ELSEVIER

International Biodeterioration & Biodegradation 55 (2005) 149–152

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

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Note

The lipolytic activity of *Thermomyces lanuginosus* strains isolated from different natural sources

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Received 11 August 2004; accepted 20 September 2004

Abstract

The ability of 144 *Thermomyces lanuginosus* wild strains isolated from biohumus, mushroom and garden composts, decayed leaves, hazelnuts, and raw coffee beans to hydrolyze synthetic (tributyrin, Tween 20, Tween 40, Tween 60, and Tween 80) and natural fatty substrates (sunflower, soybean, rapeseed and corn oil) was evaluated, and whether the lipolytic activity depended on the isolation source determined. All strains incubated at 55 °C on solid media containing 1% synthetic and 15% natural fatty substrates hydrolyzed both types of substrate. Mean lipolytic activity on natural substrates was significantly higher than on synthetic substrates. The highest mean activity index was noted after growth on sunflower oil, followed by soybean oil and tributyrin; indices on other fatty substrates were low. Strains isolated from raw coffee beans showed the highest mean index, followed by those from biohumus and garden compost; the lowest index being for strains isolated from hazelnuts. Thus, the lipolytic activity index depended on the specific fatty substrate and the source of the isolates.

Keywords: *Thermomyces lanuginosus*; Lipolytic activity; Tributyrin; Tween; Vegetable oils

1. Introduction

Among the *Eukaryota*, only thermophilic fungi can grow and develop at high temperatures of 50–60 °C (Cooney and Emerson, 1964; Magan, 1997). These fungi are widely distributed in all climatic zones. The thermophilic fungi have been isolated from self-heating plant substrates, leaving green leaves, decayed leaves, seeds, raw coffee beans, grain, groundnut, palm kernels, bagasse, peat, cocoa, composts, animal nests, hair, tobacco and tobacco products, atmosphere, different soils and man-made substrates and environments like plastics and plasticizers, aircraft fuel tanks and coal spoil tips (Shekhar Sharma, 1989; Mouchacca, 1997; Ryckeboer et al., 2003). Heat-stable enzymes produced by thermophilic fungi have potential applications in different branches of industry (Maheshwari et al., 2000; Kirk et al., 2002).

One of the most common thermophilic fungi is *Thermomyces lanuginosus* (Cooney and Emerson, 1964; Mouchacca, 1997). Among such fungi, this species has the highest maximum temperatures for growth and development. Its minimum, optimum and maximum temperatures are 25–37, 40–55 and 55–63 °C, respectively. The fungus produces cellulolytic, amylolytic, xylanolytic, proteolytic and lipolytic enzymes (Haasum et al., 1991; Hasnain et al., 1992; Puchart et al., 1999; Maheshwari et al., 2000). Lipases have been widely used for biotechnological applications in dairy industry, oil processing, production of surfactants and preparation of pharmaceuticals (Reetz, 2002).

The present study was to evaluate the ability of *T. lanuginosus* wild strains to hydrolyze synthetic and natural fatty substrates, i.e. tributyrin (glyceryl tributyrate), Tween 20 (lauric acid ester), Tween 40 (palmitic

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0964-8305/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibiod.2004.09.003

acid ester), Tween 60 (stearic acid ester), and Tween 80 (oleic acid ester), and sunflower oil, soybean oil, rapeseed oil, and corn oil. The study was also to determine whether the lipolytic activity depends on the isolation source.

2. Materials and methods

Altogether, 144 *T. lanuginosus* strains were used. They were isolated from raw coffee beans (50 strains), mushroom compost (42), biohumus (26), decayed leaves (14), garden compost (6) and hazelnuts (6) on rose bengal agar, and identified according to their macro- and micro-morphological characteristics using selected taxonomic monographs (Cooney and Emerson, 1964; Domsch and Gams, 1993). Pure cultures were incubated on PDA medium with 0.3% yeast extract, which has been proved to be the best for growth of this fungus (Haasum et al., 1991).

