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Research Article

Transition of *Sporothrix Schenckii* from Mycelial to Yeast Form and Determination of its Growth Curve

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Abstract: The transition from mould to yeast form in a standard strain of *Sporothrix schenckii* (ATCC 14284) was attained by sub-culturing mycelia or conidia on enriched culture medium, blood agar (BA). Five successive subcultures were done to obtain yeast form of *S. schenckii*. A growth curve was determined using transformed standard strain of *S. schenckii* from mycelial to yeast form. Yeast nitrogen base medium (YNB) was used to study the different phases of growth in the yeast form of *S. schenckii*. After doing the sub-culture, the optical density (OD) was recorded and then tube was incubated at 37ºC, thereafter, daily readings of OD were taken to obtain a growth curve. Lag phase was observed from time of inoculation to day one. Log phase was observed from day 2 to day 18 followed by the stationary phase till day 24. Thereafter, an unceasing decline in ODs was observed till day 34 and considered as the death phase.
Keywords: Transition, *Sporothrix schenckii*, Growth curve, Optical density

INTRODUCTION

Sporotrichosis is a sub-acute or chronic infection that affects humans and other mammals. It is caused by the dimorphic fungus *Sporothrix schenckii*. The infection is acquired after the fungal penetration through the broken skin. Within infected tissue, the fungus differentiates into yeast forms and proliferates leading to cutaneous and lymphocutaneous sporotrichosis [1]. This study describes the transition of *S. schenckii* from mycelial to yeast form *in vitro*. The demonstration of dimorphism is essential for definitive identification of *S. schenckii* [2]. This study also describes the monitoring of growth of the yeast form of *S. schenckii* in broth by turbidity method. The time required for division of a cell to give rise to two daughter cells under optimum conditions is known as generation time. When an inoculum of cells is transferred to a growth medium, a delay may take place before measureable growth occurs. This time period where cells are adjusting to new growth conditions is called lag phase [3]. Once the cells have adjusted their regulatory systems to produce maximum growth under the new conditions, the culture enters the exponential or logarithmic growth phase where cells have approximately the same generation time. Eventually the stationary phase is reached, a period when measureable growth ceases. Note that if a new viable cell was formed for each cell that lost viability or if loss and increase in cell mass were balanced, there would be no measurable increase in cell growth [3]. Some cells retain viability upon reaching the stationary phase whereas others rapidly lose viability and enter a death phase.

There is paucity of studies for transformation from mould to yeast and different growth phases in yeast form of *S. schenckii*. Therefore, it was decided to know the transition and different growth phases of *S. schenckii* using enriched culture medium and turbidity method respectively.

MATERIALS AND METHODS

This experimental study was conducted in the department of Microbiology in a tertiary care hospital. A standard strain of *S. schenckii* (ATCC 14284 / MTCC 1359) was procured from Institute of Microbial Technology (IMT), Sector 39 A, Chandigarh, India. The standard strain was provided at 25ºC (mould form) by the IMT. The transition from mould to yeast form in *S. schenckii* was attained by sub-culturing mycelia or conidia on enriched culture medium, blood agar (BA). Five successive subcultures were done to obtain yeast form of *S. schenckii*. The inoculated media was incubated at 37ºC for 5-7 days [4]. The growth was confirmed by colony characteristics and microscopic examination [5]. Yeast nitrogen base medium (YNB) was used for further growth character of *S. schenckii* [3,

6]. The YNB was prepared and distributed in 5.0 mL aliquots in test tubes. These were sterilized and refrigerated. Growth of *S. schenckii* from slope of BA was sub-cultured in 5.0 mL of YNB medium and incubated at 37ºC. On the seventh day 0.1 mL of the suspension from test tube was sub-cultured into 5.0 mL of YNB medium. This suspension was observed for transmission of 90% at 540 nm of wavelength on photo colorimeter. After sub-culturing, the optical density (OD) was recorded and then tube was incubated at 37ºC, thereafter, daily readings of OD were taken to obtain a growth curve.

RESULTS

After doing five successive subcultures of the standard strain of *S. schenckii* (mycelial) on BA, colonies acquired a creamy aspect and a yellow to tan colour (Fig. 1). The growth was confirmed by colony characteristics and microscopic examination using Gram stain and lactophenol-cotton blue (LPCB) mount (Fig. 2 & 3).

