the

effect of temperature on the growth of *T. rubrum*, the SDA medium plates having pH 7 were inoculated with *T. rubrum* in triplicate and incubated at different temperatures such as 25° C, 28° C, 30° C, 32° C, 35° C and 40° C. The extent of growth was observed at regular intervals on each plate and colony diameter was taken.

pH:

pH is another important factor which influences the growth of dermatophytes. To study the effect of pH, the SDA medium plates set with different pH such as 5, 5.5, 6, 6.5, 7.0 and 7.5 were inoculated with *T. rubrum* in triplicate and incubated at optimum temperature (32° C). The extent of growth was observed on each culture plate having different pH at regular intervals. The colony diameter of *T. rubrum* was measured on each plate.

Culture Media:

To study the effect of culture media, the *T. rubrum* culture was inoculated to the media like Sabouraud's Dextrose Agar medium 1 (SDA1) (1% peptone and 4% dextrose), SDA2 (2% peptone and 2% dextrose), Potato Dextrose Agar (PDA) and Czapek Dox Agar (CZA) in triplicate having pH 7.0 and the plates were incubated at an optimum temperature of 32° C. The extent of growth was observed on each culture plate at regular intervals and the colony diameter was taken.

Incubation time:

To study the effect of incubation time, the *T. rubrum* was inoculated on SDA medium having pH 7.0 in triplicate and plates were incubated at an optimized temperature (32° C) for different time include 5,10,15,20.25 and 30 days. After each incubation period, the culture plates were observed for the extent of growth and it was measured in terms of colony diameter.

Light:

To study the effect of light on dermatophyte growth one set of SDA1 inoculated plates with optimum pH were incubated in light and another set in dark at optimum temperature for optimum incubation period. After incubation for 30 days, the extent of growth was measured in terms of colony diameter.

2.2 Effect of Environmental parameters on Antigen production:

Fermentation media:

The *T. rubrum*, was cultured on various media such as SDB 1 (1% peptone and 4% dextrose), SDB 2 (2% peptone and 2% dextrose) (Mycosel broth), and PDB with and without hairs and nail clippings.

pH, Temperature and Incubation period:

The *T. rubrum* was inoculated to SDB 2 flasks set with different pH (5, 5.5, 6, 6.5 and 7) and were incubated at different temperatures (25°C, 28°C, 30° C, 32° C 35° C and 40°C) for 10 days to 45 days (10days, 15 days, 20days, 25days, 30days and 45days), to detect suitable pH, temperature and incubation period for abundant growth and sufficient production of antigen. The protein concentration in culture broth having different pH and incubated at different temperatures for different incubation period was estimated by Bradford method using bovine serum albumin as standard. [10]

Aeration:

One set of *T. rubrum* culture broth flasks was incubated in incubator rotary shaker adjusted to 100 rpm and 30° C temperature and another set in stationary condition at a temperature of 30° C to study the effect of aeration on fermentation.

Statistical analysis

Statistical analysis of data was performed using Graph Pad Prism Software Version 5.01. The one-way ANOVA was used to determine statistical significance between different environmental factors on the growth of *T. rubrum* culture and its antigen production. Results are mean of triplicate ± standard error. The P value of <0.05 was considered significant.

3. RESULTS

3.1 Effect of Environmental parameters on growth

The colony diameter of *T. rubrum* incubated at different temperatures, pH,

incubation period and light in different culture media showed a significant difference.

Temperature

The colony diameter of *T. rubrum* incubated at different temperatures showed a significant difference. A temperature of 32° C was found to be suitable for optimal growth at incubation time for 30 days on SDA1 medium having pH 7. The colony diameter of the *T. rubrum* at different temperatures as shown in Fig.1. It was maximum (5.7 cm) at 32° C.

pH

Colony diameter of the isolate at various pH as shown in Fig.2. The average colony diameter of the isolate at pH 7 incubated at a temperature of 32° C for 30 days was maximum (5.8 cm). Hence, the pH 7 was an optimum for the growth of *T. rubrum.*

Culture Media

The colony diameter of *T. rubrum* on different media as shown in Fig.3 and it was maximum (5.8 cm) on SDA1 medium. From this study it was found that SDA1 medium was suitable for the growth of *T. rubrum.*

Incubation period

Colony diameter of *T. rubrum* on SDA1 medium of pH 7 incubated at a temperature of 32° C for different incubation time as shown in Fig. 4 and it was maximum (5.9 cm) in the plate incubated for 30 days.

Light

Colony diameter of *T. rubrum* on SDA1 medium of pH 7 incubated at a temperature of 32° C for 30 days in the presence and absence of light as shown in Fig.5 and it was more (5.7 cm) in the culture plates incubated in the presence of light.

