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EVALUATION OF NON-CONVENTIONAL YEASTS ISOLATED FROM ROTTEN WOOD FOR HYDROLYTIC ACTIVITIES AND XYLOSE FERMENTATION

Hydrolysis of lignocellulose to fermentable sugars and their subsequent conversion to ethanol remain great challenges in the biofuel industry. Rotten wood is first colonized by bacteria and molds that possess strong hydrolases. Yeasts are also an important group of microorganisms that may participate in wood hydrolysis. Decaying wood could provide a rich natural reservoir of yeasts possessing promising hydrolytic activities, including xylanases, cellulases, β-glucosidases, or abilities essential for the fermentation of pentose sugars derived from lignocellulose degradation, especially xylose. Therefore, ***the aim*** *of this work was to screen yeasts isolated from rotten wood samples for the production of hydrolytic enzymes directed at lignocellulose components and the ability to ferment xylose, L-arabinose, and cellobiose.* ***Methods.*** *Yeast strains were isolated from 22 samples of rotten wood and identified by phenotypic characteristics according to Kurtzman et al. Hydrolytic properties and the ability of the isolated strains to ferment xylose, L-arabinose, and cellobiose were determined using conventional methods.* ***Results.*** *30 strains of yeasts and yeast-like micromycetes were isolated from 22 samples of rotten wood in the Holosiivskyi Forest, Kyiv. Based on phenotypic properties, most of the isolated yeasts belonged to ascomycetous yeasts and were represented by the following genera: Candida (8 strains), Debaryomyces (5 strains), Kluyveromyces (5 strains), Pichia (5 strains), Scheffersomyces (2 strains), Lachancea, Hanseniaspora, Saccharomyces, and Geotrichum/Galactomyces. A strain of yeast-like non-photosynthetic alga Prototheca sp. was also detected. Most of the isolated microfungi (66.6% isolates) exhibited extracellular β-glucosidase activity, two Candida tropicalis strains possessed weak pectinase and xylanase activity. None of the isolates demonstrated extracellular cellulase activity. Two yeast strains preliminarily identified as Scheffersomyces stipitis were able to ferment xylose at a concentration of 20—100 g/L over a wide temperature range up to 37 ^{0}C. Acetic acid at 0.25—1% (v/v) concentration resulted in the complete inhibition of xylose fermentation. Ethanol production from xylose up to 6 g/L was observed under the microaerobic fermentation conditions for 24 hr at the substrate concentration 40 g/L, but the subsequent fermentation resulted in decreasing ethanol concentration presumably due to ethanol re-assimilation. None of the isolated strains was capable of fermenting cellobiose or*

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L-arabinose under the microaerobic conditions. ***Conclusions.*** *This work provides the characterization of yeast microbiota of rotten wood that was represented predominantly by ascomycetous yeasts. The dominant extracellular hydrolytic activity of the isolates was β-glucosidase. This is the first report on the isolation of xylose-fermenting yeasts Scheffersomyces stipitis in Ukraine, which comprised 7% of all the microfungi isolated from rotten wood.*

Keywords: *rotten wood, yeasts, hydrolytic properties, xylose fermentation.*

The decomposition of lignocellulosic biomass, which epresents the richest source of renewable carbon on the Earth [1], is a difficult and multistage process that involves many groups of microorganisms. Lignocellulose is a complex biopolymer composed of cellulose, hemicellulose, lignin, and pectin [2]. Cellulose consists only of D-glucose units while hemicellulose is a heteropolymer whose structural components (pentoses, hexoses, and sugar acids) depend on the type of wood [3]. Lignin is the most difficult to degrade component of lignocellulose. The hydrolysis of lignocellulosic biomass usually requires a wide spectrum of enzymatic activities of the microorganisms involved. Thus, decaying lignocellulosic materials are inhabited by microbes with potentially valuable biotechnological traits [3].

