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THERMOPHILIC FUNGI WITH GLYCOSIDASE AND PROTEOLYTIC ACTIVITIES

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The directed search for extremophilic producers in order to obtain hydrolytic enzymes with increased thermal stability has an unconditional practical potential for use in the food and feed industry to improve the quality of the final product. ***The aim*** *of the work was to study the ability of collection strains of thermophilic fungi to show α-L-rhamnosidase, α-galactosidase, cellulase, β-mannanase, keratinase and caseinolytic activity.* ***Methods****. Micromycetes were grown under submerged conditions in test tubes at 42 °C for 8–14 days. Enzymatic activities were studied in the culture liquid supernatant. p-Nitrophenyl-α-D-galactopyranoside, naringin, guar gum galactomannan and Na-carboxymethylcellulose were used as substrates to determine α-galactosidase, α-L-rhamnosidase, β-mannanase and cellulase activities, respectively. Casein and crushed defatted feathers were served as substrates for the determination of proteolytic activity.* ***Results.*** *The enzymatic activity of 50 strains of micromycetes belonging to 17 species was investigated. The studied group showed high activity: 94 % of the strains had at least one, 34 % – two, 26 % – from three to five enzyme activities. The most active keratinase producers were Thielavia terrestris 1920 and 62, Rhizomucor tauricus 1909, Chrysosporium thermophilum 2050, Thermoascus thermophilus 92 and Thermoascus aurantiacus 2052 (10–26 U/mL). The highest α-L-rhamnosidase activity was observed in T. terrestris 62 (0.35 U/mL), and carboxymethylcellulase activity −in Thermomyces lanuginosus 2046. Six strains showed α-galactosidase (0.05–0.2 U/mL) and four strains − β-mannanase (5–130 U/mL) activity.* ***Conclusions.*** *As a result new strains producing proteolytic and glycolytic enzymes were isolated among thermophilic micromycetes. Soil thermophilic micromycetes can be used as producers of proteolytic and glycolytic enzymes. Of particular interest are the cultures of Acremonium thermophilum 1963, Corynascus thermophilum 2050, C. sepedonium 1899 and 65068, T. thermophilus 1946, which are capable of producing complexes of proteases and glycosidases in the culture liquid. This indicates that these strains are promising for use as destructors in various technologies processing of complex raw materials.*

Keywords: micromycetes, thermophiles, α-L-rhamnosidase, α-galactosidase, carboxymethylcellulase, β-mannanase, keratinase, proteolytic activities.

The enormous biotechnological potential of micromycetes in the field of obtaining biologically active compounds is explained by the wide range of their enzymatic activity in relation to various, including hard-to-reach, plant and animal substrates, such as cellulose and hemicellulose, rhamno- and galactoglycosides, collagen and keratin [1].

Polysaccharides, such as cellulose, hemicelluloses and pectin, which constitute up to 70 % of plant biomass, used in many industrial fields, in particular, in biofuel production, pulp and paper production, technologies for improving the quality of food for humans and animal feed [1, 2]. To process such raw materials, a whole arsenal of polysaccharide-degrading enzymes can be successfully used, belonging to 35 families of glycosylhydrolases, three families of esterases, and six − polysaccharide lyases [3]. The efficiency of raw materials processing depends on the choice of the optimal enzymatic complex to achieve the set goals. The use of α-L-rhamnosidases, α-galactosidases, cellulases and β-mannanases in various cycles of polysaccharide processing makes it possible to obtain more active and accessible flavonoids, probiotic oligosaccharides, hydrolyze hard-to-digest galactooligosaccharides, improve the quality of juices and wines, and increase the yield of monosaccharides and digestibility of feed [4–7].

Proteolytic enzymes of microorganisms are among the most demanded enzymes for the

brewing, food, textile, dairy, pharmaceutical, leather industries, for the production of detergents, as well as for the disposal of difficult-to-degradable waste containing, in particular, keratin. Microbial proteases account a two-thirds share of commercial protease around the globe [8]. Every year, as a by-product of poultry, livestock, leather industry, a huge amount of keratin-containing waste in the form of feathers, hooves, wool, etc. is thrown into the environment. The waste of chicken feathers alone amounts to about 8.5 million tons, and this amount is increasing every year. But feather-down raw materials contain about 65 % of feed protein (specialized protein – keratin), which can be converted into digestible form by using specific enzymes that can break down mechanically stable keratin proteins. Keratinases can be a cost-effective and environmentally friendly recycling method to combat keratin-containing wastes that pollute the environment [9].

Proteases and keratinases produced by thermophilic micromycetes are of considerable fundamental and practical interest. This is due to the fact that they have the unique ability to survive and synthesize enzymes into the environment at increased temperatures, while minimizing the risk of unwanted contamination of the cultivator during growing. In addition, the thermal stability of such fungal enzymes makes it possible to increase the efficiency of technological processes.

Since the catalytic properties and substrate specificity of hydrolytic enzymes vary significantly within not only different species, but also strains, and biotechnological industries need new high specificity and stable enzymes, the screening for producers among extremophilic, and especially thermophilic, microorganisms remains an urgent task.

The aim of our work was to study the glycosidase (α-L-rhamnosidase, α-galactosidase, cellulase, and β-mannanase) and proteolytic (caseinolytic and keratinase) activities of collection strains of thermophilic fungi.

