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Growth and sporulation of dermatophyte fungi at different temperature

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Abstract
The aim of the present study is to analyze the effect of temperature on growth and sporulation of *Aspergillus niger*, *Trichophyton rubrum* and *Rhizopus microsporus*. The various temperatures employed in this investigation were 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 28 °C, 30 °C, 35 °C, 37 °C and 40 °C. Superficially cut agar discs from 8-21 days old colonies were used as inoculum. Good growth of the fungal species was observed between 25 °C and 30 °C. The fungus could not grow either at 10 °C and below or 37 °C and above. Excellent sporulation was recorded at 20 °C and 25 °C, while it exhibited good sporulation at 25 °C and 28 °C. Fair sporulation of these fungal species was observed at 30° and 35 °C. At the end of incubation period, fungal colonies were thoroughly washed and subsequently filtered on previously dried and weighed paper what man's No. 42. Filter papers containing the fungal mats were subsequently withdrawn from the oven, cooled and accurately weighed.

Keywords: dermatophytes, temperature, aspergillus niger, trichophyton rubrum, rhizopus microsporus

Introduction
In Asia, *T. rubrum* and *T. mentagrophytes* are the most commonly isolated pathogens, causing Tinea pedis and unguium, as is the case in Europe. In contrast to Europe, the next most commonly isolated pathogen is *T. violaceum*, the causative agent of tineacapitis and corporis. *Microsporum ferrugineum* and *T. concentricum* are also found in Asia, especially India. *Trichophyton concentricum* causes tineaimbricata. The prevalence of tineapedis in Asia is similar to that in Europe (approximately 20%); it is also common in Australia. Tineacorporis and capitis are frequently found in children and adolescents.

In northern India, anthropophilic dermatophytes are the predominant pathogens causing tineacapitis. In a study of 153 consecutive patients with tineacapitis 90% of the patients were aged less than 15 years; 75% belonged to poor socio-economic groups and 19% had a family history of tineacapitis. *Trichophyton violaceum* (38%), *M. audouinii* (34%), *T. schoenleinii* (10%) and *T. tonsurans* (10%) were the most commonly isolated pathogens (Singal *et al.* 2001) [18]. Similarly, Madhya Pradesh was surveyed in 1986-1987, and reported presence of *T. rubrum* (64.5%), *T. mentagrophytes* (5.37%), *T. violaceum*, *E. floccsum* (18.12%), *M. gypseum* (7.52%) and *M. nanum* (Rai *et al.* 1992) [11].

Previous studies on keratinolytic microorganisms and native keratin conversion mostly focused on the ability to produce keratinolytic protease (Tsuboi *et al.* 1987) [20]. Keratinolytic enzymes are produced by fungi, actinomycetes and bacteria and have been frequently isolated from soils where keratinous materials are deposited (Kaul and Sumbali, 1997; Riffel and Brandelli, 2006) [7, 12]. Among fungi, keratinases are particularly described among dermatophytes isolated from human and animal injuries. Keratinases produced by *Microsporum*, *Trichophyton*, *Doratomyces* and *Microsporum* (Grzywnowicz *et al.* 1989; Gradisar *et al.*2000) [6, 5] and some of these enzymes are well characterized as they present great medical relevance (Deschamps *et al.* 2003) [4]. Strains of *Aspergillus fumigatus* and *Aspergillus flavus* producing keratinases were described (Santos *et al.* 1996) [17]. Among bacteria, keratinolytic activity has been widely *Bacillus* and *Streptomyces* (Lin *et al.* 1999; Kim *et al.* 2001; Bressolier *et al.* 1999) [10, 8, 2]. Only some bacteriaand keratinophilic fungi are capable of using keratin as the sole source of carbon, nitrogen, sulphur and energy.

Material and Method

Growth and sporulation of the three fungi viz., *Aspergillus niger* (MTCC 10180) *Trichophyton rubrum* (MTCC 7859) and *Rhizopus microsporus*, have been studied at different temperatures, and temperature ranges for their satisfactory growth and sporulation have been determined. Such studies may partly explain the seasonal variations in the intensities of keratinophilic diseases.

Procurement and maintenance of microbial culture

The two species of keratinophilic fungiviz. *Aspergillus niger* (MTCC 10180) *Trichophyton rubrum* (MTCC 7859),were obtained from Institute of Microbial Technology (IMTECH), Chandigarh and one species of fungi i.e. *Rhizopus microsporus* were collected from the laboratory of Society for Research Diagnostics and Treatment of Human Fungal Diseases, Jabalpur.

The fungal cultures were subcultured on Sabouraud Dextrose Agar (SDA) medium (peptone - 10 g., dextrose - 20 g., agar - 20 g., distilled water - 1000 ml) incubated for 7-21 days and *Trichophyton rubrum*and one week for *Aspergillus Niger, Rhizopus microsporus* at 28±1°C. The stock cultures of fungi were maintained on sabourd dextrose agar slant at 28±1°C.

