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*KATARZYNA JANDA*

# LIPOLYTIC ACTIVITY AND RADIAL DAILY GROWTH RATE CHANGES DURING INCUBATION OF *THERMOMYCES LANUGINOSUS* ON NATURAL AND SYNTHETIC FATTY SUBSTRATES

## ZMIANY AKTYWNOŚCI LIPOLITYCZNEJ I DZIENNEGO PRZYROSTU ŚREDNICY KOLONII *THERMOMYCES LANUGINOSUS* PODCZAS HODOWLI NA NATURALNYCH I SYNTETYCZNYCH SUBSTRATACH TŁUSZCZOWYCH

Department of Microbiology and Environmental Biotechnology
Faculty on Environmental Studies and Agriculture
Agriculture University of Szczecin
Słowackiego St. 17, 71-434 Szczecin, Poland
e-mail: kjanda@agro.ar.szczecin.pl
Head: prof. dr hab. *A. Nowak*

*The aim of the study was to compare lipolytic activity and radial growth rate changes during the incubation of Thermomyces lanuginosus strains on natural (sunflower oil, soybean oil, rapeseed oil, and corn oil) and synthetic (tributyrin, Tween 20, Tween 40, Tween 60, Tween 80, and Tween 81) fatty substrata. The general lipolytic activity index decreased on natural substrata and increased on synthetic substrata during a five-day incubation period. The general daily growth rate changes were found to be similar on both natural and synthetic fatty substrates.*

## INTRODUCTION

*Thermomyces lanuginosus* is a ubiquitous thermophilic fungus, with minimal, optimal and maximal temperatures ranging between 25-37, 40-55, and 55-63°C, respectively [1, 9]. The fungus produces a variety of enzymes, e.g. celulases, amylases, xylanases, proteases, and lipases [14, 15, 18]. Lipases have been widely used for biotechnological applications in food and dairy industry, oil processing, production of surfactants, pharmaceuticals and cosmetics [7, 14-16].

In a previous study [10], general data on the lipolytic activity of *Thermomyces lanuginosus* strains isolated from different natural sources were presented. This study was to compare lipolytic activity index and daily radial growth rate changes during the incubation of the above-mentioned strains on natural (sunflower oil, soybean oil, rapeseed oil, and corn oil) and synthetic (tributyrin, Tween 20, Tween 40, Tween 60, Tween 80, and Tween 81) fatty substrata.

## MATERIALS AND METHODS

Altogether, 144 *Thermomyces lanuginosus* strains were used. The strains were isolated from raw coffee beans (50 strains), mushroom compost (42 strains), biohumus (26 strains), decayed leaves (14 strains), garden compost (6 strains), and hazelnuts (6 strains). Methods for fungi isolation and identification were those of Janda [10].

A semiquantitative test to evaluate fungal lipolytic activity was employed. The substrata tested were as follows: sunflower oil, soybean oil, rapeseed oil, corn oil, tributyrin (glyceryl tributyrate), Tween 20 (lauric acid ester), Tween 40 (palmitic acid ester), Tween 60 (stearic acid ester), Tween 80, and Tween 81 (oleic acid esters). Oils were commercially available products, while tributyrin and tweens were provided by Sigma-Aldrich. The media by *Kunert* and Lysek [11] and *Sierra* [17] were used to evaluate fungal lipolytic activity on natural and synthetic fatty substrates, respectively. The original concentrations of natural and synthetic fatty substrata in the media were 1.5 and 1%, respectively. The media containing fatty substrata were homogenized, autoclaved at 121°C for 15 min., and poured into Petri dishes. Five-day fungal cultures on PDA with 0,3% yeast extract were used as inoculum. Small aerial mycelium/spore pieces were picked up from this medium with a syringe needle and inoculated in the center of the test media dishes. The inoculated dishes were incubated at 55°C for 5 days in the dark. The experiment was performed in triplicate.

