Notas Científicas

Growth and germination of some thermophilic fungi isolated from eucalypt wood chips

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Abstract - The main fungal species present in self-heated chip piles of eucalypt wood in Brazil were Aspergillus fumigatus, Chrysosporium thermophilum, Dactylomyces thermophilus, Penicillium bacillisporum, Rhizomucor pusillus and Thermoascus aurantiacus. This study determined the micelial growth and spore germination of these species at several temperatures on PDA medium ranging from 20 °C to 70 °C. Optimum growth temperatures were observed at 30 °C for A. fumigatus and P. bacillisporum; 40 °C for R. pusillus and 50 °C for D. thermophilus, C. thermophilum and T. aurantiacus. Growth was not observed at 60 °C and 70 °C. Incubation of spores on water-agar medium at 20 °C, 30 °C, 40 °C and 50 °C showed that only A. fumigatus, P. bacillisporum and R. pusillus could germinate at 20 °C.

Index terms: Microorganism, physiology, temperature, thermophilism.

Crescimento e germinação de alguns fungos termófilos isolados de cavacos de madeira de eucalipto

Resumo - Os fungos termófilos encontrados em pilhas de cavacos de eucalipto auto-aquecidas no Brasil foram *Aspergillus fumigatus*, *Chrysosporium thermophilum*, *Dactylomyces thermophilus*, *Penicillium bacillisporum*, *Rhizomucor pusillus* e *Thermoascus aurantiacus*. Este estudo determinou o crescimento micelial e a germinação de esporos em uma faixa de temperaturas que variou de 20 °C a 70 °C. Temperaturas ótimas foram observadas a 30 °C para *A. fumigatus* e *P. bacillisporum*; 40 °C para *R. pusillus* e 50 °C para *C. thermophilum*, *D. thermophilus* e *T. aurantiacus*. Não houve crescimento nas temperaturas de 60 °C e 70 °C. A incubação dos esporos em ágar-água a 20 °C, 30 °C, 40 °C e 50 °C mostrou que somente *A. fumigatus*, *P. bacillisporum* e *R. pusillus* germinaram a 20 °C.

Termos para indexação: Fisiologia, microorganismos, termofilia, temperatura.

Introdution

Thermophilism is a characteristic of some fungi growing under temperatures above those observed in common environment. Such ability is related to physiological ability developed to adapt themselves to extreme conditions of temperature (TUOMELA et al., 2000). Various substrates can be used by thermophilous fungi: organic matter during composting; coal residues; kernels; and wood chip (SMITH; OFOSU-ASIEDU, 1972; SHARMA, 1989; MOUCHACCA, 1997).

The phenomenon of self-heating of wood chip piles started to be registered during the 1960's. Rothrock et al. (1961), evaluating storage of *Pinus caribaea* wood, observed temperatures reaching 60 °C inside chip piles during first weeks to five months after piling. Other studies were performed on the process of thermophilism towards understanding the self-heating process, fungal physiology and fungi effects on wood properties (TANSEY, 1971; SCHIMIDT, 1969). The effects usually are dependent on wood type, environment and storage conditions and the fungal population present (FEIST et al., 1973; HULME; HATTON, 1976; OFOSU-ASIEDU; SMITH, 1973). The presence of thermophilic fungi were reported in pile wood chips of eucalypts in Brazil (AUER, 1986), but information on their physiology is still unpublished. Therefore, the present work aimed to determine growth characteristics and spore germination of six thermophilic fungi under different temperatures.

Samples of eucalypt wood chips were collected from self-heated piles at Mogi Guaçu, SP, under temperatures over 40 °C, after four months of outside storage. Chips were obtained from 1.0-1.5 m deep holes made in piles and stored in plastic bags for transportation to the laboratory.

In order to obtain isolates from the wood chips, small fragments were removed from flamed chips and then placed into Petri dishes containing PDA medium (potato extract 39 g; dextrose, 20 g; powdered agar, 18 g; distilled water 1000 mL) and incubated at 50 °C. Three isolates were obtained each of *Aspergillus fumigatus* Fres., *Dactylomyces thermophilus* Sopp., *Penicillium bacillisporum* Swift, *Rhizomucor pusillus* (Lindt.) Schipper, *Chrysosporium thermophilum* (Apinis) van Klopotek, and *Thermoascus aurantiacus* Miehe. Cultures were identified at Instituto de Botânica, São Paulo, Brazil.

For the mycelial growth assay, 6 mm-diameter PDA plugs with mycelium were taken from 7-days-old single culture of each species and placed in the center of dishes containing 25 mL PDA medium. Three isolates of each fungus, in triplicate, were grown in incubators at 20, 30, 40, 50, 60, and 70 ± 2 °C, in dark conditions. At 60 and 70 °C, dishes were placed into glass jars containing cotton plugs soaked with sterile distilled water, to reduce desiccation of cultures. Petri dishes were examined daily and colony diameters were measured at right angles to each other when the margin reached 1.0 to1.5 cm from the dish border. The rates of growth were determined by dividing the average diameter of the colony by the time taken to reach that size. The optimum temperatures were considered those with the maximum growth rates. Data of colony growth were analysed by ANOVA analysis (PIMENTEL-GOMES; GARCIA, 2002).

