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Influence of incubation temperature on growth of fifteen thermophilous fungi

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Growth response of fifteen thermophilous fungi isolated from north Indian soils was studied at eight different incubation temperatures ranging from 15° - 65°C. On the basis of their growth responses to different incubation temperatures, these fungi could be categorized into two groups : thermotolerant (≤ 20-50°C or above) and thermophilic (≥ 20-50°C or above). The growth rate of different fungi varied from 0.30-1.90 mm/h at their optimum temperature. Five species belonged to slow growing, three to moderately growing, and seven to fast growing group. *Emericella nidulans* was the slowest and *Myceliophthora fergusii* was the fastest growing species. Based on temperature optima, the 15 fungi tested belonged to three different groups *i.e.* two having 35°C, eleven having 45°C and the other two having 55°C as their optimum temperature.

Key words : Growth, temperature, thermophilic, thermotolerant

INTRODUCTION

A prerequisite for complete control and exploitation of thermophilic fungi is the detailed data describing how their behaviour is influenced by the most outstanding variable of the environment, the temperature. It is presumed that a microorganism grows most rapidly at a temperature at which the various metabolic processes that contribute to growth operate optimally (Farrell and Rose, 1967). Mathematical models are being employed as a tool in mycology in order to describe fungal growth and to characterize the microorganism behaviour in defined circumstances (Edelstein *et al.*, 1983; Farina *et al.*,1997).

Thermophilic fungi are characterized by their growth optima at elevated temperatures. Because of the interest of this laboratory on the biodegradation of cellulosic and lignocellulosic materials by the thermophiles, we were curious to screen thermophilic and thermotolerant fungi for their degradative abilities. Before this programme could be installed, it was obligatory to establish optimal growth conditions for the chosen fungus/fungi. Also, to exploit them in various industrial uses, which they offer, the optimum temperature for their growth had to be found out. Keeping in view, the importance of temperature as a criterion to assign them position whether these are thermophilic (i.e. true thermophiles) or thermotolerant, fungi isolated from various soils of north India were screened for their growth response to eight different incubation temperatures.

MATERIALS AND METHODS

Organisms

The organisms used in this study were isolated from north Indian soils and the identifications were confirmed from the International Mycological Institute, UK. The fungi used are: *Absidia corymbifera, Rhizomucor pusillus, Rhizopus microsporus, Chaetomium thermophile, Emericella nidulans, Myriococcum albomyces, Thermoascus aurantiacus, Aspergillus fumigatus, Humicola grisea, Humicola insolens, Malbranchea surfurea, Myceliophthora fergusii, Stilbella thermophila, Thermomyces lanuginosus and Torula themophila.*

Medium

Yeast starch agar (YpSs) medium was used throughout this study and consisted of (g/l): soluble starch, 15.0; yeast extract, 4.0; K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 0.5; agar, 20.0; dissolved in distilled and tap water (3.1), 1000 ml. The medium was

autoclaved at 121°C for 30 min. The *P*H of the medium before sterlization was 6.5. Petri dishes 90 X 15 mm, were filled with 30 ml of the medium and dried overnight at 40°C before use.

Conditions of incubation

Before incubation, the inoculated plates were kept in polyethylene bags, to prevent contamination and drying of the medium at higher incubation temperatures. The bags were opened twice daily to aerate the cultures. All incubators were at the required temperatures with a variation of ±1°C. The incubation temperatures were 15°, 20°, 25°, 35°, 45°, 55°, 60° and 65°C. For temperatures above 35°C a beaker of sterilized water was also placed in each of the bucket containing the inoculated plates to avoid desiccation.

Growth measurements

The measurement of the diameter of the colony was used as a parameter to determine the rate of growth of a fungus following Trinci (1971). All plates of YpSs were centrally inoculated with a 8 mm mycelial disc of the test organism cut from the advancing mycelial edge of a colony growing on the solid medium. A single circular colony was produced on each plate, whose diameter was measured in two perpendicular directions at 12 h intervals. Five replicates of each isolate at each temperature were grown. All measurements for a single isolate at a particular time and temperature were averaged to give the average diameter and standard error for each plate. Conditions of temperature leading to the maximum increase in colony diameter in a given time period were taken as optimum for the growth of the organism. The diametrical growth rate (Kd) of a colony in mm/h was calculated from the expression:

$$Kd = D/T$$

Where D = the experimentally determined average diameter of the fungus colony in mm exclusive of the diameter of the inoculum (8.0 mm) and T = total time period in h.