Yeasts are an important group of microorganisms found in various lignocellulosic substrates and valuable players in the hydrolysis of plant biomass [3, 4]. Many yeasts and yeast-like fungi isolated from decaying biomass secrete extracellular hydrolytic enzymes directed at lignocellulose components, e.g. cellulases, xylanases, pectinases, and β-glucosidases [3]. Although cellulolytic activity is rarely found among yeasts [4, 5], xylanase and pectinase are much more frequently detected [3].

Another promising trait exhibited by a number of yeasts inhabiting lignocellulosic substrates is the ability to ferment sugars which are among the main lignocellulose components. The most efficient ethanol producer among yeasts is *Saccharomyces cerevisiae* [6], however, its incapability to ferment pentoses, especially xylose, impedes the efficient conversion of lignocellulose into ethanol as xylose is the second most abundant carbohydrate in lignocellulose after glucose [7]. A number of recent studies have described new yeast species with the ability to efficiently ferment xylose, cellobiose, and even L-arabinose [7—10]. Many such yeasts are isolated from lignocellulosic substrates or from the gut of wood-feeding insects. Considering the frequent detection of non-conventional yeasts with various valuable extracellular hydrolases or the ability to ferment pentose sugars in various decaying plant materials, the aim of this work was to screen yeasts isolated from rotten wood samples for the production of hydrolytic enzymes directed at lignocellulose components and the ability to ferment xylose, L-arabinose, and cellobiose.

Materials and methods. *Isolation of yeasts and yeast-like microfungi from rotten wood.* Rotten wood samples were collected in the Holosiivskyi Forest, Kyiv in sterile plastic tubes and analyzed on the day of collection. 1g of samples was inoculated in tubes containing 10 mL of an enrichment medium of the following composition (g/L): Yeast Nitrogen Base — 6.7, xylose — 10.0, sodium propionate — 2.5, chloramphenicol — 0.2, pH 3.5. Cultivation was carried out at 26 °C for 2 weeks or until the appearance of visible microbial growth. Each morphotype of the obtained colonies was microscopically examined and those belonging to yeasts or yeast-like microfungi were purified by streaking on YPD agar at least 3 times.

Phenotypic identification of isolates. The characterization of morphological and physiological traits of the yeasts and yeast-like microfungi was performed according to Kurtzman et al. [11]. Yeast macromorphological (morphology of the colonies and growth in broth media) and micromorphological (size and morphology of yeast vegetative cells, spore formation, mode of asexual reproduction, filament formation) charac-

teristics were described. Fermentation of sugars was carried out in Dunbar tubes containing 2% corresponding carbon source at 26 °C for 3—4 weeks. The aerobic assimilation of 36 carbon sources was performed on the Yeast Nitrogen Base agar (YNB) for 3 weeks at 26 °C. The yeast inoculation was performed using a multi-point inoculator. Assimilation of nitrogen sources (potassium nitrate and sodium nitrite) was studied in broth Yeast Carbon Base medium (YCB) for 3 weeks at 26 °C. Yeast's ability to grow at 37 °C, on 50% glucose agar, in a medium containing 10% NaCl and 5% glucose, and to produce extracellular amyloid compounds was examined.

Determination of the hydrolytic activity of isolated yeasts. The cellulolytic activity was determined on agar YPD supplemented with 0.5 % carboxymethylcellulose. Plates were incubated at 25—26 °C for 5 days. Yeast colonies were removed from the plates by rinsing with distilled water, and the agar was stained with 0.03% Congo Red followed by destaining with 1M NaCl. The formation of the hydrolysis zone around colonies indicated the presence of cellulolytic activity [12].

The xylanase activity of isolated was examined according to Strauss et al. [12] with some modifications on YNB agar containing 1% xylan. Plates were incubated at 26 °C for up to 7 days and flooded with iodine solution. The appearance of a hydrolysis zone around yeast colonies indicated the presence of xylanase activity.