3.2 Effect of Environmental parameters on Antigen production

Effect of pH, temperature, light, culture media and aeration on antigen production was studied to optimize the fermentation conditions. The incubation of *T. rubrum* culture both in the presence and absence of light at different temperatures (25° C, 28° C, 30° C, 32° C, 35° C and 40° C) for different incubation time (5,10,15,20,25 and 30day) showed a significant difference in the protein concentration. Optimal mycelia growth and protein production in SDB 2 were observed at pH 6·8-7·0 incubated at 30-32°C temperature for 30 days in the presence of light. The protein concentration was highest under these optimized conditions and it was found to be 3000-3200 µg/ml.

The effect of aeration on the growth of *T. rubrum* on SDB 2 having pH 7 and incubated at different temperatures (25° C, 28° C, 30° C, 32° C ,35° C and 40° C) for different incubation time (5,10,15,20.25 and 30 days) showed a significant difference in protein concentration in the culture filtrate. The protein concentration in the culture filtrate that was under shaking found to be 3200 µg/ml as shown in Fig. 6.

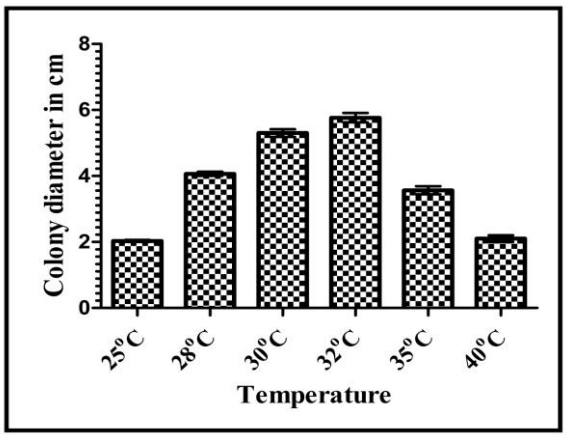

Fig.1 Effect of temperature on *T. rubrum*'s growth
Note: Values are mean of triplicate ± standard error (SE)

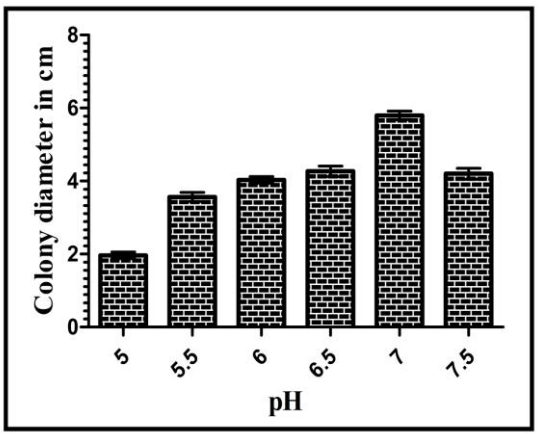

Fig.2 Effect of pH on *T. rubrum*'s growth
Note: Values are mean of triplicate ± standard error (SE)

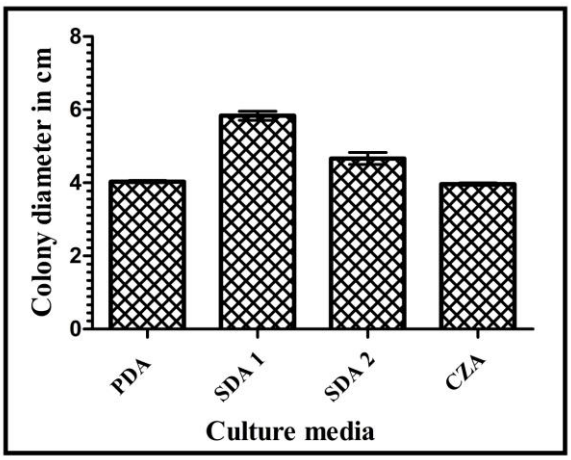

Fig. 3 Effect of culture media on *T. rubrum*'s growth
Note: Values are mean of triplicate ± standard error (SE)

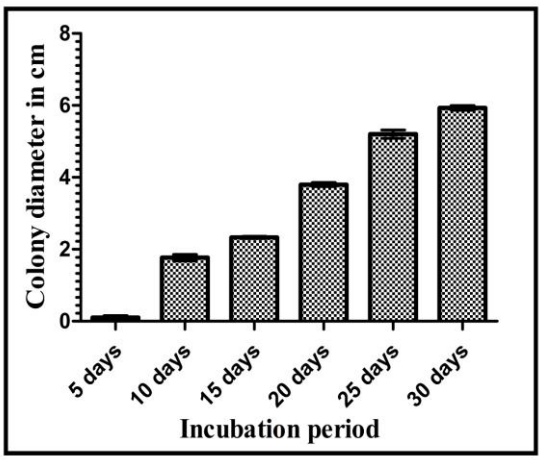

Fig. 4 Effect of incubation time on *T. rubrum*'s growth
Note: Values are mean of triplicate ± standard error (SE)