Materials and methods. The objects of the study were 50 strains of thermophilic micromycetes isolated from various natural and anthropogenic sources (Table 1). The strains are stored in the collection of live cultures of the Department of Physiology and Taxonomy of Micromycetes of the Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

Micromycetes were grown for 14 days on wort agar, after which they were grown under submerged conditions on several types of liquid nutrient medium. To detect glycosidase activities, the strains were grown under submerged conditions at a temperature of 42 °C, with a rotation speed of 220 rpm for 8 days on medium of the following composition (g/L): maltose (or rhamnose) – 5.0; KH_2PO_4 – 1.6; $MgSO_4 \times 7H_2O$ – 0.75; $ZnSO_4 \times \times 7H_2O$ – 0.25; $(NH_4)_2SO_4$ – 0.5; yeast autolysate – 0.15; soy flour – 10.0; pH 5.0. To study keratinase and caseinolytic activities, micromycetes cultures were grown under submerged conditions at 42 °C for 14 and 8 days on medium of the following composition (g/L): crushed defatted feathers – 5.0; sucrose – 13.3; $NaNO_3$ – 2.0; KH_2PO_4 – 1.0; KCl – 0.5; $MgSO_4 \times 7H_2O$ – 0.5; $FeSO_4 \times 7H_2O$ – 0.01; pH 7.0–7.2.

After the end of fermentation, the biomass was separated by filtration. Enzyme activities were determined in the culture liquid supernatant. α-Galactosidase activity was determined using *p*-nitrophenyl-α-D-galactopyranoside ("Sigma-Aldrich") [10]. α-L-Rhamnosidase activity was determined by the Davis method [11] using naringin as a substrate. The unit (U) of enzymatic activity was defined as the amount of the enzyme that hydrolyzes 1 μmol of the substrate in 1 min under the experimental conditions.

β-Mannanase activity are estimated using 1 % solution of guar gum galactomannan ("Sigma-Aldrich") as substrate in 0.1 M phosphate citrate buffer, pH 5.0. The reaction mixture contained 0.25 mL of supernatant and 0.25 mL of substrate solution. The reaction mixtures are then incubated at 40 °C for 30 min. 2.5 ml of dinitrosalicylic acid reagent solution was added to the reaction mixture after incubation. The reaction mixtures were then boiled at 100 °C for 5 min. Absorbance was measured at 540 nm [12].

Carboxymethylcellulase (CM-cellulase) activity of the strains was determined as described above, but 1 % Na-carboxymethylcellulose 25–75 mPas («Millipore») solution was used as a substrate, and glucose was used as a standard [12].

One unit (U) of CM-cellulase or β-mannanase activity is the amount of enzyme required to liberate, under the assay conditions, 1 μmol/min reducing sugar expressed as glucose (mannose) equivalent.

Caseinolytic activity was determined by the Anson method as modified by Petrova [13]. The unit of activity was expressed in units that corresponded to the amount of μmol of tyrosine released during enzymatic hydrolysis of casein for 1 min under experimental conditions.

Table 1

Thermophilic fungi isolated from various sources

No.	Specific name of the strain	Selection source and year
1	*Acremonium thermophilum* 1963	Kyiv region, forest soil, 1998
2	*Chrysosporium thermophilum*	Zhytomyr region, waste wood, 1998
3	*C. thermophilum* 2050	Volyn region, soil, 1998
4	*Corynascus sepedonium* 65068	Poltava region, soil, 2001
5	*C. sepedonium* 1899	Crimea, soil, 1998
6	*Coprinus delicatus* 2053	Zhytomyr region, compost pit, 1998
7	C. *delicatus* 2007	Zhytomyr region, compost pit, 1998
8	*Melanocarpus albomyces* 62366	Sakhalin, wood processing plant, 1998
9	*M. albomyces* 2048	Kherson region, soil, 1970
10	*M. albomyces* 2056	Volyn region, soil, 1998
11	*M. albomyces* 1937	Kyiv, soil, 2002
12	*M. albomyces* F-62164	Mykolaiv region, soil, 2000
13	*M. albomyces* 2057	Kremenchuk, refinery, 2007
14	*Malbranchea cinnamomea* 1985	Kherson, soil, 2002
15	*Rhizomucor pussillus* 1979	Poltava region, soil, 2000
16	*Rhizomucor tauricus* 1909	Koktebel, soil, 1998
17	*R. tauricus* 61908	Mykolaiv region, soil, 2000
18	*R. tauricus* 23	Mykolaiv region, soil, 2000
19	*Rhizomucor miehei* 61992	Kyiv region, rotten grain, 2000
20	*Scytalidium thermophilum* 1911	Mykolaiv region, soil, 2000
21	*S. thermophilum* 1738	Mykolaiv region, soil, 2000
22	*Thielavia terrestris* 62	Mykolaiv region, soil, 2000
23	*T. terrestris* 60	Mykolaiv region, soil, 2000
24	*T. terrestris* 1920	Mykolaiv region, soil, 2000
25	*Talaromyces emersonii* 1944	Ivano-Frankivsk region, forest soil, 2000
26	*T. emersonii* 2043	Zaporizhzhia, forest soil, 2002
27	*T. emersonii* 143	Poltava region, pulp pit, 1972
28	*T. emersonii* 2044	Moldova, sawdust waste, 1996
29	*Talaromyces thermophilus* 2047	Zaporizhzhia, sawdust waste, 1996
30	*Thermomyces lanuginosus* 1982	Poltava region, soil, 1998
31	*T. lanuginosus* 2046	Volyn region, soil, 1996
32	*T. lanuginosus* 2023	Kherson region, compost pit, 2001
33	*T. lanuginosus* 63298	Poltava region, soil, 2005
34	*T. lanuginosus* 63299	Poltava region, soil, 2005
35	*T. lanuginosus* F-63286	Poltava region, soil, 2005
36	*T. lanuginosus* 2023	Kherson region, compost pit, 2001
37	*Thermoascus aurantiacus* 2045	Moldova, soil, 1996
38	*T. aurantiacus* 2049	Kherson region, compost, 2001
39	*T. aurantiacus* 2051	Zaporizhzhia, wood working waste, 1996
40	*T. aurantiacus* F-62111	Mykolaiv region, soil, 2000
41	*T. aurantiacus* 2022	Mykolaiv region, soil, 2000
42	*T. aurantiacus* 64802	Mykolaiv region, soil, 1999
43	*T. aurantiacus* 2052	Poltava region, soil, 2000
44	*Thermoascus crustaceous* 8 T	Sakhalin, sawdust waste, 2000
45	*T. crustaceous* 65599	Kyiv region, grain, 2006
46	*T. crustaceous* 65561	Israel, soil, 2005
47	*Thermoascus thermophilus* 1946	Sakhalin, pulp and paper mill, 1998
48	*T. thermophilus* 92	Sakhalin, pulp and paper mill, 2005
49	*T. thermophilus* 91 T Cad	Sakhalin, pulp and paper mill, 2005
50	*T. thermophilus* 89 T Cad	Sakhalin, pulp and paper mill, 1998