RESULTS AND DISCUSSION

After an initial lag phase, increase in diameter was linear with time for all species. Detailed data for these species is given in Fig. 1. At any single temperature, growth rate of different species differed considerably (Table 1). Many colonies, especially those growing at extremes of their temperature ranges, did not completely filled the dishes by the end of the test period (120 h) therefore, measurements of growth after 120 h of incubation were not recorded in these cases.

Table 1: Growth rate (mm/h)[a] of fifteen thermophilous fungi at different incubation temperatures

Fungi	Incubation temperature (°C)								Optimum temperature	Category
	15	20	25	35	45	55	60	65		
Zygomycetes										
Absidia corymbifera	-	0.18	0.30	0.64	0.85	0.44	-	-	45	F
Rhizomucor pusillus	-	-	0.18	0.60	0.85	0.34	0.03	-	45	F
Rhizopus microsporus[b]	0.50	0.11	0.30	0.98	1.10	0.85	-	-	45	F
Ascomycetes										
Chaetomium thermophile	-	-	0.03	0.37	1.13	0.51	0.02	-	45	F
Emericella nidulans[b]	0.03	0.09	0.17	0.30	0.22	0.02	-	-	35	S
Myriococcum albomyces	-	-	0.20	0.54	0.63	0.22	-	-	45	M
Thermoascus aurantiacus	-	0.04	0.21	1.70	1.30	0.98	0.02	-	35	F
Hyphomycetes										
Aspergillus fumigatus[b]	0.06	0.09	0.16	0.35	0.38	0.22	-	-	45	S
Humicola grisea	-	0.02	0.08	0.16	0.26	0.32	-	-	55	S
Humicola insolens	-	0.05	0.12	0.17	0.27	0.35	-	-	55	S
Malbranchea sulfurea	-	-	0.12	0.36	0.54	0.39	-	-	45	M
Myceliophthora fergusii	-	-	0.18	0.73	1.90	-	-	-	45	F
Stilbella thermophila	-	-	0.03	0.12	0.37	0.07	-	-	45	S
Thermomyces lanuginosus	-	0.03	0.11	0.29	0.71	0.36	0.02	-	45	M
Torula thermophila	-	-	0.05	0.36	1.13	0.32	-	-	45	F

[a]Data are mean of five replications.
[b]thermotolerant

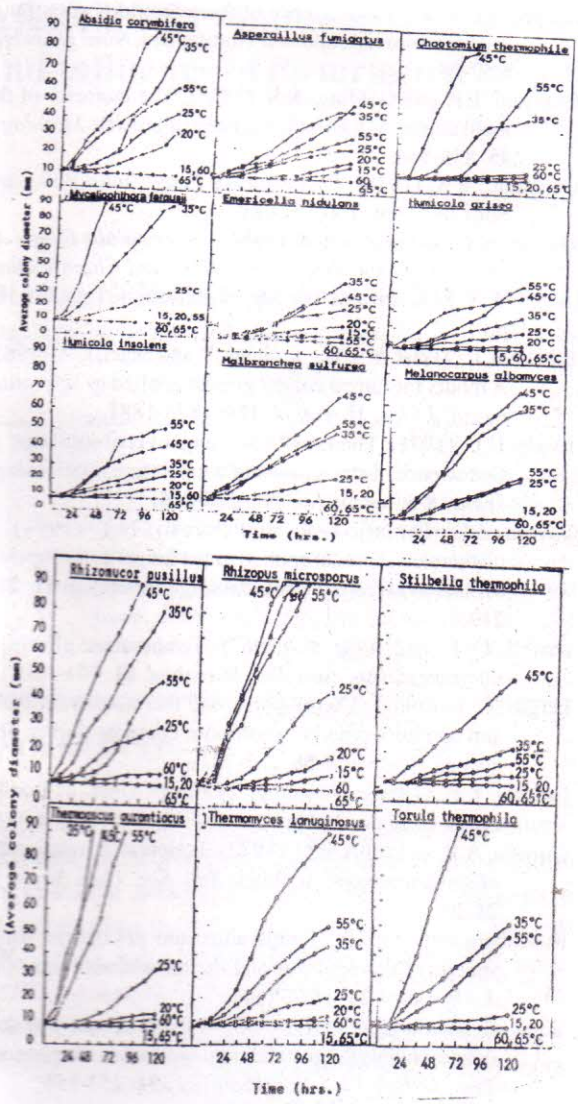

Fig. 1. Average diameter of fungal colonies at different tempera tures as a function of time