Pectinase activity was determined on YNB agar containing 1% citrus pectin [5]. Plates were incubated at 26 °C for up to 7 days and flooded with iodine solution [13]. The appearance of a hydrolysis zone around yeast colonies indicated the presence of pectinase activity.

The detection of β-glucosidase activity was carried out on an agar medium containing 0.5 % arbutin, 1% yeast extract, and 2% agar, and ammonium ferric citrate solution was added after sterilization [11]. Tubes were incubated at 26 °C for 5—7 days. The development of dark purple-brownish color of the medium indicated the presence of extracellular

Table 1. **Phenotypic identification of microorganisms isolated from rotten wood**

Strain	Yeast species/genus	Strain	Yeast species/genus
w1.1	*Debaryomyces hansenii*	w11.2	*C. tropicalis*
w1.2	*Pichia* sp.	w11.3	*Pichia membranifaciens*
w2	*D. hansenii*	w12	*Pichia* sp.
w3	*D. hansenii*	w13.1	*Geotrichum/Galactomyces* sp.
w4	*D. hansenii*	w13.2	*Candida* sp.
w5	*Kluyveromyces lactis* var. *drosophilarum*	w15	*P. memranifaciens*
w6.1	*D. hansenii*	w16	*Lachancea fermentati*
w6.2	*Kluyveromyces* sp.	w17.1	*Candida* sp.
w7.1	*Pichia* sp.	w17.2	*Hanseniaspora* sp.
w7.2	*Prototheca* sp.	w18	*Scheffersomyces stipitis*
w9	*K. lactis var.drosophilarum*	w20.1	*S. stipitis*
w10.1	*Saccharomyces paradoxus*	w20.2	*Candida* sp.
w10.2	*Candida tropicalis*	w20.3	*Candida* sp.
w10.3	*Kluyveromyces lactis* var. *drosophilarum*	w21.1	*Candida* sp.
w11.1	*K. lactis* var. *drosophilarum*	w21.6	*Candida* sp.

β-glucosidase, while the change to light-medium brown was considered weak activity.

Preliminary screening of yeasts fermenting xylose, L-arabinose and cellobiose. The ability of the isolated yeasts to ferment xylose, L-arabinose and cellobiose was examined in Dunbar tubes containing nutrient broth of the following composition (g/L): yeast extract — 5.0, the corresponding sugar — 20.0. Yeasts grown on YPD agar for 48 hr at 26 °C were suspended in the sterile saline solution with the final cell concentration of 10^8 CFU/mL and used for the inoculation. Dunbar tubes were cultivated at 26 °C for up to 4 weeks. The ability of yeasts to ferment xylose, L-arabinose, and cellobiose was qualitatively assessed by the amount of carbon dioxide gas accumulated in the closed arm of the fermentation tube: «—» — the absence of fermentation; «+» — weak fermentation, «++» — the accumulated gas displaces the medium from the closed arm of the tube by ½, «+++» — the accumulated gas displaces the medium by 2/3, «++++» — the accumulated gas displaces the medium completely from the closed arm of the tube. The ability of yeasts to ferment xylose (20 g/L) was also qualitatively determined at the elevated temperatures of 32 °C and 37 °C and in the presence of 0.25—1% (v/v) acetic acid.

Ethanol production from xylose by batch fermentation. Yeast culture grown on YPD agar at 26 °C for 24 h was inoculated into YPX broth containing 40 g/L xylose instead of glucose and cultivated at 28 °C on a rotor shaker at 200 rpm. The obtained inoculum was added into 250 mL flasks containing 80 mL of YPX broth. The initial cell concentration was 1—1.5 x 10^7 CFU/mL. Batch fermentation was carried out under static conditions (without shaking) in flasks stoppered with glass fermentation traps containing 40% sulphuric acid and also under the submerged microaerobic conditions on a rotor shaker at 120 rpm at 26 °C for 5—7 days. Samples were withdrawn every 24 hr for biomass and ethanol determination. Yeast growth was monitored by CFU counting on YPD agar plates using the serial dilutions method.