Keratinase activity was determined using keratin-containing raw materials (crushed defatted feathers) as substrate. The increase in the absorption of the filtrate of the test sample relative to the controls at 280 nm was taken as the degree of protein release [14]. The unit of keratinase activity was defined as the amount of the enzyme causing an increase in absorption by 0.01 for 3 hours of incubation.

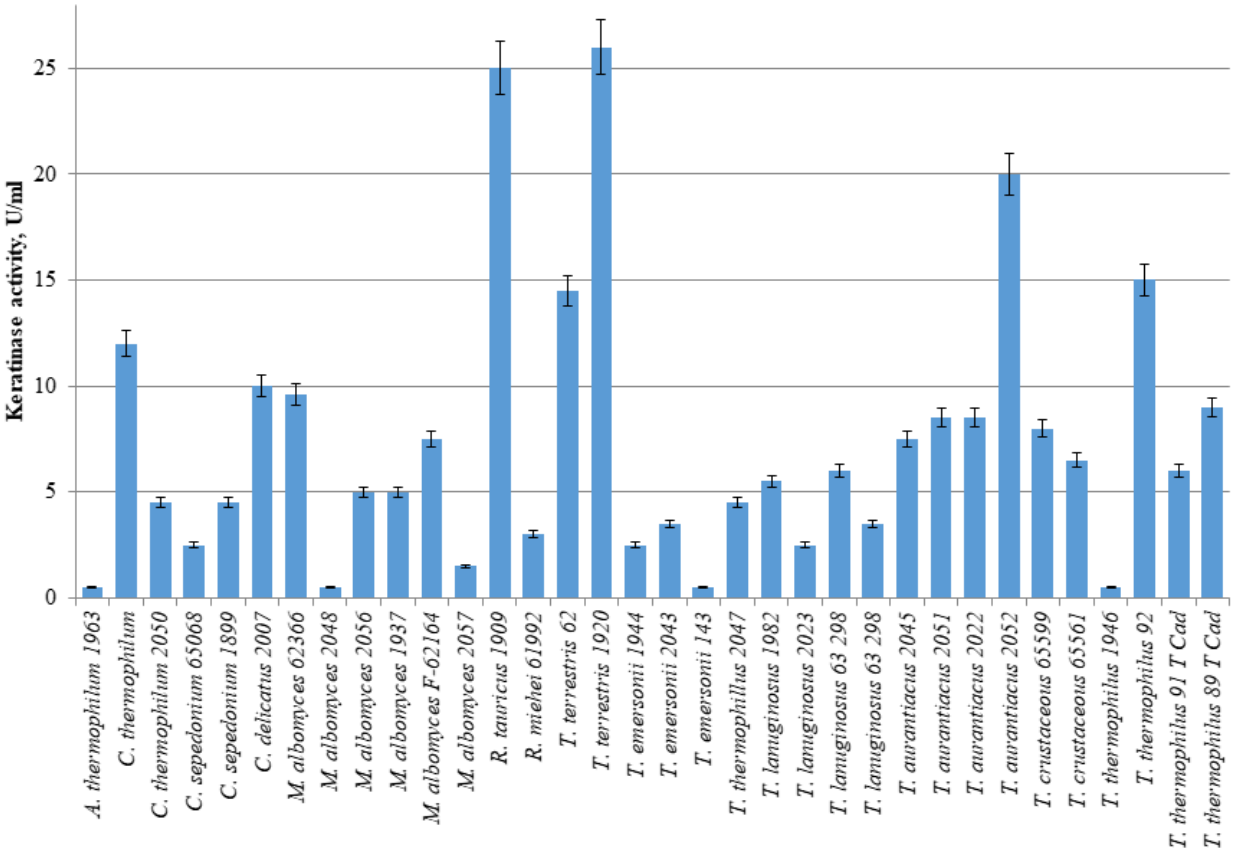
All experiments were replicated 3–5 times. Statistical analysis of the results of the experimental series was carried out by standard methods using Student's t-criterion. The results, presented graphically, were obtained using Microsoft Office Excel 2007.

