Lipase activity of yeasts and yeast-like fungi functioning under natural conditions

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It was ascertained that lipase activity was exhibited by the following species of yeasts and yeast-like fungi: *Rhodotorula rubra*, *Rhodotorula minuta*, *Candida lipolytica*, *Candida parapsilosis*, *Candida valida*, *Debaryomyces vanriji*, *Debaryomyces hansenii*, *Kluyveromyces marxianus*, *Pichia burtonii*, *Pichia kluyveri*, *Geotrichum fermentans*. A quantitative analysis showed that the strains of *Geotrichum fermentans* and *Candida lipolytica* possessed the highest lipase activity – 8.2 ± 0.09 and 7.9 ± 0.03 u/ml, respectively.

The yeasts and yeast-like fungi were found to be able to adapt themselves to substrates rich in fat under anthropogenic impact.

Key words: lipase, yeasts, yeast-like fungi

INTRODUCTION

Yeasts and yeast-like fungi are a specific group of microorganisms widespread on various substrates. Their ecological peculiarities are little investigated in Lithuania. It is assumed that yeasts and yeast-like fungi can adapt to substrates rich in fat under conditions of anthropogenic impact. This characteristic has become urgent due to utilization of industrial waste. The main index of their activity is excreted lipolytic enzymes.

Significant attention is paid to yeasts of the family *Saccharomycetaceae* as producers of lipases. It is established that under certain conditions *Saccharomyces cerevisiae*, *Debaryomyces hansenii*, *Debaryomyces kloeckeri* and *Lipomyces starkeyi* produce lipases [1–4]. The highest lipolytic activity of the strain *Hansenula anomala* [5] was observed after 48 h growth in the medium consisting of 1% glucose, 0.5% peptone, 0.3% yeast extract and corn extract (pH 5.5; 28 °C). When *Saccharomycopsis lipolytica* [6] was cultivated, conditions for lipase synthesis most optimal in the medium containing oil (pH 8; 30 °C). Producers of lipases are yeasts of the family *Cryptococcaceae*: *Candida antarctica*, *C. deformans*, *C. rugosa* and *C. lipolytica* [7–10]. Yeast-like fungi of the genus *Geotrichum* are reported also to be producers of lipases [11].

The main objective of the work was primary screening of the lipolytic activity of yeasts and yeast-like fungi and a quantitative analysis of the most active strains.

MATERIALS AND METHODS

To isolate yeasts and yeast-like fungi, we used a glucose–ammonium medium of the following composition (g/l): glucose – 20; $(NH_4)_2SO_4$ – 5; KH_2PO_4 – 0.85; K_2HPO_4 – 0.15; $MgSO_4$ – 0.5; NaCl – 0.1; $CaCl_2$ – 0.1, and agar – 20.

Identification of yeasts and yeast-like fungi was performed taking into account the morphological, physiological and biochemical peculiarities following the Kreger-van Rij identification system [12]. For quantitative screening of lipase activity of yeasts and yeast-like fungi, the following media were used (%):

1. Olive oil – 1.0; K_2HPO_4 – 0.25; $(NH_4)_2SO_4$ – 0.13; $(NH_2)_2CO$ – 0.13; $MgSO_4 \times 7H_2O$ – 0.05; yeast extract – 0.05; agar – 1.5, and the pH was adjusted with Na_2CO_3 (pH 7 and pH 9.5).

2. Tributirate – 1.0; K_2HPO_4 – 0.25; NaH_2PO_4 – 0.25; $(NH_4)_2SO_4$ – 0.1; $MgSO_4 \times 7H_2O$ – 0.05; yeast extract – 0.05; agar – 1.5; Victoria Blue – 0.01 (pH 5.5 and pH 7).

A quantitative analysis of lipolytic activity was performed by the titration method using olive oil as a substrate [13].

RESULTS AND DISCUSSION

There were 155 strains of yeasts and yeast-like fungi isolated from different substrates: natural and synthetic polymeric materials, waste and sewage of various industrial production and natural substrates. It was established that according to their morphological, phy-

Table. **Qualitative screening of lipase activity of yeasts and yeast-like fungi on different media (number of strains)**

Generic names of yeasts and yeast-like fungi	Number of strains investigated	Medium with tributyrate (pH 5.5; 27 °C)	Medium with tributyrate (pH 7; 27 °C)	Medium with olive oil (pH 7; 27 °C)	Medium with olive oil (pH 9.5; 27 °C)
Saccharomyces Meyen ex Reess	13	1	1	1	0
Rhodotorula Harrison	12	4	3	2	2
Candida Berkhout	25	5	3	3	1
Cryptococcus Kutzing	2	0	1	1	0
Arthroascus von Arx	1	1	1	0	0
Geotrichum Link	4	1	1	1	1
Lodderomyces van der Walt	1	0	0	0	0
Hansenula H. et P. Sydov	4	0	0	0	0
Citeromyces Santa maria	1	0	0	0	0
Zygosaccharomyces Barker	9	1	1	0	0
Kloeckera Janke	1	0	0	0	0
Aureobasidium Viala et Bayer	2	1	1	1	0
Debaryomyces Lodder et Kreger-van Rij	8	4	1	3	2
Hanseniaspora Zikes	4	0	0	0	0
Kluyveromyces van der Walt	16	2	1	0	0
Lipomyces Lodder et Kreger-van Rij	5	1	1	1	0
Pichia Hansen	38	3	2	2	1
Saccharomycodes Hansen	2	0	0	0	0
Saccharomycopsis Schionning	4	2	1	2	1
Torulaspora Lindner	3	0	0	0	0
Total	155	26	18	17	8

siological and biochemical peculiarities the isolated strains belonged to 20 genera (Table) and 49 species.