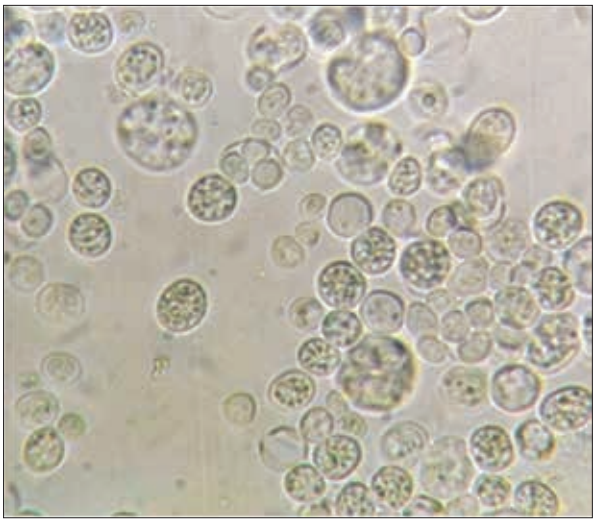

Fig. 1. Cells of yeast-like non-photosynthetic alga *Prototheca* sp. grown on malt agar, 5 days

Determination of ethanol concentration. Ethanol concentration in the medium was determined by gas chromatography-mass spectrometry using an Agilent 6890N/5973inert (Agilent Technologies, USA) capillary column HP-INNOWax (30m×0.25mm×0.25mm) (J&W Scientific, USA). Separation was performed with a temperature gradient of 20 °C/min from 40 to 120 °C, the carrier gas was helium, and the flow rate through the column was 1 mL/min.

All the experiments were performed in triplicate, and the results are respresented as means ± standard deviations.

Results. From 22 samples of rotten wood collected in the Holosiivskyi Forest, Kyiv in 2020—2021, 30 strains of yeasts and yeast-like microfungi were isolated. The preliminary identification based on the phenotypic traits of the isolates was performed. Almost all isolated strains were represented by ascomycetous yeasts and belonged to the genera *Debaryomyces, Pichia, Kluyveromyces, Candida, Lachancea, Hanseniaspora, Scheffersomyces, and Saccharomyces* (Table 1).

One isolated strain was identified as yeast-like non-photosynthetic alga *Prototheca* sp. (Fig. 1). The largest number of isolates were represented by

Table 2. **Extracellular hydrolytic activity of strains isolated from rotten wood**

Strain	Extracellular hydrolytic activity*				Strain	Extracellular hydrolytic activity*			
	β-glu	xyl	cel	pect		β-glu	xyl	cel	pect
w1.1					w11.2				
w1.2					w11.3				
w2					w12				
w3					w13.1				
w4					w13.2				
w5					w15				
w6.1					w16				
w6.2					w17.1				
w7.1					w17.2				
w7.2					w18				
w9					w20.1				
w10.1					w20.2				
w10.2					w20.3				
w10.3					w21.1				
w11.1					w21.6				

Note: black color indicates positive reaction, gray color indicates weak hydrolytic activity, white color indicates the lack of hydrolytic actitvity; * β-glu — β-glucosidase activity, xyl — xylanase activity, cel — cellulolytic activity (CMC hydrolysis), pect — pectinase activity

Table 3. **Screening for fermentation of xylose, L-arabinose, and cellobiose**

Strain	Sugar fermentation			Strain	Sugar fermentation		
	xylose	cellobiose	L-arabinose		xylose	cellobiose	L-arabinose
w1.1	—	—	—	w11.2	—	—	—
w1.2	—	—	—	w11.3	—	—	—
w2	—	—	—	w12	—	—	—
w3	—	—	—	w13.1	—	—	—
w4	—	—	—	w13.2	—	—	—
w5	—	—	—	w15	—	—	—
w6.1	—	—	—	w16	—	—	—
w6.2	—	—	—	w17.1	—	—	—
w7.1	—	—	—	w17.2	—	—	—
w7.2	—	—	—	w18	++++	—	—
w9	—	—	—	w20.1	++++	—	—
w10.1	—	—	—	w20.2	—	—	—
w10.2	—	—	—	w20.3	—	—	—
w10.3	—	—	—	w21.1	—	—	—
w11.1	—	—	—	w21.6	—	—	—

Note: «—» — the lack of fermentation, «++++» — fermentation of sugar (xylose), the accumulated gas displaces the medium completely from the closed arm of the tube.