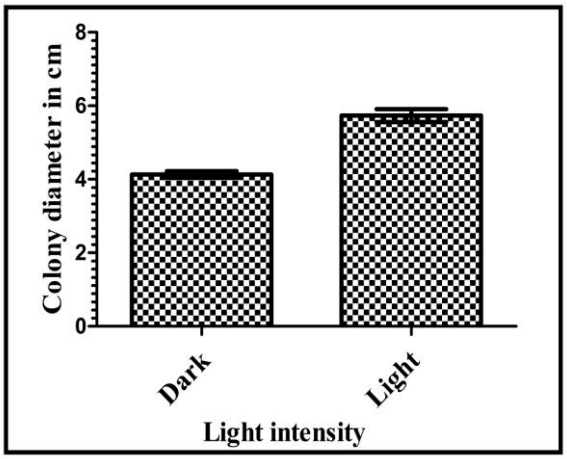

Fig. 5 Effect of light intensity on *T. rubrum*'s growth
Note: Values are mean of triplicate ± standard error (SE)

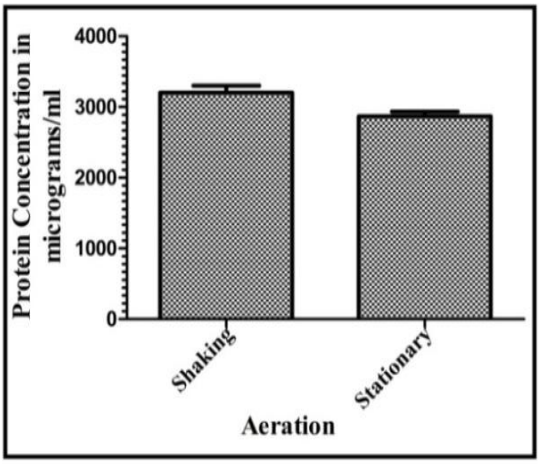

Fig 6 Effect of aeration on protein production of *T. rubrum*
Note: Values are mean of triplicate ± standard error (SE)

4. DISCUSSION

Environmental conditions like pH, temperature, light, culture media and aeration play an important role in the dermatophytes growth and antigen production. [11,12] In our study, the effect of different environmental conditions on *T. rubrum's* growth was detected in terms of colony diameter (in cm) and on antigen production in terms of protein concentration in the culture filtrates. Every fungus has fixed cardinal temperatures at which they show growth. Usually, most of the fungi grow at the temperature range of 25^{o} C to 35^{o} C. [11, 12] It showed maximum growth at 32^{o} C, at this temperature the colony diameter was found to be 5.7 cm. This was in concordance with studies done by Kadhim et al., they found that 32^{o} C was the optimum temperature for *T. rubrum's* growth [12] and Sharma et al. showed that 33^{o}C temperature was most suitable for *T. rubrum's* growth. [13] The pH is another important factor which influences dermatophytes growth. Like temperature, every fungus has pH range at which they show growth. In general, fungi grow best at pH range of 4.2-9.3. In the present study, *T. rubrum*'s growth was studied over a wide pH range. It showed maximum growth at pH 7.0-7.2. This result was comparable to the studies conducted by Sharma et al. [11] The type of culture media also has a great impact on the growth of fungi. It has been reported that dermatophytes grow well in media rich in nitrogen source and carbon source. [12] Several studies have reported that Sabouraud's Dextrose medium (SDA and SDB) is suitable for the growth of dermatophytes. In the present study,

Sabouraud's Dextrose medium was found to be suitable for *T. rubrum*'s growth and its antigen production. Our result was in agreement with studies of Kadhim et al. They also got more growth on SDA medium. [14] We used two types of Sabouraud's Dextrose media, one with (SDA1) Peptone-1% and Glucose-4%, another with (SDA 2) Peptone-2% and Glucose-2%. *T. rubrum* showed maximum growth on SDA1 and antigen production in SDB 2 with hairs and nails. The more protein yield in SDB 2 is because of more amounts of peptone, hairs and nails. This might have induced the production of a keratinase enzyme and other proteins. Incubation time is another factor known to influence the growth and antigen production of *T. rubrum.* We found more growth and protein yield at 30 days of incubation. We also found a significant difference in the colony diameter and protein concentration in the Sabouraud's Dextrose media which have been inoculated with *T. rubrum* and incubated in the presence and absence of light. Aeration also plays a role in the antigen production; the protein concentration was higher in culture broth under shaking than in stationary culture broth. Proper shaking during growth provide aeration and it in turn increase metabolic rate and thereby growth and antigen production.

5. CONCLUSION

The study of effect of different environmental factors on *T. rubrum's* growth and its antigen production may help in better understanding of the pathogenicity, proper management and appropriate treatment of dermatophytosis. It may further help in the study of host immune responses against *T. rubrum.*

Conflict of Interest: The authors declare that there are no conflicts of interest.

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