The results of qualitative screening of lipolytic activity revealed that while cultivating yeasts and yest-like fungi in the acidified (pH 5.5) tributirate medium at 27 °C, even 26 strains showed lipolytic activity. The most active yeasts were from the genera *Candida*, *Rhodotorula*, *Kluyveromyces*, *Pichia* and *Saccharomycopsis*. When yeasts and yeast-like fungi were cultivated in a neutral (pH 7) tributirate medium at 27 °C, 18 strains exhibited lipolytic activity. The most active lipase producers were observed from genera *Rhodotorula*, *Candida*, *Pichia* and *Geotrichum*. In a neutral olive oil medium (pH 7; 27 °C), lipolytic activity was exhibited by 17 strains of yeasts and yeast-like fungi, meanwhile in the alkaline medium (pH 9.5; 27 °C), only 8 strains showed this enzymatic activity.

Based on the results of qualitative screening, for quantative analysis of lipase production the following strains were chosen: *Rhodotorula rubra* (Demme) Lodder, *Rhodotorula minuta* (Saito) Harrison, *Candida lipolytica* (Harrison) Diddens et Lodder, *Candida parapsilosis* (Ashford) Langeron, *Candida valida* (Leberle) van Uden et Buckley, *Debaryomyces vanriji* Abadie, Pignal et Jacob, *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij, *Kluyveromyces marxianus* (Hansen) van der Walt, *Pichia burtonii* Boidin, Pignal, Lehodey, Vey et Abadie, *Pichia kluyveri* Bedford ex Kudriavzev and *Geotrichum fermentans (*Diddens et Lodder) von Arx.

The results showed that after cultivation of yeasts and yeast-like fungi in the liquid medium with olive oil (pH 7) at 27 °C, the highest lipolytic activity was exhibited by the strains of *Geotrichum fermentans* and *Candida lipolytica*; the activity of the *G. fermentans* strain was 8.2 ± 0.09 u/ml and that of the

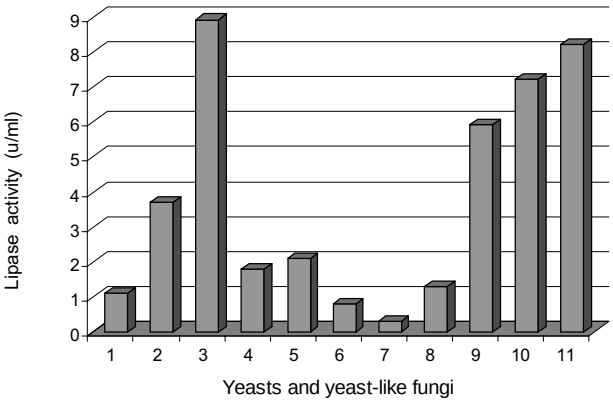

Figure. Lipase activity (u/ml) of yeasts and yeast-like fungi after a 5-day growth in the liquid medium with olive oil (pH 7, 27 °C): 1 – *Rhodotorula rubra*; 2 – *Rhodotorula minuta*; 3 – *Candida lipolytica*; 4 – *Candida parapsilosis*; 5 – *Candida valida*; 6 – *Debaryomyces vanriji*; 7 – *Debaryomyces hansenii*; 8 – *Kluyveromyces marxianus*; 9 – *Pichia burtonii*; 10 – *Pichia kluyveri*; 11 – *Geotrichum fermentans*

C. lipolytica strain 7.9 ± 0.03 u/ml. The lower activity was observed in *Pichia kuyveri* and *Pichia burtonii* cases – 7.2 ± 0.2 and 5.9 ± 0.03 u/ml, respectively. The lowest lipolytic activity was determined for the strain *Debaryomyces hansenii* and was equal only to 0.3 ± 0.01 u/ml. The activity of other strains did not exceed 4 u/ml (Figure).

The obtained results indicated that yeasts and yeast-like fungi under anthropogenic impact could adapt themselves to substrates rich in fat. Lipase activity was high in yeast-like fungi from the genus *Geotrichum*. It was possibly determined by the morphological, physiological and biochemical peculiarities of the microorganisms. Moreover, microbial metabolic processes and the amount of enzymes catalyzing them greatly depended on growth medium composition and growth conditions.

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A. Paškevičius

GAMTINĖMIS SĄLYGOMIS FUNKCIONUOJANČIŲ MIELIŲ IR MIELIAGRYBIŲ LIPAZINIS AKTYVUMAS

S a n t r a u k a

Išaiškinta, kad lipaziniu aktyvumu pasižymėjo šios mielių ir mieliagrybių rūšys: *Rhodotorula rubra*, *Rhodotorula minuta*, *Candida lipolytica*, *Candida parapsilosis*, *Candida valida*, *Debaryomyces vanriji*, *Debaryomyces hansenii*, *Kluyveromyces marxianus*, *Pichia burtonii*, *Pichia kluyveri*, *Geotrichum fermentans*. Kiekybinės analizės rezultatai parodė, kad didžiausiu lipaziniu aktyvumu pasižymėjo *Geotrichum fermentans* ir *Candida lipolytica* rūšių štamai, kurių aktyvumas buvo atitinkamai 8,2 ± 0,09 vnt/ml ir 7,9 ± 0,03 vnt/ml.

Gauti tyrimų rezultatai parodė, kad antropogeninio poveikio sąlygomis mielės ir mieliagrybiai prisitaiko prie riebalų turtingų substratų.