Table 4. **Xylose fermentation by yeasts *S. stipitis* w18 and w20.1 at elevated temperatures***

Yeast strain	26 °C Xylose concentration, g/L				32 °C Xylose concentration, g/L				37 °C Xylose concentration, g/L			
	20	40	70	100	20	40	70	100	20	40	70	100
S. stipitis w18	4 days	4 days	5 days	5 days	4 days	4 days	5 days	5 days	4 days	4 days	5 days	5 days
S. stipitis w20.1	4 days	4 days	5 days	5 days	4 days	4 days	5 days	5 days	4 days	4 days	5 days	6 days

Note: * the time necessary for the completion of xylose fermentation in Dunbar tubes (++++).

Table 5. **Effect of acetic acid on xylose fermentation by yeasts *S. stipitis* w18 and w20.1**

Yeast strain	Acetic acid concentration,% (v/v)			
	0	0.25	0.5	1.0
S. stipitis w18	++++	—	—	—
S. stipitis w20.1	++++	—	—	—

species *Debaryomyces hansenii* (5 strains), *Kluyveromyces lactis* var. *drosophilarum* (4 strains), and *Candida* sp. (6 strains).

As rotten wood is known to be a rich source of microorganisms with a broad spectrum of hydrolytic activities [3], the ability of the isolated yeasts to hydrolyze such substrates as arbutin, xylan, pectin, and carboxymethylcellulose was determined (Table 2). The majority of the isolates exhibited strong or weak extracellular β-glucosidase activity. However, only two strains *C. tropicalis* w10.2 and w11.2 possessed weak extracellular pectinase and xylanase activities. None of the studied microorganisms exhibited cellulolytic properties.

The ability of yeasts to ferment sugars released during hydrolysis of lignocellulosic substrates is a valuable biotechnological trait as the major ethanol producer from lignocelluloses — yeasts *Saccharomyces cerevisiae* are incapable of fermenting pentoses, e.g. xylose or L-arabinose [6]. Screening of the isolated strains for yeasts fermenting xylose, L-arabinose, or cellobiose resulted in the detection of two strains identified as *Scheffersomyces stipitis* with a xylose-fermenting activity (Table 3). All studied isolates lacked the ability to ferment L-arabinose or cellobiose.

The yeasts *Scheffersomyces stipitis* are known as efficient ethanol producers from xylose, but they also often lack the ability to withstand stressful conditions. Both isolated yeast strains *S. stipitis* w18 and w20.1 retained their xylose-fermenting activity at substrate concentrations up to 100 g/L at elevated temperatures (32 °C and 37 °C) (Table 4). However, acetic acid, one of the main inhibitory compounds resulting from lignocellulose hydrolysis, completely inhibited fermentation of xylose by both strains at concentrations 0.25—1% (v/v) (Table 5).

Ethanol production from xylose by yeasts *S. stipitis* w18 and w20.1 was determined during batch oxygen-limiting fermentation under static conditions and on a rotary shaker at 120 rpm. Ethanol production from 40 g/L xylose under static conditions was very low (2.2—2.8 g/L ethanol after 7 days of cultivation) (data not shown). During cultivation on a rotor shaker, ethanol production from 40g/L xylose was much higher, with the maximum ethanol concentrations (5.5—6.1 g/L) and biomass values achieved by the end of the first day of fermentation (Fig. 2). However, ethanol levels in the fermentation medium considerably decreased after 24 h fermentation, which can be explained by re-assimilation of ethanol by yeast cells [14].