The lipolytic activity on natural substrates resulted in the change of the medium color from salmon to green or blue due to the liberation of fatty acids and pH decrease. Subsequently, the lipolytic activity on tweens was observed as a visible precipitation zone around the colony due to the formation of crystals of the calcium salt of the fatty acid liberated by lipolytic enzymes. The lipolytic activity on tributyrin was observed as a clearing zone around the colony. Colony diameters and precipitation/clearing/color-changed zones were measured at 24-hour intervals during a 5-day incubation period [6].

The lipolytic activity index (LAI) was the hydrolysis zone diameter/colony diameter ratio [5, 6]. The LAI = 1 meant no lipolytic activity around the colony. The lipolytic activity underneath the colony was not taken for consideration. The daily growth rate (DGR) was the daily colony diameter increase (mm/24 h). LAI and DGR values were calculated for each incubation day. Statistical analysis (means and standard deviation calculations, simple linear correlations) was performed with the Statistica 5.1 program at $p \leq 0.05$.

## RESULTS

The changes in lipolytic activity index (LAI) and daily growth rate (DGR) during the incubation of *Thermomyces lanuginosus* strains on natural and synthetic fatty substrata are presented in tables I and II, respectively.

As regards fungal lipolytic activity on natural substrata, the highest LAI values were observed during the first two days of incubation on sunflower oil. During the first two days, high LAI values also were observed on rapeseed oil. The lipolytic activities on these oils considerably decreased during longer incubation. A high LAI value was noticed during the first day of incubation on corn oil. However, no lipolytic activity around the colonies on this oil was detected during longer incubation (LAI = 1). The low lipolytic activity was observed up to the 3rd incubation day on rapeseed oil. No lipolytic activity (LAI = 1) around the colony on this oil was detected during longer incubation.

The highest DGR values were observed on rapeseed oil. The DGR values increased up to the 3rd day and decreased during longer incubation. Similar DGR changes were found for the other natural fatty substrata. The lowest DGR values were observed on sunflower oil.

Table I. Changes in lipolytic activity index (LAI) and daily growth rate (DGR) during the incubation of *Thermomyces lanuginosus* strains on natural fatty substrates
Zmiany indeksu aktywności lipolitycznej (LAI) i dziennych przyrostów średnicy kolonii (DGR) *Thermomyces lanuginosus* podczas hodowli na naturalnych susbtratach tłuszczowych

| Incubation day | Corn oil | | Rapeseed oil | | Sunflower oil | | Soybean oil | |
|---|---|---|---|---|---|---|---|---|
| | LAI | DGR | LAI | DGR | LAI | DGR | LAI | DGR |
| 1 | 1.6 ± 0.5 | 4.0 ± 1.1 | 1.3 ± 0.2 | 7.9 ± 1.1 | 2.1 ± 1.0 | 4.1 ± 0.9 | 1.6 ± 0.6 | 6.8 ± 0.7 |
| 2 | 1.0 ± 0.0 | 9.1± 3.2 | 1.2 ± 0.1 | 15.8 ± 2.1 | 2.1 ± 0.6 | 6.0 ± 3.6 | 1.7 ± 0.5 | 7.8 ± 2.9 |
| 3 | 1.0 ± 0.0 | 9.8 ± 4.5 | 1.1 ± 0.0 | 17.1 ± 1.8 | 1.8 ±0.4 | 6.4 ± 2.1 | 1.5 ± 0.5 | 7.2 ± 4.8 |
| 4 | 1.0 ± 0.0 | 9.9 ± 1.2 | 1.0 ± 0.1 | 10.6 ± 3.9 | 1.7 ± 0.4 | 3.3 ± 1.9 | 1.3 ± 0.4 | 4.4 ± 1.4 |
| 5 | 1.0 ± 0.0 | 9.7 ± 2.4 | 1.0 ± 0.1 | 11.8 ± 2.8 | 1.7 ± 0.4 | 3.2 ± 1.3 | 1.2 ± 0.3 | 4.6 ± 2.9 |