Preparation of microbial suspension

Fungal suspension were prepared from 21 days old culture of *T. rubrum* and *R. microsporus*one week old culture of *A. niger* grown on Sabouraud dextrose agar medium. The petri dishes were flooded with 8 to 10 ml of distilled water and conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595}nm) to obtain a final concentration of approximately 10^5 spores / ml. Each of the plates was homogenized to ensure uniform distribution of the inoculums and air-dried to remove surface moisture. They were then left at least for three hours before inoculation at a temperature at which the growth was to be observed to remove the lag effect. The various temperatures employed in this investigation were 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 28 °C, 30 °C, 35 °C, 37 °C and 40 °C. Superficially cut agar discs from 8-21 days old colonies were used as inoculum. The period of incubation was one week for *Aspergillus Niger, Rhizopus microsporus* and three week for *Trichophyton rubrum*. At the end of incubation period, fungal colonies were thoroughly washed and subsequently filtered on previously dried and weighed paper what man's No. 42. Filter papers containing the fungal mats were subsequently withdrawn from the oven, cooled and accurately weighed. In general there was no significant difference between the replicates, and therefore, the mean of the 3 values has been recorded as a measure of growth under differing treatments. Changes in pH of the medium as well as the degree of sporulation of the species were noted and the results of the experiment are delineated in table 1.

Table 1: Growth and sporulation of fungi at different temperatures

S.No.	Fungal species	Dry weight in mg at different temperatures									
		25^{0}C	**28^{0}C**	**20 ^{0}C**	**30^{0}C**	**15^{0}C**	**35^{0}C**	**37^{0}C**	**40^{0}C**	**10^{0}C**	**5^{0}C**
1	*A. Niger*	118	110	60	98	51	41	0	0	0	0
2	*T. rubrum*	70	64	52	23	40	0	0	0	0	0
3	*R. microsporus*	83	85	10	80	32	0	0	0	0	0

A. Niger= Aspergillus Niger, T. rubrum =Trichophyton rubrum, R. microsporus = Rhizopus microsporus

Table 1, shows that best growth of *Aspergillus niger*, *Trichophyton rubrum* and *Rhizopus microsporus*, were accomplished at 25 °C. Good growth of the organism was observed between 25 °C and 30 °C. The fungus could not grow either at 10 °C and below or 37 °C and above. Excellent sporulation was recorded at 20 °C and 25 °C, while it exhibited good sporulation at 25 °C and 28 °C. Fair sporulation of these fungal species was observed at 30° and 35 °C. The above results show that these could grow and sporulate satisfactorily between 25 °C and 28 °C.

As is apparent from the tables, an exhilirating experience of the present studies was the fact, that the maximum growth coordinated with excellent sporulation and the manifestations were in delightful harmonic balance. Hence an obvious emerging corollary is the inevitable choice of 25 °C ± 1 as an ideal temperature for executing the biochemical need and the nutritional requirements of the organisms under the stress of experimentation.

Temperature plays an important role in influencing the growth and sporulation of fungi (Cochrane, 1963) [3]. Sharma (1983) [19] studied the effect of different temperature on the growth and sporulation of *Gymnoascusreessii, Microsporum gypseum, Trichophyton simii, Cephaliophora irregularis* and *Chrysosporium tropicum*. It was reported that these fungi grew well at temperature between 15°C to 30°C. Abarca *et al*. (1990) [1]. studied the effect of temperature on 17 strains of genus Epidermophyton and found that 28 °C and 31 °C temperature was found to be most suitable for optimum growth of most of the strains. Mehra and Jaitly (1995) [13] found that 28 °C temperature found to be suitable for optimum growth of some common fungi from city waste. Michael *et al*. (1998) [14] investigated the effect of the ecological factors like pH, temperature and ionic strength on *Candida milleri*. Relative humidity also plays huge role in fungal growth and sporulation along with optimum range of temperature. Knight (1976) [9] investigated the effect of temperature and humidity on the growth and sporulation of *Trychophyton mentagrophytes* on human stratum corneum *in vitro* and found that 24 °C to 36 °C temperature was the best for the growth along with 97% relative humidity. Ninomiya (2000) [16] reported the effect of temperature, humidity and minor injury to the penetration of dermatophytes into human stratum corneum. The results showed that 35°C temperature with 95% - 100% humidity were most suitable for penetration of dermatophytes. Morishita *et al*. (2003) [15] investigated the effect of temperature, humidity, minor injury and washing on penetration of dermatophytes in human stratum corneum and observed that 35 °C temperature with 95% - 100% humidity were most suitable for penetration of dermatophytes into human stratum corneum.

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