Discussion. Plant biomass is an important ecological niche for various microorganisms. As its main component cellulose, the major source of polysaccharides on Earth is very resistant to microbial degradation, the first microorganisms to begin colonization of lignocellulosic substrates are usual-

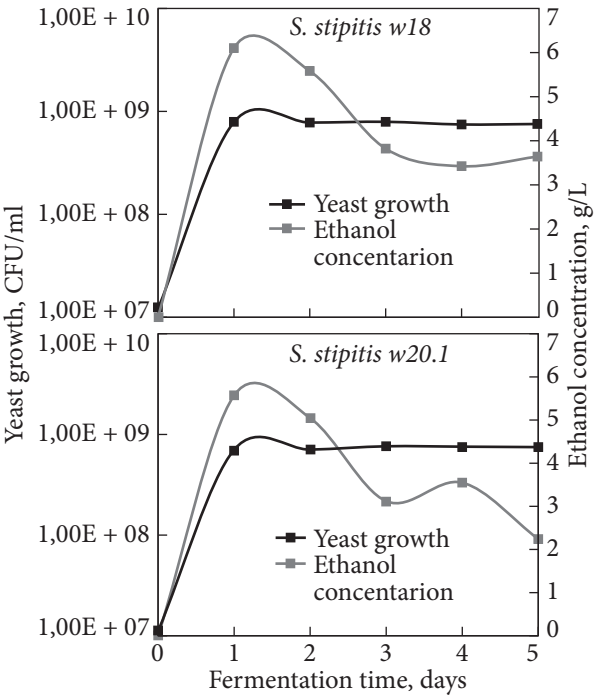

Fig. 2. Ethanol production from xylose by *S. stipitis* w18 and w20.1 strains

ly filamentous fungi possessing efficient hydrolytic enzymes [1]. Yeasts are also an important group of microorganisms that can form associations with other microorganisms and are consistently found in various lignocellulosic substrates [3].

In this work, 30 strains of yeast and yeast-like microfungi have been isolated from 22 samples of rotten wood. 29 out of 30 strains were represented by ascomycetous fungi, predominantly genera *Debaryomyces*, *Kluyveromyces, Pichia,* and *Candida. D. hansenii* was the most frequently isolated yeast species (5 strains) followed by *K. lactis* var. *drosophilarum* (4 strains). *D. hansenii* is commonly found in rotten wood and other plant materials [3, 7, 8]. Interestingly, *Kluyveromyces* yeasts are comparatively rarely isolated from decaying biomass [4], although yeasts *K. lactis* var. *drosophilarum* are associated with insects and the places of their habitat [15]. *Pichia* yeasts which were frequently isolated in this work are also among the most common inhabitants of rotten wood and decaying plant materials [4, 16]. *S. stipitis* are xylose-fermenting yeasts that are commonly associated with various lignocellulosic substrates [7, 17, 18] and wood-feeding insects [9, 18] and have been detected in various geographical locations, including Europe, North and Central America [18]. However, to our knowledge, this is the first report on the isolation of *S. stipitis* in Ukraine and, very likely, on the territory of the former Soviet Union.

One strain of yeast-like non-photosynthetic alga *Prototheca* was detected. This microorganism is widely present in various natural sources, including tree slime fluxes and sediments. It is also known as a causative agent of human and cattle infections [19]. In general, the yeast microbiota of rotten wood in this work represents typical ascomycetous yeasts found in different lignocellulose-related habitats. Although in some works, both ascomycetes and basidiomycetes are found in the samples of rotten wood [4] and tree bark [16], and predominantly ascomycetous yeasts were isolated when xylose-containing enrichment medium was applied [7], no basidiomycetous yeasts were isolated in this study.