A semiquantitative test to evaluate fungal lipolytic activity was employed using the following: tributyrin (glyceryl tributyrate), Tween 20 (lauric acid ester), Tween 40 (palmitic acid ester), Tween 60 (stearic acid ester) and Tween 80 (oleic acid ester), all from Sigma-Aldrich, and the commercially available food products sunflower, soybean, rapeseed and corn oil. The media of Sierra (1957) and Kunert and Lysek (1987) were used to evaluate fungal lipolytic activity on respectively synthetic and natural fatty substrates. The concentrations of the synthetic and natural substrates in the media were 1% and 1.5%, respectively. The media with fatty substrates were homogenized, sterilized and poured into Petri dishes. Small pieces of aerial mycelium/spores were transferred with a syringe needle from 5-day *T. lanuginosus* cultures on potato dextrose agar (PDA) containing 0.3% yeast extract to the centre of the triplicate test plates, which were incubated at 55 °C for 5–10 days in the dark.

Lipolytic activity on the Tween media was evident as a visible precipitation zone round the colony resulting from the formation of crystals of the calcium salt of the fatty acid liberated; on tributyrin as a clearing zone; and on natural substrates as a change in colour of the medium from salmon to green or blue due to liberation of fatty acids and a consequent decrease in pH. Colony diameters and precipitation/clearing/colour-changed zones were measured at 24-h intervals throughout incubation.

The lipolytic activity index was determined from the ratio of the diameter of the hydrolysis zone:colony diameter ratio (Ho and Foster, 1972; Hellgren and Vincent, 1980). For each substrate and strain, the mean activity index values average for the whole incubation period was used in statistical analysis. One-way ANOVA and least significant difference (LSD) tests were employed, using the Statistica 5.1 program (signficance level, $p \leqslant 0.05$).

3. Results and discussion

Lipolytic activity in *T. lanuginosus* has been the subject of many studies, and lipases produced by the fungus have many practical applications in industry (Schmidt-Dannert, 1999; Sharma et al., 2001; Saxena et al., 2003). However, previous studies have been performed only with single-source strains. This is the first study, however, in which lipolytic activity among strains isolated from various sources has been compared.

The study included 1188 observations: 684 observations on synthetic and 504 on natural fatty substrates. All strains showed lipolytic activity during growth on all substrates tested, but the mean lipolytic activity (Table 1) when the strains were grown on natural substrates (1.41 ± 0.54) was significantly higher than on synthetic substrates (1.18 ± 0.31). The highest mean lipolytic activity index for a specific substrate was recorded after growth on sunflower oil (Table 1), followed by soybean oil and tributyrin. On the other substrates, especially rapeseed and corn oils and on Tween 80 and 20, the values were much lower. The differences in lipolytic activity between the first three substrates mentioned and the others are clearly demonstrated in Table 2.

It is reasonable to seek explanations for the differences in lipolytic activity in the differences in fatty acid composition of the substrates tested. Sunflower, soybean and corn oil show similarities in their fatty acid composition, with more linoleic acid (18:2) dominating over oleic acid (18:1) and other fatty acids, but there is a much higher quantity of linoleic acid (18:3) in rapeseed oil (Ratledge, 1997). It has been shown that the more the double bonds in 18-carbon fatty acids the higher the inhibitory effect towards fungi (Garg et al., 1985; Garg

Table 1
Mean lipolytic activity indices of *T. lanuginosus* grown on natural and synthetic fatty substrates

Substrate	Mean lipolytic activity index	Number of observations	SD*
Sunflower oil	1.90	126	0.60
Soybean oil	1.50	144	0.51
Tributyrin	1.41	198	0.44
Tween 40	1.14	126	0.19
Tween 60	1.13	90	0.20
Rapeseed oil	1.10	108	0.14
Corn oil	1.08	126	0.27
Tween 80	1.05	144	0.10
Tween 20	1.03	126	0.05

*Standard deviation.