*Mean ± standard deviation*

Table II. Changes in lipolytic activity index (LAI) and daily growth rate (DGR) during the incubation of *Thermomyces lanuginosus* strains on synthetic fatty substrates
Zmiany indeksu aktywności lipolitycznej (LAI) i dziennych przyrostów średnicy kolonii (DGR) *Thermomyces lanuginosus* podczas hodowli na syntetycznych susbtratach tłuszczowych

| Incubation day | Tributyrin | | Tween 20 | | Tween 40 | | Tween 60 | | Tween 80 | | Tween 81 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LAI | DGR | LAI | DGR | LAI | DGR | LAI | DGR | LAI | DGR | LAI | DGR |
| 1 | 1.4 ± 0.3 | 3.0 ± 0.8 | 1.0 ± 0.0 | 5.6 ± 1.1 | 1.0 ± 0.0 | 8.0 ± 1.7 | 1.0 ± 0.0 | 9.3 ± 1.8 | 1.0 ± 0.0 | 6.9 ± 2.7 | 1.0 ± 0.0 | 4.1 ± 0.9 |
| 2 | 1.5 ± 0.4 | 1.2 ± 0.6 | 1.0 ± 0.0 | 9.7 ± 2.1 | 1.0 ± 0.0 | 13.3 ±1.3 | 1.0 ± 0.0 | 14.4 ± 1.5 | 1.0 ± 0.0 | 9.2 ± 2.8 | 1.0± 0.0 | 5.1 ± 1.3 |
| 3 | 1.6 ± 0.6 | 1.6 ± 0.6 | 1.0 ± 0.0 | 10.0 ± 3.3 | 1.0 ± 0.0 | 11.7 ± 2.2 | 1.0 ± 0.0 | 12.4 ± 1.1 | 1.1 ± 0.2 | 10.0 ± 3.4 | 1.3± 0.2 | 6.3 ± 1.5 |
| 4 | 1.5 ± 0.5 | 2.5 ± 1.3 | 1.0 ± 0.0 | 9.1 ± 2.5 | 1.2 ± 0.2 | 9.7 ± 3.1 | 1.2 ± 0.2 | 13.2 ± 1.5 | 1.1 ± 0.1 | 11.2 ± 2.9 | 1.5± 0.1 | 7.8 ± 1.8 |
| 5 | 1.6 ± 0.5 | 2.8 ± 1.0 | 1.0± 0.0 | 6.7 ± 2.2 | 1.3 ± 0.2 | 6.8 ± 3.8 | 1.4 ± 0.1 | 9.6 ± 2.2 | 1.1 ± 0.1 | 9.7 ± 4.0 | 1.4 ± 0.2 | 8.7 ± 2.0 |