Yeasts isolated from decaying biomass frequently possess a set of hydrolytic enzymes directed at the decomposition of lignocellulosic substrates, e.g. cellulose, hemicelluloses, pectin, and cellobiose [3]. The screening of yeast strains isolated in this work for extracellular hydrolytic enzymes revealed a high prevalence of β-glucosidase-positive strains although only two out of 30 isolates possessed weak pectinase and xylanase extracellular activities and none — cellulase activity. Such a phenomenon can be partly explained by the taxonomy of the isolated yeasts predominantly belonging to *Ascomycota.* In several reports regarding yeasts isolated from lignocellulosic substrates, the cellulase and xylanase activities were predominantly exhibited by basidiomycetous yeast *Aureobasidium* sp. [16, 20], or by other basidiomycetous yeasts [20, 21]. In contrast, the β-glucosidase activity is more widely

distributed in various taxonomic groups of yeasts [22], which is consistent with our findings.

As yeast *S. cerevisiae* commonly used for ethanol production lacks the capacity to ferment pentoses formed after hydrolysis of lignocellulose [6], a search for new efficient natural pentose-fermenting yeasts goes on. No cellobiose or L-arabinose-fermenting yeast strains were detected in rotten wood samples in this work. Two strains *S. stipitis* w18 and w20.1 capable of D-xylose fermentation were found.

Xylose-fermenting yeasts have been found in nature all over the world: rotten wood in the Amazonian forests [7], rotten wood, decaying fruit and soil in Japan [23], and gut of wood-feeding insects in Guatemala [9]. A high diversity of cellobiose-fermenting yeasts was found in rotten wood in Brazilian rainforests [8]. There are extremely few reports regarding L-arabinose-fermenting yeasts [24].

The cost of ethanol production from lignocellulosic materials is still high, partly due to the different temperature optima of enzymatic hydrolysis and fermentation stages that require the separation of these two processes [25]. Thus, the use of thermotolerant xylose-fermenting yeasts in simultaneous hydrolysis and fermentation could provide several advantages for biofuel production costs. Yeasts *S. stipitis* are perhaps the best-studied natural xylose-fermenting yeasts. Their temperature optimum for ethanol production from xylose is strain-variable; there are reports of the best performance at temperatures of around or below 30 °C [26, 27] and even the loss of the fermentation ability at 37 °C [26]. In our qualitative experiments, yeast strains *S. stipitis* w18 and w20.1 have been shown to maintain their ability to ferment xylose at 32 °C and 37 °C, which makes them potentially promising producers of ethanol.

During lignocellulose hydrolysis, several compounds that inhibit the growth and fermenting activity of microorganisms are usually released. Their composition and concentration depend upon the fermentation substrate and the mode of hydrolysis, although acetic acid is commonly one of the main inhibitors as it is released as a result of hemicellulose hydrolysis [2]. The isolated *S. stipitis* strains lost their xylose-fermenting activity in the presence of 0.25—0.5% acetic acid, which is consistent with the data about the lack of tolerance of *Pichia (Scheffersomyces) stipitis* to even low levels of acetic acid [28].

Conclusions. This work provides characterization of yeast microbiota of rotten wood represented predominantly by ascomycetous yeasts of genera *Pichia, Debaryomyces, Kluyveromyces, Scheffresomyces,* and *Candida*. This is the first report on the isolation of xylose-fermening yeasts *S. stipitis* in Ukraine. Two isolates *S. stipitis* possessed the ability to ferment xylose at elevated temperatures and produced up to 6 g/L ethanol from 40 g/L xylose. Decaying wood proved to be an important natural resource of β-glucosidase-positive and xylose-fermenting yeasts.

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ВИЗНАЧЕННЯ ГІДРОЛІТИЧНИХ ВЛАСТИВОСТЕЙ ТА ФЕРМЕНТАЦІЇ КСИЛОЗИ НЕТРАДИЦІЙНИХ ДРІЖДЖІВ, ІЗОЛЬОВАНИХ З МