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THE GROWTH OF *THERMOMYCES LANUGINOSUS* (TSIKL.) ISOLATES FROM GARDEN COMPOSTS AND COFFEE BEANS ON CELLULOSE SUBSTRATES AND XYLAN AT VARIOUS WATER ACTIVITY

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Abstract: The study was to determine the effect of water activity (0.850; 0.900; 0.950; 0.995; and 0.999 a_w) on the growth of *T. lanuginosus* on solid media containing different cellulose substrates (crystalline cellulose, carboxymethyl cellulose – CMC, filter paper, and sawdust) and xylan. The growth of isolates from coffee beans and garden composts were compared. All isolates did not grow on media with $a_w < 0.950$. On media with $a_w > 0.950$, the hydrolysis zones were only observed on xylan and CMC. The highest daily growth and hydrolysis zone rates were mostly obtained at 0.995 a_w and the lowest values were observed at 0.950 a_w. The coffee beans isolates at 0.950 a_w had the CMC hydrolysis coefficient 1.7-times higher than that for xylan. The fungal growth (FG) coefficient data indicate that the coffee beans isolates were able to utilize CMC and crystalline cellulose for growth and the highest growth rate was obtained at 0.999 a_w. Subsequently, the compost isolates were able to grow on all substrates but the highest growth rate was obtained on CMC at 0.950 and 0.999 a_w. Thus, coffee beans and composts provide *T. lanuginosus* isolates with various growth and hydrolytic zone rates in the range of 0.950−0.999 a_w.

INTRODUCTION

Thermomyces lanuginosus (Tsikl.) is a thermophilic fungus, which occurs in a variety of environments (compost, saw, municipal solid waste, animal excrements, sewage sludge, soil, indoor and outdoor air, etc.) in all climatic zones [7]. Oil plant seeds including coffee beans have also been reported to be one of the natural sources of this fungus [14].

T. lanuginosus produces a number of enzymes (including thermostable enzymes) such as xylanase and other hemicellulases, lipase, α-amylase, glucoamylase, phytase, trehalase, invertase and others [21, 28]. There is a vast body of papers on xylanase produced by *T. lanuginosus* [4, 12, 18, 28]. These enzymes have potential applications in paper, detergent,

drug and food industries [11]. With better understanding of enzyme activity mechanisms new potential industrial applications have emerged [1, 5]. For instance, a recent finding has shown that xylanases can be used in organic solvent solutions; greatly expanding the potential applications and economic impact of biocatalysis [15, 26]. However, there is no consensus regarding the production of cellulases by *T. lanuginosus* [2, 3, 8, 13, 16, 18, 22, 25, 29].

Water activity has been found to be an important factor affecting fungal growth and enzyme activity [9, 27]. In the available literature, little data on the influence of water activity on *T. lanuginosus* growth and enzyme activity and properties have been found. Kamra and Satyanarayama [16] studied the xylanase and cellulase production by this fungus at 0.950 a_w in solid-state fermentation. Gogou *et al.* [10] determined the thermal stability of *T. lanuginosus* xylanases at different water activity.

The aim of the present study was to determine the influence of water activity on the growth of *T. lanuginosus* on solid media containing different cellulose substrates or xylan. The growth of isolates from coffee beans and garden composts were compared and discussed.

MATERIAL AND METHODS

Nine *Thermomyces lanuginosus* isolates (4 from coffee beans and 5 from garden composts) were used in the study. The basal medium by Tansey [29] containing 2 g $NH_4H_2PO_4$, 0.6 g KH_2PO_4, 0.4 g K_2HPO_4, 0.89 g $MgSO_4 \times 7H_2O$, 0.5 g yeast extract, 4 mg adenine, 8 mg adenosine, and 100 µg thiamine-HCl per 1 dm^3 distilled water was chosen for the experiment. The basal medium was supplemented with 1% crystalline cellulose, carboxymethyl cellulose (CMC), birch wood xylan or sawdust as the main carbon sources. Sterilized filter paper discs (MN 619 G) were placed on the surface of the basal medium in Petri dishes. The medium without supplements served as control.

The water activity of the media was adjusted with sodium chloride to 0.999, 0.995, 0.950, 0.900, and 0.850 a_w [19]. The a_w was measured with a DE 202 Aqua Lite meter.

Ten days old fungal cultures on MEA slants at 37°C were used for preparing spore suspensions. 5 ml of sterile physiological saline were added to each slant. The slants were then vigorously shaken with a vortex for 3 minutes. Each plate was centrally inoculated with 5 µl of spore suspension. The incubation was carried out for 10 days at 40°C.

Colony and hydrolysis zone ("halo" around or underneath the colonies) diameters were measured after 2, 4, 6, 8 and 10 days of incubation with a ruler. On CMC medium hydrolysis zones were developed with 10% copper acetate solution.

The daily growth rates (mm/day) and hydrolysis zone were calculated from the linear regression equation, $r = a \cdot t + b$, in which: r – colony radius (mm); t – incubation time (day); a – daily growth rate; and b – growth retardation time (lag phase; λ). The calculations were performed in the Excel program. According to Dantigny *et al.* [6], the lag time has no biological significance, because its calculation results from macroscopic observations of mycelium. It was the reason why the analysis of b values was abandoned in this study.

The hydrolysis index (H) is the ratio of daily hydrolysis zone rate to colony growth rate. The H values between 0 and 1 indicates that the hydrolysis zone is underneath the colony, whereas the H values > 1 means the hydrolysis zone radius greater than the colony radius.

The FG (Fungal Growth) index is the subtraction of the daily growth rate on a given substrate minus the daily growth rate on the control medium. The negative FG values

indicate that the daily growth rate on a substrate was smaller than the daily growth rate on control medium (devoid of the substrate).

The experiment was verified three times. The statistical significance of the differences in daily growth and hydrolysis zone rates was evaluated with one-way ANOVA test at $p < 0.05$.

RESULTS

The *T. lanuginosus* isolates did not grow on media with $a_w < 0.950$ (Table 1).

Table 1. Daily growth rates (mean ± standard deviation) of *Thermomyces lanuginosus* coffee and compost isolates on different substrates and on control medium at different water activity a_w

Substrates	Water activity a_w	Daily growth rate [mm/day]	
		Coffee isolates	Compost isolates
CMC	0.850	0 ± 0	0 ± 0
	0.900	0 ± 0	0 ± 0
	0.950	0.30 ± 0.35*	1.16 ± 0.54
	0.995	2.71 ± 0.86*	2.99 ± 0.30
	0.999	2.50 ± 0.81*	3.00 ± 0.14
Sawdust	0.850	0 ± 0	0 ± 0
	0.900	0 ± 0	0 ± 0
	0.950	0.34 ± 0.18*	0.70 ± 0.19
	0.995	2.20 ± 0.22	2.12 ± 0.30
	0.999	1.52 ± 0.74*	1.83 ± 0.18
Filter paper disc	0.850	0 ± 0	0 ± 0
	0.900	0 ± 0	0 ± 0
	0.950	0.40 ± 0.46*	1.00 ± 0.20
	0.995	1.81 ± 0.76*	2.35 ± 0.23
	0.999	1.71 ± 0.83*	2.12 ± 0.11
Crystalline cellulose	0.850	0 ± 0	0 ± 0
	0.900	0 ± 0	0 ± 0
	0.950	0.41 ± 0.36*	0.89 ± 0.23
	0.995	2.46 ± 0.41*	2.06 ± 0.38
	0.999	2.70 ± 0.36*	2.34 ± 0.41
Xylan	0.850	0 ± 0	0 ± 0
	0.900	0 ± 0	0 ± 0
	0.950	0.44 ± 0.49*	1.13 ± 0.35
	0.995	2.05 ± 0.65*	2.43 ± 0.18
	0.999	1.86 ± 0.88*	2.14 ± 0.12
Control	0.850	0 ± 0	0 ± 0
	0.900	0 ± 0	0 ± 0
	0.950	0.97 ± 0.52	1.08 ± 0.38
	0.995	2.30 ± 0.43*	1.88 ± 0.61
	0.999	1.80 ± 0.17	1.92 ± 0.36

* statistically significant differences between coffee and compost isolates at a given substrate and water activity a_w

In coffee beans isolates the highest daily growth rates on all media were observed at 0.995 a_w, while the lowest rates were found at 0.950 a_w. In compost isolates the relationship between daily growth rates and water activity was slightly different from that of coffee beans isolates. The growth rates on CMC, crystalline cellulose and on control medium at 0.999 a_w were found to be equal or even higher than those at 0.995 a_w. In both fungal isolates (on all media) the differences in daily growth rates between 0.995 and 0.950 a_w were statistically significant at $p < 0.05$. Statistically significant differences in daily growth rates between 0.995 and 0.999 a_w were observed on xylan (coffee beans and compost isolates), CMC (coffee beans isolates), crystalline cellulose (coffee beans and compost isolates), sawdust (coffee beans and compost isolates) and on control medium (coffee beans isolates).

At 0.950 a_w on all media the daily growth rates for compost isolates were found to be higher than those for coffee beans isolates. Except for control medium the differences were statistically significant at $p < 0.05$. At 0.995 a_w the daily growth rates on CMC, filter paper and xylan for compost isolates were found to be higher than those for coffee beans isolates, while the growth rates on crystalline cellulose, sawdust and on control medium were higher for coffee beans isolates. Except for sawdust, these differences were statistically significant at $p < 0.05$. On all substrates (except for crystalline cellulose) at 0.999 a_w the daily growth rates for compost isolates were found to be higher than those for coffee beans isolates. These differences were statistically significant at $p < 0.05$.

Hydrolysis zones were only observed on CMC and xylan media (Table 2). The hydrolysis zone rates were found to be the lowest at 0.950 a_w. At all a_w values the hydrolysis zone rates for compost isolates were found to be higher than those for coffee beans isolates. However, only the differences at 0.950 a_w were statistically significant at $p < 0.05$.

The highest hydrolysis coefficient (H) values were observed on CMC and xylan at 0.950 a_w (Fig. 1). At this a_w the H values for coffee beans isolates were found to be much higher than those for compost isolates.

Table 2. Hydrolysis zone rates (mean ± standard deviation) of *Thermomyces lanuginosus* coffee and compost isolates on different substrates and at different water activity

Substrates and media	Water activity a_w	Hydrolysis zone rate [mm/day]	
		Coffee isolates	Compost isolates
CMC	0.850	0 ± 0	0 ± 0
	0.900	0 ± 0	0 ± 0
	0.950	1.54 ± 1.28*	1.84 ± 1.11
	0.995	2.65 ± 0.84	3.02 ± 0.31
	0.999	2.63 ± 0.84	2.94 ± 0.30
Xylan	0.850	0 ± 0	0 ± 0
	0.900	0 ± 0	0 ± 0
	0.950	0.93 ± 0.99*	1.88 ± 0.36
	0.995	2.05 ± 0.65	2.21 ± 0.59
	0.999	1.81 ± 0.87	2.01 ± 0.47

* statistically significant differences between coffee and compost isolates at a given substrate and water activity a_w

The differences in FG coefficient values between coffee beans and compost isolates are illustrated in Fig. 2. In coffee beans isolates at 0.950 a_w all FG values were found to be negative (daily growth rates on control medium was higher than on a given substrate). In compost isolates at this a_w only three negative FG values were observed, i.e. on sawdust, filter paper and crystalline cellulose. In coffee isolates at 0.995 a_w negative FG values were observed on sawdust, filter paper and xylan, whereas in compost isolates all FG values at this a_w were found to be positive. In compost isolates positive FG values (except for sawdust) were also observed at 0.999 a_w, whereas in coffee beans isolates positive values at this a_w were found on CMC, crystalline cellulose and on xylan (slightly > 0). In coffee beans isolates the highest FG value was observed on crystalline cellulose at 0.999 a_w. At this a_w the FG value on CMC were also high. In compost isolates, subsequently, the highest FG values were found on CMC at 0.995 and 0.999 a_w.

DISCUSSION

In the available literature, xylan or crystalline cellulose have been used as the substrates for fungal growth [3–5, 12, 15, 24]. Both cellulases and xylanases are extracellular enzymes and, therefore, their diffusion to the media and substrate hydrolysis caused the formation of hydrolysis zones. In the present study, the *T. lanuginosus* isolates grew on all media used but hydrolysis zones were only observed on xylan and CMC. The daily growth and hydrolysis zone rates along with H and FG coefficients have all been found to be dependent on water activity (a_w).

The influence of water activity on fungal growth has been a subject of many studies [9, 19−20, 27]. Very little data on the effect of a_w on the *T. lanuginosus* growth and activity are available in the literature [16]. In this study, the *T. lanuginosus* isolates did not grow on media with a_w < 0.950. This finding has confirmed that the species is not

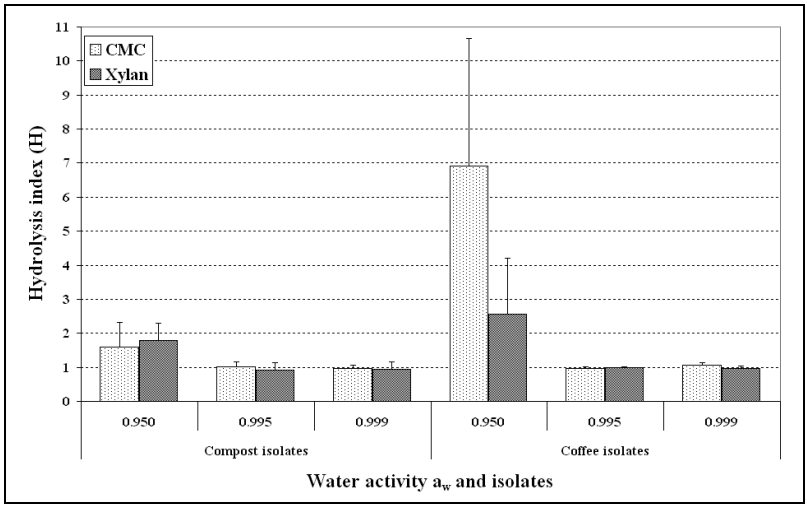

Fig. 1. Average H (hydrolysis) coefficient values (mean ± standard deviation) for *Thermomyces lanuginosus* coffee and compost isolates on CMC and xylan at different water activity a_w

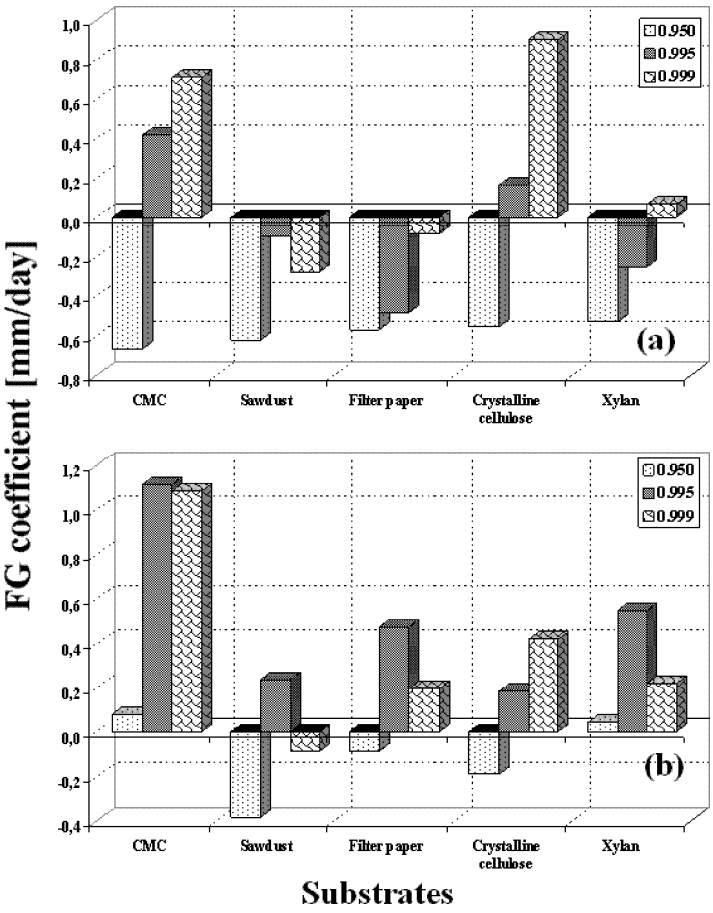

Fig. 2. Average FG (fungal growth) coefficient values for *Thermomyces lanuginosus* coffee (a) and compost (b) isolates on different substrates and at different a_w

xerophilic or xerotolerant by nature. However, some important observations have been performed in the range of 0.950−0.999 a_w.

The highest daily growth and hydrolysis zone rates were mostly obtained at 0.995 a_w, while the lowest values were observed at 0.950 a_w. As a result of the H and FG coefficient analysis and the comparison of coffee and compost isolates, however, the data could be presented in another way. Figure 1 shows the highest H coefficient values on xylan and CMC at 0.950 a_w. The coffee isolates had much higher H coefficients than the compost isolates did. The results indicate that the coffee beans isolates produced endocellulases and, to a lower degree, xylanases at 0.950 a_w. It is explainable, since coffee beans with their hulls provide the environment rich in cellulose and hemicelluloses and with low a_w [23].

Figure 2 displays the differences between coffee beans and compost isolates in FG coefficients on different substrates. This coefficient only relates to fungal growth. The FG profile for coffee beans isolates was found to be different from that for garden compost isolates. In coffee beans isolates the FG values were mostly negative. The negative FG

values meant the lower daily growth rates than that for the control medium (growth inhibition). High positive FG values were observed for CMC and crystalline cellulose at 0.995 and 0.999 a_w. On the contrary, in compost isolates the FG values were found to be mostly positive (with two exceptions). The data indicate that the coffee beans isolates were able to utilize CMC and crystalline cellulose for growth and the highest growth rate was obtained at 0.999 a_w. Subsequently, the compost isolates were able to grow on all substrates with high growth rates obtained on CMC at 0.950 and 0.999 a_w. The high growth rates of compost isolates on cellulose substrates and xylan are explainable, since composts contain various cellulose and hemicellulose wastes.

Thus, coffee beans and composts provide *T. lanuginosus* isolates with various growth and hydrolytic zone rates in the range of 0.950−0.999 a_w. Some isolates can be used in solid state fermentation (0.950 a_w), while others are more useful in bioprocesses requiring high water activity. From this point of view, it is purposeful to continue the isolation of *T. lanuginosus* from various sources, including dry plant materials.

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WZROST SZCZEPÓW *THERMOMYCES LANUGINOSUS* (TSIKL.) WYIZOLOWANYCH Z KOMPOSTU I ZIAREN KAWY NA SUBSTRATACH CELULOZOWYCH I NA KSYLANIE PRZY RÓŻNEJ AKTYWNOŚCI WODY

Badano wpływ różnych aktywności wody a_w (0,850; 0,900; 0,950; 0,995; 0,999) na wzrost grzyba *Thermomyces lanuginosus* (Tsikl.) na pożywkach zawierających różne źródła celulozy (celulozę krystaliczną, karboksymetylocelulozę – CMC, bibułę filtracyjną i trociny) oraz ksylan. W badaniach wykorzystano szczepy *T. lanuginosus* wyizolowane z zapleśniałego ziarna kawy i kompostu. Całkowita inhibicja wzrostu dotyczyła pożywek o aktywności wody $a_w < 0{,}950$. Strefa hydrolizy obserwowana była jedynie na pożywkach o aktywności wody $a_w > 0{,}950$, zawierających ksylan lub CMC. Największe wartości dziennego przyrostu kolonii i strefy hydrolizy obserwowano przy aktywności wody w pożywce wynoszącej 0,995, a najmniejsze przy $a_w = 0{,}950$. Szczepy *T. lanuginosus* wyizolowane z kawy, na pożywkach o $a_w = 0{,}950$ charakteryzowały się 1,7 razy wyższym współczynnikiem hydrolizy celulozy w stosunku do ksylanu. Na podstawie współczynnika wzrostu grzybów (FG) stwierdzono, że szczepy z kawy są zdolne do przetwarzania celulozy krystalicznej i CMC na pożywkach o wysokiej aktywności wody (0,999). Szczepy z kompostu rosły na wszystkich badanych źródłach celulozy i ksylanu, przy czym najlepsze parametry wzrostu uzyskiwano na pożywkach o aktywności wody 0,950 i 0,999.