Results. 50 strains of micromycetes, represented by 17 species of 12 genera, were objects of this research. Most of them were soil representatives, although strains isolated from compost pits, waste from woodworking and pulp and paper enterprises, and oil-contaminated soils have been also investigated.

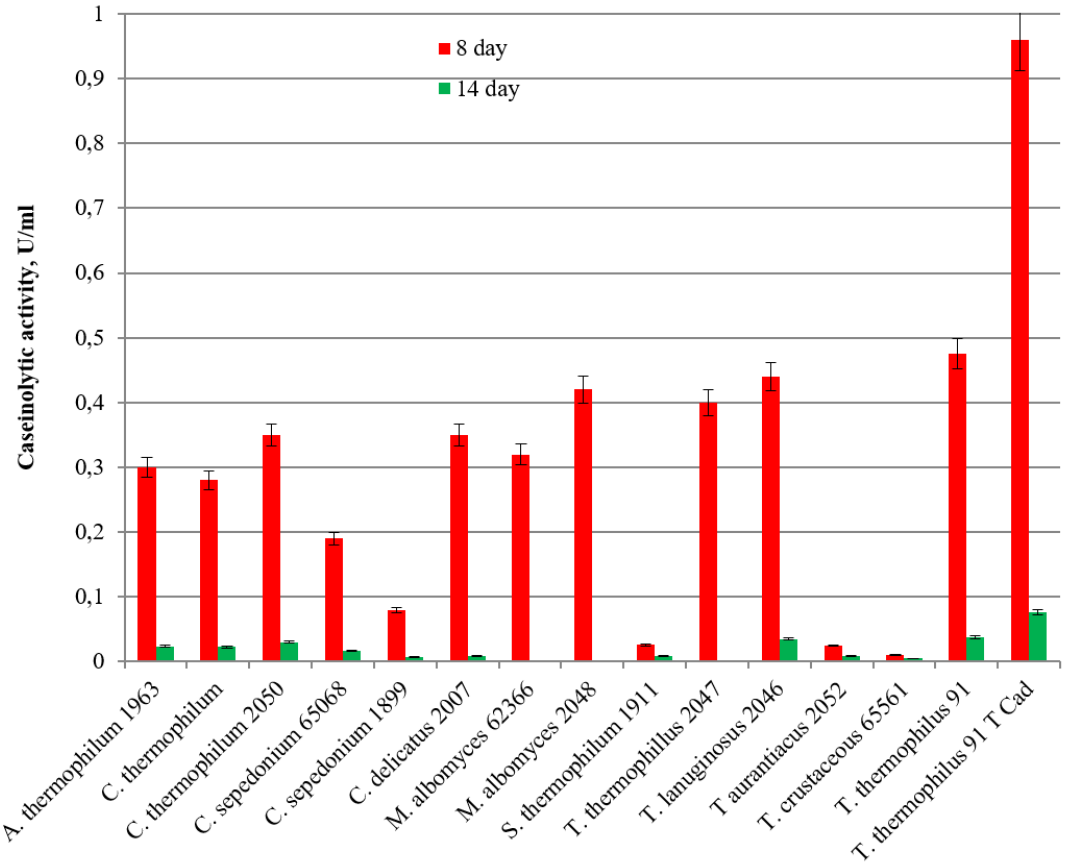
Some proteolytic and glycosidase activities were studied in the culture liquid of micromycetes. To study the proteolytic activity, the cultures were grown for 14 days. The maximum keratinase activity was observed on the 14th day, and the caseinolytic activity − on the 6–8th day (Fig. 1, 2). 68 % of the strains exhibited keratinase activity. The most active were representatives of the species *C. thermophilum*, *R. tauricus*, *T. terrestris*, *T. aurantiacus* and *T. thermophilus*. Keratinase activity was noted in all studied representatives of the species *M. albomyces* and *T. thermophilus*. In this case, the soil micromycetes were in no way inferior to the crops isolated from industrial sources in terms of the level and the prevalence of activity.

Caseinolytic activity was observed in the culture liquid of only 30 % of the studied strains. However, in all cases it was extremely low. Almost all the strains that showed caseinolytic activity also showed keratinase activity, with the exception of *S. thermophilum* 1911 and *T. lanuginosus* 2046.

The studies of glycosidase activities showed that the most common were α-L-rhamnosidase and CM-cellulase (31 and 19 strains, respectively) (Fig. 3, 4). The highest α-L-rhamnosidase activity was noted in *T. terrestris* 62 (0.35 U/mL), and CM-cellulase activity − in *T. lanuginosus* 2048 (16 U/mL). α-Galactosidase activity was shown by 6 strains (0.05–0.2 U/mL), and β-mannanase − by 4 strains (5–130 U/mL).



F i g. 1. Keratinase activity of thermophilic fungi on the 14th day of cultivation



F i g. 2. Caseinolytic activity of thermophilic fungi on the 8th and 14th days of cultivation

In general, the most promising can be noted two strains of the species *C. sepedonium*, which demonstrated the ability to synthesize all the studied enzymes with high rates of activity and also the representatives of the species *A. thermophilum, S. thermophilum, T. lanuginosus, T. thermophilus, T. terrestris*.

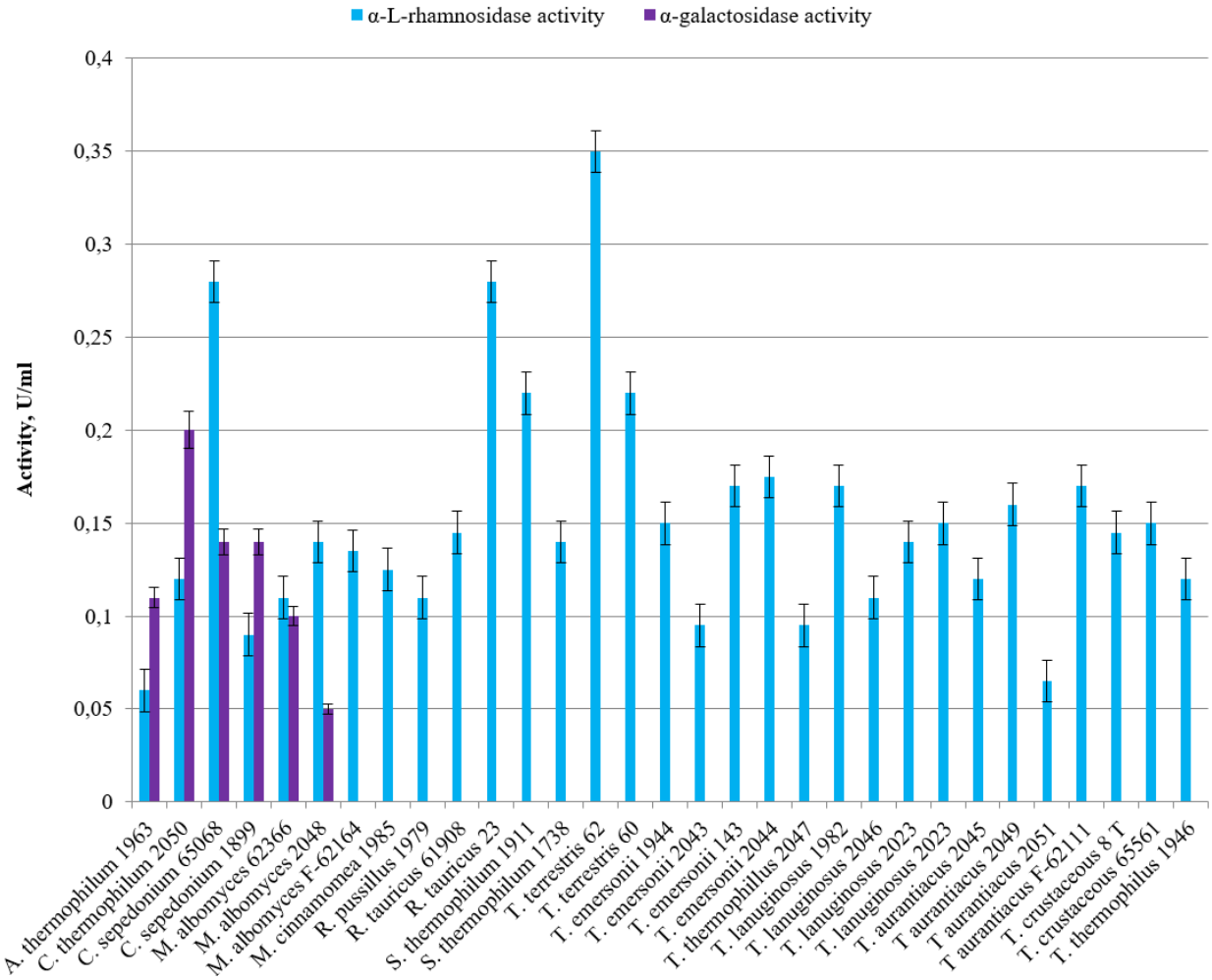
Discussion. The study of extremophiles allows us to discover and recognize more and more ways of adaptation of organisms and proteins, which gives a possibility to approach individualized proteins designed for various stressful conditions. Such protein-enzymes can help in solving many problems, in particular, waste disposal, water purification, and biofuel production [15, 16].

Filamentous fungi have great potential in the field of microbial synthesis due to their ability to produce a wide range of hydrolytic enzymes. Glycoside hydrolases and proteases of micromycetes are widely used in areas requiring the transformation of plant cell wall polymers and poorly soluble proteins.

Thermophilic fungi can exist at high temperatures due to the special physiological and biochemical characteristics. Thermostable enzymes of fungi are characterized by increased ionic interaction and a stronger hydrophobic core and have advantages over enzymes of mesophilic fungi [15]. Activity of thermophilic enzymes at increased temperatures improves the efficiency of technological processes [8].

Thermophilic fungi represent a taxonomically rather small but widespread group of microscopic fungi. The most studied producers of hydrolytic enzymes among thermophilic and thermotolerant micromycetes are *Thermoascus auranticus, T. lanuginosus, Aspergillus flavus, Aspergillus niger, Rhizomucor pusillus, Rhizopus microsporus* var. *chinensis, Rhizopus schipperae, Achaetomium umbonatum, Chaetomium atrobrunneum, Chaetomium virescens, C. sepedonium, Emericella desertorum, Emericella similis, Rhexothecium globosum, Talaromyces assiutensis, Talaromyces eburneus, Thielavia arenaria, Thielavia expansa, Myceliophthora lutea, Paecilomyces clavisporus, Salilagenidium thermophilum,* etc [1, 4, 17–20].

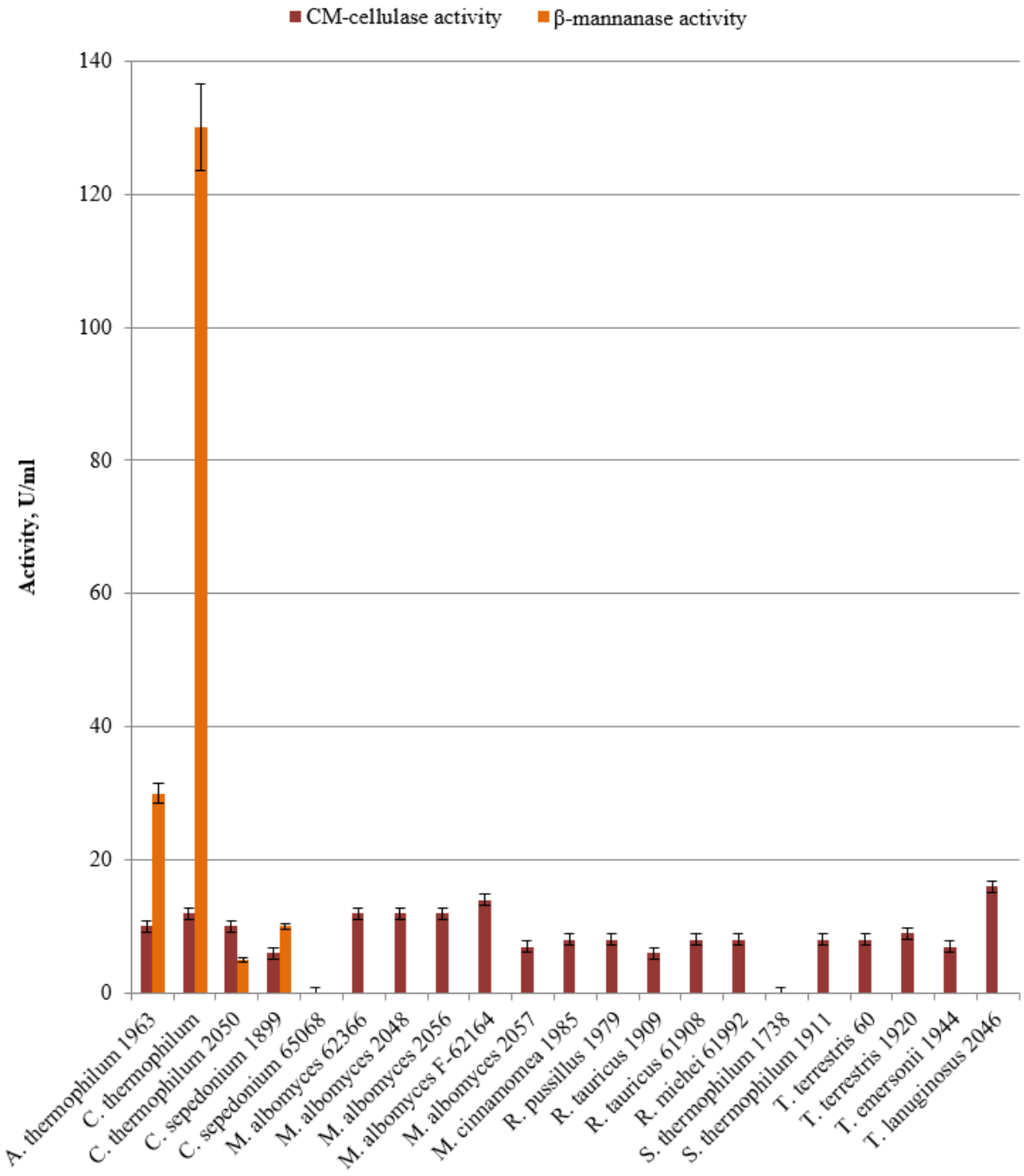
In our work, we studied the glycosidase activity of 50 strains of micromycetes of 17 species from the collection of live cultures of the Department of Physiology and Taxonomy of Micromycetes. The strains were isolated during 1970–2005 years.



F i g. 3. α-Galactosidase and α-L-rhamnosidase activities of thermophilic micromycetes (6 days of cultivation, 42 °C)

Unfortunately, we do not have data on the hydrolase activity of these strains at the time of their isolation, and we cannot assess how long the isolated cultures retain their activity during storage. However, it can be noted that some strains (*M. albomyces* 2048 and F-62164, *C. thermophilum* 2050, *C. sepedonium* 1899, *R. tauricus* 1909, *T. terrestris* 62 and 1920, *T. lanuginosus* 2046, *T. aurantiacus* 2052), which were kept in the collection for more than 20–50 years, showed activity at a level above average.

The studied group demonstrated a sufficiently high enzymatic activity: 94 % of the strains showed at least one activity, 34 % – two activities, 26 % – from three to five activities. Most of the strains had keratinase activity (34 strains), followed by α-L-rhamnosidase (30 strains), CM-cellulase (19 strains), and caseinolytic (15 strains) activities. And only six and four strains exhibited α-galactosidase and β-mannanase activities, respectively. The widest range of studied activities was observed in strains of the genera *Acremonium, Chrysosporium, Corynascus, Melanocarpus, Thermomyces* and *Thermoascus*, which are common representatives of thermophilic fungi with polysaccharide-degrading activity [19–21]. The most active species were *A. thermophilum, C. sepedonium, R. tauricus, S. thermophilum, T. lanuginosus, T. thermophilus, T. terrestris*, isolated from the soils of various regions of Ukraine and waste of pulp and paper production. Among the representatives of these species, active producers of glycosidases have been described. Thus, *T. lanuginosus* is widely known for its hydrolytic activity; soil strains of this species are characterized by high activity of xylanases, cellulases, pectinases, α-galactosidases [22–24]. Producers of cellulose-degrading enzymes have been described among *S. thermophilum* and *R. tauricus* [25, 26]. The CM-cellulase activity (12–16 U/mL) and β-mannanase activity of *C. sepedonium* (130 U/mL) revealed in the culture liquid of *M. albomyces, C. thermophilum,*



F i g. 4. β-Mannanase and CM-cellulase activity of thermophilic micromycetes (6 days of cultivation, 42 °C)

T. lanuginosus strains indicate the biotechnological prospects of these fungi. The selection of effective substrates and inductors in the future will help to increase the activity of these strains and compete with such producers as *Trichoderma reesei* (5–30 U/mL), *Pycnoporus coccineus* (111 U/mL), *Schizophyllum commune* (142 U/mL) [27–29]. β-Mannanase activity of *C. sepedonium* 1899 and 65068 exceeds the activity of such producers as *Penicillium oxalicum* GZ-2 (84 U/mL), *Aspergillus sojae* (64 U/mL) *R. miehei* (105 U/mL), but inferior to *T. lanuginosus* (354 U/mL) [4].

As a result of screening, we first detected α-L-rhamnosidase activity in representatives of *A. thermophilum*, *C. thermophilum*, *C. sepedonium*, *M. albomyces, M. cinnamomea, R. pussillus, R. tauricus, S. thermophilum, T. emersonii, T. lanuginosus, T. aurantiacus, T. crustaceous*, and α-galactosidase – in *T. thermophilus*. By the level of α-L-rhamnosidase activity, the most active strains of *C. sepedonium, R. tauricus* and *T. terrestris* are comparable to such producers as *Aspergillus flavipes, Penicillium decumbens, Rhizopus nigricans* [30].

As for keratinases synthesized by thermophilic fungi, there is very little information in the literature, since most keratinophilic fungi are mesophiles, although *Microsporum gypseum* and some species of *Chrysosporium* are thermotolerant and thermophilic. So, optimal keratinase production by *Chrysosporium keratinophilum* occurs at 90 °C [31, 32]. While under optimal conditions, keratinophilic fungi, *Scopulariopsis brevicaulis* and *Trichophyton mentagrophytes* [32], result in keratinase activity to the levels 3.2 and 2.7 U/mL with the ability to degrade 79 and 72.2 % of chicken feathers, respectively. Also, a strain of *Aspergillus fumigatus* was isolated from decaying wood in a hot spring, which is capable of cleaving chicken feather keratin as the only source of carbon and nitrogen and is known as a producer of several proteinases, such as elastase, fibrinogenolytic protease and collagenase [31]. We first showed keratinase activity for *C. thermophilum*, *C. delicates*, *M. albomyces*, *M. cinnamomea*, *R. tauricus*, *R. miehei, S. thermophilum, T. emersonii, T. crustaceous* and *T. thermophilus* species. We have shown a high keratinase activity of *C. thermophilum, R. tauricus, T. thermophilus, T. terrestris, T. auranticus* (10–26 U/mL). Strains of these species have repeatedly acted as producers of proteolytic enzymes [16, 17, 33], although they were an order of magnitude inferior activity to representatives of *Aspergillus terreus* [34]. The high keratinase activity of studied thermophilic fungi testifies to the high potential of this group for the search for producers of keratinolytic enzymes, which are in demand by the processing and feed industry.

As a result of this work, a number of promising new producer strains were selected: *R. tauricus* 1909 and *T. terrestris* 1920 with high keratinase activity, *C. sepedonium* 65068, *R. tauricus* 23 and *T. terrestris* 62 with high α-L-rhamnosidase activity, *C sepedonium* 1899 and 65068 with high β-mannanase activity, as well as a number of *M. albomyces* and *T. lanuginosus* strains with cellulase activity. It should also be noted that some of the cultures (*A. thermophilum* 1963, *C. thermophilum* 2050, *C. sepedonium* 1899 and 65068, *T. thermophilus* 1946) produced a complex of protease and glycosidase activities in the culture liquid, which may indicate that these strains are promising for use in technologies for processing raw materials of mixed composition, feed production and waste processing.

Conclusions. As a result among thermophilic micromycetes of new strains producing proteolytic and glycolytic enzymes were isolated. Soil thermophilic micromycetes can be used as producers of proteolytic and glycolytic enzymes. Of particular interest are the cultures of *A. thermophilum* 1963, *C. thermophilum* 2050, *C. sepedonium* 1899 and 65068, *T. thermophilus* 1946, which are capable of producing complexes of proteases and glycosidases in the culture liquid. This indicates that these strains are promising for use as destructors in various technologies processing of complex raw materials.

ТЕРМОФІЛЬНІ ГРИБИ З ГЛІКОЗИДАЗНОЮ ТА ПРОТЕОЛІТИЧНОЮ АКТИВНІСТЮ

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Резюме

Спрямований пошук екстремофільних продуцентів та отримання шляхом мікробіологічного синтезу гідролітичних ензимів з підвищеною термостабільністю має безумовний практичний інтерес з огляду на їхнє подальше використання у харчовій промисловості для покращення якості кінцевої продукції. Біотехнологічний потенціал термофільних мікроміцетів пов'язаний, насамперед, з широким діапазоном їхньої ензиматичної активності щодо різноманітних важкодоступних субстратів, особливо за підвищених температур. **Метою** роботи було дослідити здатність музейних термофільних штамів мікроміцетів проявляти α-L-рамнозидазну, α-галактозидазну, целюлазну, β-мананазну, кератиназну та казеїнолітичну активність. **Методи.** Мікроміцети вирощували у пробірках у рідкому поживному середовищі при 42 °С в умовах перемішування протягом 8–14 днів. Ензиматичні активності досліджували у супернатанті культуральної рідини. Для визначення α-галактозидазної активності використовували *n*-нітрофеніл-α-Д-галактопіранозид, для α-L-рамнозидазної – наринґін, для β-мананазної – галактоманан гуару, для целюлазної –

Na-карбоксиметилцелюлозу. Для визначення протеолітичних активностей як субстрат використовували казеїн та знежирене куряче пір'я. **Результати.** Було досліджено ензиматичну активність 50 штамів 17 видів мікроміцетів. Було показано, що ензиматична активність культур не залежала від джерела виділення та часу зберігання. Вивчена група продемонструвала досить високу ензиматичну активність: 94 % штамів виявляли хоча б одну активність, 34 % – 2 активності, 26 % – від 3-ох до 5-ти активностей. Найбільш поширеною серед вивчених штамів була кератиназна активність (34 штами), далі – α-L-рамнозидазна (30 штамів), карбоксиметилцелюлазна (19 штамів) і казеїнолітична (15 штамів) активності. Найактивнішими продуцентами кератинази виявилися *Thielavia terrestris* 1920 та 62, *Rhizomucor tauricus* 1909, *Chrysosporium thermophilum*, *Thermoascus thermophilus* 92 та *Thermoascus aurantiacus* 2052 (10–26 од/мл). Найвищу α-L-рамнозидазну активність відмічено у *T. terrestris* 62 (0,35 од/мл), а карбоксиметилцелюлазну – у *Thermomyces lanuginosus* 2046. α-Галактозидазну активність проявили тільки 6 штамів (0,05–0,2 од/мл), а β-мананазну – 4 штами (5–130 од/мл). В цілому, найбільш активними виявилися 2 штами виду *Corynascus sepedonium*, які проявили здатність продукувати у культуральну рідину всі досліджені активності. Також на підставі отриманих даних перспективними щодо пошуку продуцентів глікозидаз та протеаз можна вважати представників видів *Acremonium thermophilum, T. lanuginosus, T. thermophilus, T. terrestris*. **Висновки.** В результаті роботи серед термофільних мікроміцетів було виділено ряд нових штамів-продуцентів протеолітичних та гліколітичних ензимів: *R. tauricus* 1909 та *T. terrestris* 1920 – з високою кератиназною активністю, *C. sepedonium* 65068, *R. tauricus* 23 та *T. terrestris* 62 – з високою α-L-рамнозидазною активністю, *C. sepedonium* 1899 та 65068 – з високою β-мананазною активністю, а також ряд штамів *Melanocarpus albomyces* та *T. lanuginosus* – з карбоксиметилцелюлазною активністю. Особливий інтерес у майбутньому можуть мати культури *A. thermophilum* 1963, *C. thermophilum* 2050, *C. sepedonium* 1899 та 65068, *T. thermophilus* 1946, які продукують в культуральну рідину комплекс протеаз та глікозидаз, що може свідчити про перспективи використання цих штамів в якості деструкторів в технологіях переробки відходів цілої низки виробництв, обробки сировини змішаного складу для отримання поживних кормів.

Ключові слова: мікроміцети, термофіли, α-L-рамнозидазна, α-галактозидазна, карбоксиметилцелюлазна, β-мананазна, кератиназна та казеїнолітична активності.

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