The effect of temperature on spore germination was tested by using a procedure adapted from Gattani (1954). The latent period germination, which is the minimum time taken for a given percentage of the spores to form germ-tubes or the time taken for germ-tubes to reach a certain length (HAWKER, 1967) was assayed. Spores were obtained from sporulating cultures produced on PDA, incubated at 50 °C, in dark conditions. A suspension of spores was prepared and aliquots of 10^8 spores transferred into Petri dishes containing 25 mL of water-agar (agar, 18 g; distilled water, 1000 mL). The spores were spread with the use of a glass rod, and incubated at 20, 30, 40 and 50 ± 2 °C. Three isolates of each fungus and four replicates were used. The formation of germ-tubes was observed under a light microscope with 100 x magnification, at 2 hour intervals. Spores were considered germinated when the length of germ-tubes reached or exceeded the size of the largest dimension of the spore.

Aspergillus fumigatus and *P. bacillisporum* grew better at 30 °C, typically showing higher growth rates at temperatures different from those observed in self-heated piles (AUER, 1986). *R. pusillus* grew better at 40 °C whereas *D. thermophilus*, *C. thermophilum* and *T. aurantiacus* at 50 °C, and none of these grew at 60 and 70 °C. Data were presented in Table 1 because some fungi grew only on three temperatures giving few points that could be fitted in a response curve to temperature.

Table 1. Mycelial growth rate (mm/hour) of thermophilic fungi in PDA medium at different temperatures

Fungi	Temperature (± 2°C)			
	20	30	40	50
Aspergillus fumigatus	0.28b[1]	0.75[a]	0.61[a]	0.19b
Chrysosporium thermophilum	-	0.45b	0.53b	0.68[a]
Dactylomyces thermophilus	-[2]	0.43b	0.99[a]	1.26[a]
Penicillium bacillisporum	0.12b	0.30[a]	0.28b	0.10b
Rhizomucor pusillus	0.35b	1.51[a]	2.24[a]	2.02[a]
Thermoascus aurantiacus	-	0.32c	3.84a	5.34a

[1]Average of three isolates, three replicates per isolate; [2]no growth. Means followed by same letter, in lines, do not differ significantly (P<0.05).

Optima growth temperatures observed in this study revealed some differences from other reports (CRISAN, 1973; ROSENBERG, 1975). Chapman (1974), using yeast extract-starch-agar supplemented with salts (YpSs medium), verified fast growth rates at 40 °C in *Rhizomucor miehei* (COONEY; EMERSON, 1964) Schipper and *Sporotrichum thermophile* Apinis (= *C. thermophilum*). Crisan (1973) revising cardinal temperatures of thermophiles related 45-53 °C for *R. pusillus*; 40-46 °C for *T. aurantiacus*; 40-50 °C for *S. thermophile*; 37-43 °C for *A. fumigatus*; 37-40 °C for *Penicillium piceum* Raper e Fennell and 42 °C for *Penicillium* sp. Using a modified yeast extract-starch-agar, Kuthubutheen (1983) observed 1.02 mm/hour in *R. pusillus* and 1.28 mm/hour in *T. aurantiacus*, under 45 ºC.

Significant variation on growth was not observed among isolates of each tested fungi showing that isolates

grew in a similar way in each temperature. Differences between our results and information on the literature may be explained by the specificity of isolates, of amino acids and sugars concentration and other aspects in culture media.

Cooney e Emerson (1964) postulated that thermophilic fungi are microorganisms that require a temperature at least of 20 °C for growth and supporting temperatures of at least 50 °C. This range could favor certain thermotolerant fungi. Emerson (1968) discussed thermophilism as a characteristic of organisms which grow at temperature of 40 °C or higher. Based on these concepts, *C. thermophilum*, *D. thermophilus*, *R. pusillus*, and *T. aurantiacus* could be considered as thermophiles while *A. fumigatus* and *P. bacillisporum* could be thermotolerant fungi.

Lower latent periods were observed at two temperatures: 20 °C for *A. fumigatus* and *C. thermophilum*; 40 °C for the others. In many cases, the best temperature for growth was equal or inferior the shorter period spent to germination (Tables 1 and 2). Hawker (1967) stated that the temperature that promotes growth is usually lower than those necessary for germination. This was observed only with *P. bacillisporum*.

Table 2. Latent period of germination (hour) of thermophilic fungi on water-agar at different temperatures

Fungi	Temperature (± 2 °C)			
	20	**30**	**40**	**50**
Aspergillus fumigatus	20-22[1]	6-8	8-10	58-60
Chrysosporium thermophilum	-	12-14	-	-
Dactylomyces thermophilus	-[2]	20-22	12-14	24-26
Penicillium bacillisporum	44-46	8-10	6-8	-
Rhizomucor pusillus	68-70	12-14	2-4	12-14
Thermoascus aurantiacus	-	20-22	12-14	24-26

[1]Average of three replicates; [2]no germination.

The ability of *A. fumigatus*, *P. bacillisporum*, and *R. pusillus* spores to germinate at 20 °C can be related to the initial process of thermogenesis inside wood chip pile. *D. thermophile* and *T. aurantiacus* would participate during self-heating process, when temperatures reach the necessary level for the germination to occur (COCHRANE, 1958).

The participation of fungi on the thermogenesis is probably due to their ability to use wood chip as substrate. Some soluble components such as cellulose, starch, lipids and monosaccharides could be utilized as carbon source for growth (SHARMA, 1989). A small fraction of the carbon is incorporated into their cells. Some of the energy is used for microbial metabolism and the rest is released as heat (TUOMELA et al., 2000).

Our results indicated that thermotolerant fungi are *A. fumigatus*, *P. bacillisporum* and *R. pusillus*, based on growth rates and spore germination. Further characterization of other fungi whether they are thermophilic or not would require additional work, including temperature range between 50 to 60 ºC to give a better definition of optimum temperature or maximum growth temperature. This complimentary information would be useful to distinguish between thermotolerant and thermophilic fungi.

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