Based on colony diameter, the growth responses of fifteen fungi to eight different incubation temperatures, viz. 15°, 20°, 25°, 35°, 45°, 55°, 60°, and 65°C, yielded 3 thermotolerant (≤ 20°-50°C or above) and 12 true thermophiles (≥ 20°-50°C or above) based on the definition as proposed by Cooney and Emerson (1964) (Table 1) *Aspergillus fumigatus, Emericella nidulans Rhizopus microsporus* grew between 15° and 55°C, but they could not grow at 60°C and were classified as thermotolerant fungi. *Chaetomium thermophile* and *Rhizomucor pusillus* grew between 25° and 60°C and *Thermoascus aurantiacus,* and *Thermomyces lanuginosus* grew between 20° and 60°C. *Myceliophthora fergusii* could not grow even at 55°C. The upper temperature limit for the remaining fungi was at or near 55°C.

The optimum temperature of two fungi (*Emericella nidulans* and *Thermoascus aurantiacus)* was 35°C; of eleven fungi (*Absidia corymbifera, Aspergillus fumigatus, Chaetomium thermophile, Malbranchea sulfurea, M. fergusii, Myriococcum albomyces, Rhizomucor pusillus, Rhizopus microsporus, Stibella thermophila, Thermomyces lanuginosus* and *Torula thermophila)* was 45°C and of two fungi *i.e Humicola grisea* and *H. insolens* was 55°C. All the fifteen fungi were able to grow between 25°-55°C, only four fungi viz., *Chaetomium thermophile, Rhizomucor pusillus, Thermoascus aurantiacus* and *Thermomyces lanuginosus* were able to grow at 60°C and none was able to grow at 65°C. Thus the upper temperature limit of these fungi lie below 65°C.

The growth rate at the optimum temperature of 15 fungi varied from 0.30-1.90 mm/h. *E. nidulans* was the slowest and *M. fergusii* was the fastest growing fungi. The fungi having growth rate of 0.30-0.38, 0.54-0.71 and 0.85-1.90 mm/h were categorized as slow, moderate and fast growing, respectively. Comparison of growth rate at the optimum temperatures of these fungi show that five (*E. nidulans, A. fumigatus, H. grisea, H. insolens and S. thermophila)* are slow growing. Three species *(M. albomyces, M. sulfurea and T. lanuginosus)* belong to moderately growing group and seven species namely *A. corymbifera, C. thermophile, M. fergusii, R. pusillus, R. microsporus, T. aurantiacus and T. thermophila* belong to the fast growing group. A comparison of our results with the literature revealed that the temperature responses of *T. lanuginosus, H. grisea, R. pusillus* are more close to those reported by Fergus (1964), Evans (1971) and Singh and Sandhu (1982) than Apinis (1963) and Qureshi and Johri (1972). The temperature variations reported above may be due to strain variations, nutritional requirements and temperature study at wider temperature intervals in the region of fastest growth.

The fastest growth rate for all the fungi occurred at temperatures of 35°- 45°C, whereas slower rate occurred at extremes of temperatures (Table 1). The growth response of these fungi to different temperatures was determined on the basis of colony diameter because it has been shown to be a reliable method to determine the rate of growth of fungi (Trinci, 1971). But according to Hawker (1950) and Cochrane (1958), the colony diameter alone does

not account for density, height and depth of the colony. However, it has been justified for studies in which only one environmental variable for example temperature is studied (Brancato and Golding, 1953; Cochrane, 1958; Evans, 1971; Trinci, 1971; Tansey, 1972; Singh and Sandhu, 1982; Aneja *et al.*, 1989).

The length of lag phase for growth of microorganisms at different temperatures is affected by the temperature of incubation of inoculum. A shorter lag phase is expected at growth temperatures nearer that of the inoculum and is especially significant for organisms adapted to extreme environments (Farrell and Rose, 1967). The lag phase for various species reported here varied from 12-24 h at their optimum temperatures (Fig. 1). Disagreement exists in the literature concerning the appropriate parameter to choose to obtain a linear plot of growth rate on agar (Cochrane, 1958), and suggestions include radius, diameter, area and various reciprocals of these to be plotted against time. Data from mesophilic fungi usually give a linear plot when diameter of colony growth is plotted against time. Our data (Fig. 1) reveal that the thermophilic fungi responded similarly. These observations suggest that data from growth responses to different temperatures is useful evidence in support of morphological studies of taxonomic relationships.

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