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Determination of Growth Curve of *Sporothrix schenckii* in Mycelial Phase

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Abstract: *A growth curve was determined using a standard strain of* Sporothrix schenckii *(ATCC 14284). Yeast nitrogen base medium (YNB) was used to study the different phases of growth in the mycelial form of* Sporothrix schenckii. *After doing the sub-culture, the optical density (OD) was recorded and then tube was incubated at 25ºC, 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 14 followed by the stationary phase till day 16. Thereafter, an unceasing decline in ODs was observed till day 34 and considered as the death phase.*
Keywords: Sporothrix schenckii, *lag phase, log phase, stationary phase, death phase*

I. Introduction

Sporotrichosis is a varied disease caused by a single dimorphic fungal species, *Sporothrix schenckii* (*S. schenckii*). Sporotrichosis can be subacute or chronic, cutaneous or subcutaneous and is often associated with lymphangitis with or without lymph node enlargement [1]. This study describes the monitoring of growth of the filamentous form of fungi *S. schenckii* in broth by turbidity method. The time for a division cycle to be completed is the time for a generation of growth to take place and is known as 'generation time', if the growth in culture is synchronized, all cells are in exactly the same phase of the division cycle and will divide simultaneously. With synchronized cell division, the total cell number immediately increases from the initial number (N_0) to 2 x N_0. If the cells remain synchronized, another generation will simultaneously raise the number to 2 x 2 N_0 [2]. 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 [2]. 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 [2]. Some bacteria retain viability upon reaching the stationary phase whereas others rapidly lose viability and enter a death phase.

So far the scrutiny of literature goes, there has been no study that shows the various growth phases in *S. schenckii*. Therefore, it was decided to know the different growth phases of S. schenckii using turbidity method.

II. Material 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, Sector 39 A, Chandigarh, India. Yeast nitrogen base medium (YNB) was used for the growth of *S. schenckii* [3]. 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 Sabouraud's dextrose agar (SDA) was sub-cultured in 5.0 mL of YNB medium and incubated at 25ºC (Fig.1). 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 25ºC, thereafter, daily readings of OD were taken to obtain a growth curve.

Figure 1; tube (A) shows smooth and wrinkled, white to creamy colonies and tube (B) shows brown to black colonies of *S. schenckii* at room temperature (25ºC)

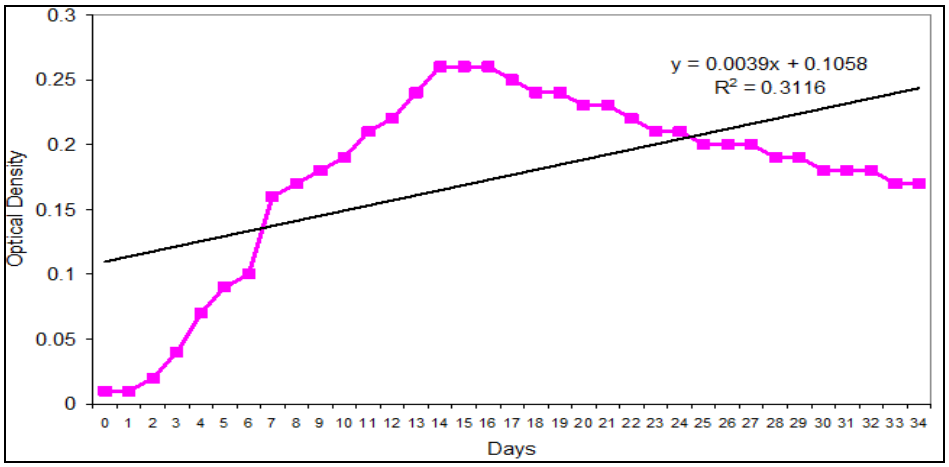
III. Results

A standard strain of *S. schenckii* was subjected to study. 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 8 it was 0.17 and peak density at day 14 with OD value of 0.26, however, at day 34, the optical density was 0.17, 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 mycelial form of *S. schenckii* (Graph 1). With increasing time an increase in optical density was observed. A moderate correlation between time and optical density was observed which was also significant statistically (r=0.56; p<0.001).

Table 1; optical densities for normal growth curve of *S. schenckii* (mycelial phase)

Days	Optical Density	Days	Optical Density
0	0.01	18	0.24
1	0.01	19	0.24
2	0.02	20	0.23
3	0.04	21	0.23
4	0.07	22	0.22
5	0.09	23	0.21
6	0.10	24	0.21
7	0.16	25	0.20
8	0.17	26	0.20
9	0.18	27	0.20
10	0.19	28	0.19
11	0.21	29	0.19
12	0.22	30	0.18
13	0.24	31	0.18
14	0.26	32	0.18
15	0.26	33	0.17
16	0.26	34	0.17
17	0.25		

r=0.56; p<0.001



Graph 1; normal growth pattern of *S. schenckii* (mycelial phase)

IV. Discussion

In the present study, growth of *S. schenckii* was measured by turbidity method using photoelectric colorimeter at 540 nm of wavelength. In the mycelial phase of *S. schenckii***,** a moderate correlation between time and optical density was observed which was significant statistically ($r=0.56$; $p<0.001$). On day of inoculation i.e. on day 0 and day 1 the OD was 0.01 but it had initiated rising form day 2 (Table1 & Graph 1). The period from inoculation to the measurable growth is considered as the lag phase. This lag time is period where cells are adjusting to new growth conditions. 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. In this study, a slight incline was observed after day 2, but after day 6 there was sharp incline was observed and it reached at peak on day 14 (Table1 & Graph 1). This phase is considered as the log phase. From day 14 to day 16 there was no incline in growth and the constant OD (0.26) was observed. Therefore, the period from day 14 to day 16 is considered as stationary phase because there is no measurable increase in cell growth. On day 17, a slight decline in the growth was observed and it was continued till day 34 (Table1 & Graph 1). This phase is 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. It was observed that while incline of optical density was quite sharp depicted by a heavy slope the decline after achieving the peak value was slightly slower. In another study, peak absorbance was observed 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) [4].

In the present study, the peak absorbance was observed on day 14 and remained stationary upto day 16. The time taken by the filamentous form to achieve the peak absorbance was almost three times than the study done by Granade et al. [4]. This difference in time may be due to the incubation temperature. Granade et al. incubated the filamentous fungi at 37°C but in the present study it was 25°C which is usually required for the filamentous form of fungi [4]. Further authors clarified that the cuvette spectrophotometry is more sensitive than in situ microspectrophotometry. According to the study, in situ microspectrophotometry required ≥ 20 µg of mycelium per ml to give a significant absorbance value. In contrast, cuvette spectrophotometry required ≥ 4.0 µg/ml, indicating that this method was somewhat more sensitive than in situ microspectrophotometry [4].

V. Conclusion

Such procedures are well established in bacteria and easy to follow, but in fungi, there is no such standardization. It is essential to know the standard growth curve of various fungi in scientific studies particularly pertaining in evaluation of antifungal compounds.

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