*Mean ± standard deviation*

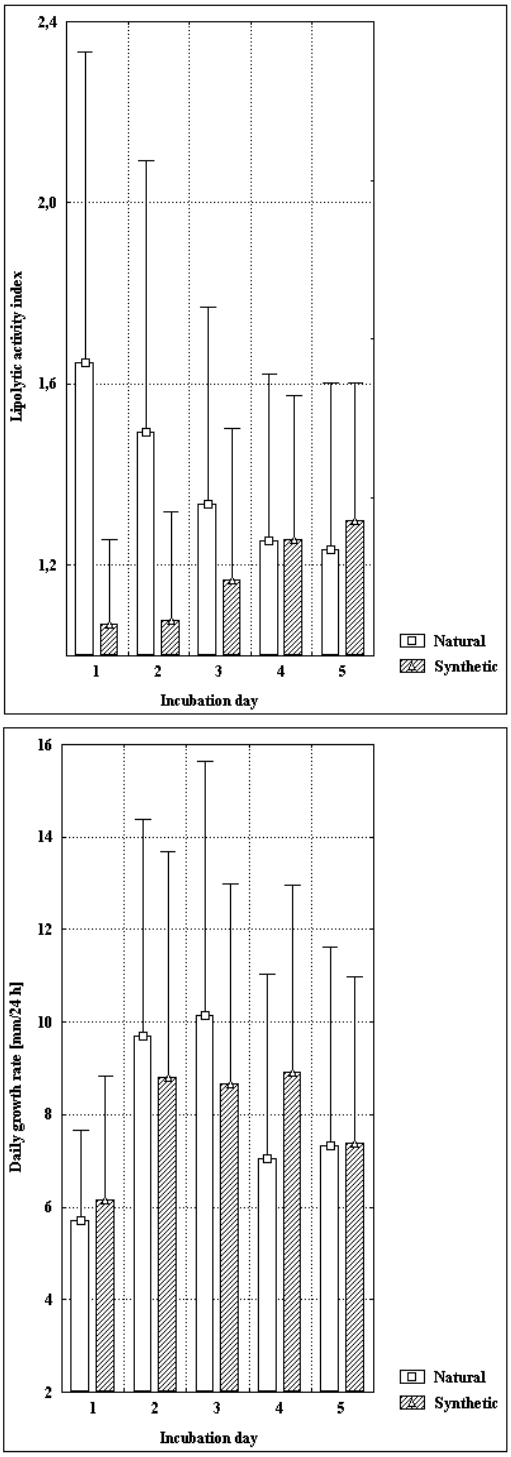


Fig. 1. General (mean) changes in lipolytic activity index (LAI) and daily growth rate (DGR) during the incubation of *Thermomyces lanuginosus* strains on natural and synthetic fatty substrates. Zmiany indeksu aktywności lipolitycznej (LAI) i dziennych przyrostów średnicy kolonii (DGR) szczepów *Thermomyces lanuginous* podczas hodowli na naturalnych i syntetycznych substratach tłuszczowych.

As regards fungal lipolytic activity on synthetic substrata, the highest LAI values were observed on tributyrin. On this substrate, the LAI values were found to be high during the whole incubation period. The LAI changes for tweens were found to be different from that for tributyrin. Except for Tween 80, no lipolytic activity was observed around the colonies up to the 3rd day of incubation. Lipolytic activities on tweens occurred between the 4th and 5th day of incubation.

The highest DGR values were observed on Tween 60 and Tween 40, and the lowest values were on tributyrin. Three DGR patterns (types of changes during a 5-day incubation period) could be distinguished. In the first pattern, DGR values first increased up to 2nd day and decreased during longer incubation (Tween, 20, Tween 40, and Tween 60). In the second pattern, DGR values continuously increased during incubation (Tween 80 and Tween 81). Finally, in the third pattern DGR values were low during the whole incubation period (tributyrin).

General (mean) changes in lipolytic activity index (DGR) and daily growth rate (DGR) during the incubation of *Thermomyces lanuginosus* strains on natural and synthetic fatty substrata are illustrated in Figure 1. The general LAI change on natural substrata considerably differed from that on synthetic substrata. The LAI values on natural substrata decreased, while the LAI values on synthetic substrata increased during the incubation period. The general DGR changes were found to be similar on natural and synthetic substrata.

Negative correlations between DGR and LAI values were found for fungal growth on both natural ($r = -0.45$) and synthetic ($r = -0.38$) substrata.

## DISCUSSION

In general, one LAI pattern (type of changes during a 5-day incubation period) and one DGR pattern were observed on natural fatty substrata. The LAI decrease was characteristic during incubation. The high standard deviations resulted from the differences in lipolytic activity among strains isolated from different sources [10]. The quantitative differences in lipolytic activity and daily growth rate changes were also found to be considerable on individual natural substrata. In addition, the negative correlation between fungal lipolytic activity and daily growth rate was observed. *Ilnicka-Olejniczak* et al. [6] and *Magan* et al. [13] also observed negative relationships between these parameters. *Janda* [10] suggested that the differences in fungal lipolytic activities could be explained with fatty acid compositions of the substrata used. It was shown that the more double bonds in 18-carbon fatty acids the higher inhibitory effect against fungi examined [3, 4]. Thus, the low lipolytic activity on rapeseed oil could be explained with a high quantity of linoleic acid released during oil hydrolysis. On this oil, however, the DGR change and values were found to be the clearest and highest, respectively. This suggests that the fungal growth was good on the test medium and rather not inhibited by the fatty substratum or/and its hydrolysis products. The problem should be explained in further experiments.

No data on the effect of different synthetic fatty substrata on the *Thermomyces lanuginosus* growth have been found in the available literature. However, results obtained for other fungal species [2, 5, 8, 12, 21, 22] demonstrated that the effects of synthetic fatty substrata on the fungi were strain-, species- and substrate-specific (from highly stimulatory to highly inhibitory). In the present study, the *Thermomyces lanuginosus* lipolytic activity on tributy-

rin was the highest and rather constant during incubation. Subsequently, the lipolytic activity on tweens had one pattern (except for Tween 20, on which no activity around the colonies was observed) but was associated with three DGR patterns. This reflects the differentiated response of the examined strains to individual synthetic fatty substrata. Further studies on determination of the factors affecting the *Thermomyces lanuginosus* lipolytic activity are of great significance, since the species is widely used for biotechnological purposes.

K. Janda

LIPOLYTIC ACTIVITY AND RADIAL GROWTH RATE CHANGES DURING INCUBATION OF *THERMOMYCES LANUGINOSUS* ON NATURAL AND SYNTHETIC FATTY SUBSTRATES

Summary

The study was to compare lipolytic activity and radial growth rate changes during the incubation of *Thermomyces lanuginosus* strains on natural (sunflower oil, soybean oil, rapeseed oil, and corn oil) and synthetic (tributyrin, Tween 20, Tween 40, Tween 60, Tween 80, and Tween 81) fatty substrata. The strains were isolated from different natural sources and incubated at 55°C on solid media containing natural and synthetic fatty substrata. The general (mean) lipolytic activity index decreased on natural substrata and increased on synthetic substrata during a five-day incubation period. The general daily growth rate changes were found to be similar on both natural and synthetic fatty substrata. However, the lipolytic activity index and daily growth rate changes were found to be highly differentiated on synthetic fatty substrata. Further studies on determination of the factors affecting the *Thermomyces lanuginosus* lipolytic activity are needed, due to the species is widely used for biotechnological purposes.

K. Janda

ZMIANY AKTYWNOŚCI LIPOLITYCZNEJ I DZIENNEGO PRZYROSTU ŚREDNICY KOLONII *THERMOMYCES LANUGINOSUS* PODCZAS HODOWLI NA NATURALNYCH I SYNTETYCZNYCH SUBSTRATACH TŁUSZCZOWYCH

Streszczenie

Celem badań było porównanie zmian aktywności lipolitycznej i dziennego przyrostu średnicy kolonii podczas hodowli szczepów *Thermomyces lanuginosus* na naturalnych (olej słonecznikowy, sojowy, rzepakowy i kukurydziany) i syntetycznych (trójbutyryna, Tween 20, Tween 40, Tween 60, Tween 80 i Tween 81) substratach tłuszczowych. Szczepy wyizolowano z naturalnych źródeł i hodowano w temperaturze 55°C na pożywkach stałych zawierających wyżej wymienione substraty tłuszczowe. Wartości średnie współczynnika aktywności lipolitycznej miały tendencję malejącą i rosnącą podczas hodowli odpowiednio na substratach naturalnych i syntetycznych. Zmiany średnich wartości dziennego przyrostu średnicy kolonii były podobne na obu rodzajach substratów. Jednakże zróżnicowanie zmian współczynnika aktywności lipolitycznej i dziennego przyrostu średnicy kolonii na substratach syntetycznych było znaczne. Dalsze badania mające na celu określenie czynników wpływających na aktywność lipolityczną *Thermomyces lanuginosus* mają duże znaczenie z biotechnologicznego punktu widzenia.

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