Fig. 1: Successive subcultures for conversion of *S. schenckii* from mycelial to the yeast form at 37°C on blood agar

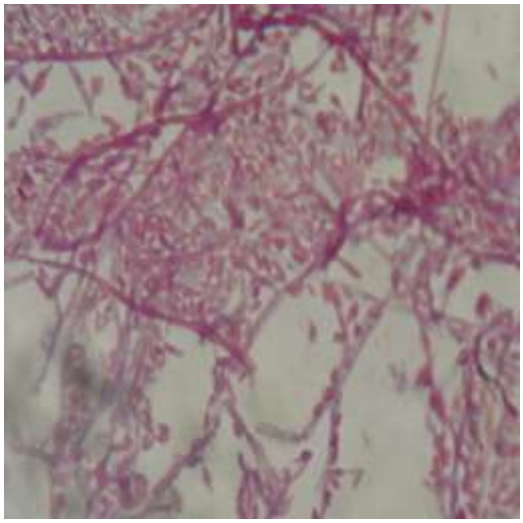

Fig. 2: Gram stained smear of *S. schenckii* during transformation from mycelial to yeast phase

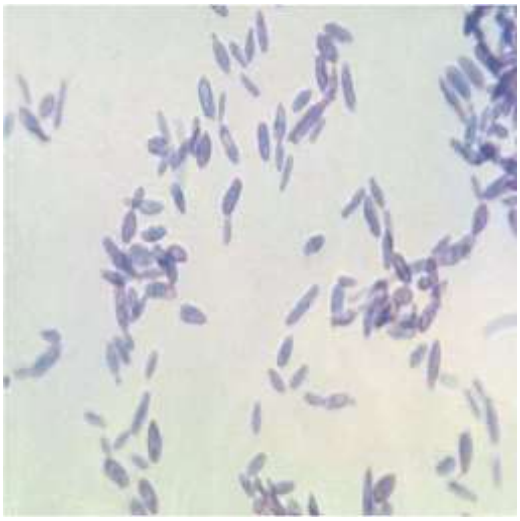

Fig. 3: Lactophenol-cotton blue (LPCB) mount of *S. schenckii* shows cigar shaped yeast cells

The growth was measured by taking OD at 540 nm of wavelength on photo colorimeter upto 34 days using YNB medium. At day 0 the optical density was 0.01, at day 10 it was 0.07 and peak density at day 18 with OD value of 0.11. This peak value sustained till day 24, however, at day 34, the optical density was 0.07, thus indicating a steady rise and slower decline (Table 1).

A growth curve was plotted against time and observations to see the different phases of yeast form of *S. schenckii* (Fig. 4). With increasing time an increase in optical density was observed. A strong correlation between time and optical density was observed which was also significant statistically ($r=0.7$; $p<0.001$).

Table 1: Optical densities for normal growth curve of *S. schenckii* (yeast form)

Days	Optical Density	Days	Optical Density
0	0.01	18	0.11
1	0.01	19	0.11
2	0.02	20	0.11
3	0.03	21	0.11
4	0.03	22	0.11
5	0.04	23	0.11
6	0.05	24	0.11
7	0.05	25	0.10
8	0.06	26	0.10
9	0.06	27	0.10
10	0.07	28	0.09
11	0.07	29	0.09
12	0.08	30	0.08
13	0.08	31	0.08
14	0.09	32	0.08
15	0.09	33	0.07
16	0.09	34	0.07
17	0.10		

r = 0.701; p < 0.001

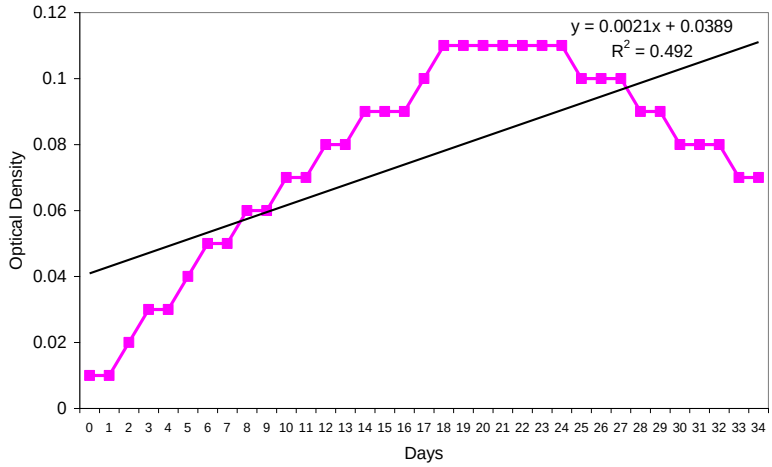

Fig. 4: Normal growth pattern of *S. schenckii* (yeast form)

DISCUSSION

Transition from mould to yeast should be demonstrated in all the isolates to differentiate pathogenic and nonpathogenic species of genus *Sporothrix*. Temperature at 37°C, medium rich in amino acids and vitamins, and humid atmosphere with 5-10% carbon dioxide, are three essential factors for transition of *S. schenckii* from mycelial to yeast form [5].

Garrison *et al*. transformed mycelial to yeast form after separation of hyphae in brain heart infusion (BHI) broth at 37°C for 96 h (4 days). They had done the ultrastructure study during the conversion from mycelium to yeast form of *S. schenckii* [4]. Howard described the ontogenic relationship of the yeast form of *S. schenckii* to its mycelial form after induction of mycelial to yeast form conversion in tissue culture [7]. After 120 h (5 days) two different morphological transformations were observed during mycelial to yeast form transition in tissue culture systems, the formation of budding, club-shaped structures at the tips of the hyphae or on the lateral hyphal branches, and the formation of chains of oidia and subsequent fragmentation of the chains into their constituent yeast form elements [7].

In the present study, complete transition was acquired after five successive subcultures in enriched medium (BA) at 37°C for 5 to 7 days (Fig. 1). The long time interval might be due to the culture medium used in the study. The enriched medium was used in the present study while Garrison *et al.* used enrichment medium and Howard used suspensions of mononuclear cells from mice [4, 7]. On the other hand, Garrison *et al*. and Howard used the clinical isolate for transition while in this study a standard strain was used. However, the results of the present study of the light microscopy of in vitro mycelial to yeast form conversion of *S. schenckii* were quite similar to the findings obtained by Howard [7] when the conversion process was studied in

tissue culture systems by conventional light microscopy.

In the present study, growth of *S. schenckii* was measured by turbidity method using photoelectric colorimeter at 540 nm of wavelength. A perusal of growth curve for *S.schenckii* yeast form shows achievement of peak activity between day 18 to day 24 followed by a declining trend. In the yeast phase**,** a strong correlation between time and optical density was observed which was also significant statistically ($r=0.7$; $p<0.001$). On day of inoculation i.e. on day 0 and day 1 the OD was 0.01 but it had started rising form day 2 (Table 1 & Fig-4). The period from inoculation to the measurable growth is considered as the lag phase, where cells adjust to new growth conditions. After the lag phase, culture enters the exponential or logarithmic growth phase [3]. In this study, a slight incline was observed on day 2, but after that a gradual rise was observed and it reached at peak on day 18 (Table 1 & Fig-4). This phase was considered as the log phase. From day 18 to day 24 there was no incline in growth and the same OD (0.11) was observed. Therefore, the period from day 18 to day 24 was considered as stationary phase because there was no measurable increase in cell growth. On day 25, a slight decline (0.10) in the growth was observed and was continued till day 34 (0.07) (Table 1 & Fig. 4). This phase was considered as a death phase. A variety of reasons may cause cell to lose viability such as production of autolysins under these conditions and exhaustion of nutrients [8]. At day 0 the optical density was 0.01, at day 18 it was 0.11. This peak value sustained till day 24, however, at day 34, the optical density was 0.07, thus indicating a gradual incline and decline.

Some authors studied the growth parameter of *S. schenckii* in mycelial form [3, 9]. Granade *et al*. were observed the peak absorbance after 120 h (on day 5) of incubation at 37°C, for filamentous fungi, *T. mentagrophyte, R. oryzae* and *S. schenckii* and remain stationary through an additional 72 h (upto day 8) [9]. Bareja *et al*. were observed the peak absorbance on day 14 and it remained stationary upto day 16 [3]. In the present study, the peak absorbance was observed on day 18 and remained stationary upto day 24. The time taken by the yeast form to achieve the peak absorbance was three times and 1.3 times more than the study done by Granade *et al*. [9] and Bareja *et al*. [3] respectively. In this study, the stationary phase remained for seven days (day 18 to day 24) while in other studies it was 3 days. This difference in time may be due to the different environmental conditions like incubation temperature (37°C) and yeast form. Granade *et al*. incubated the filamentous fungi (mould form) at 37°C while in another study it was 25°C [3, 9].

CONCLUSION

It has been found that successive subcultures are required for transition of mycelial form to yeast form in enriched medium. On the other hand, in the growth curve, there is gradual incline and decline with long log and stationary phase in yeast form as compared to mycelial form. In bacteria the growth curve can be prepared easily because such procedures are well established but in fungi there is no such standardization. Therefore, it is necessary to know the standard growth curve for scientific studies of various fungi.

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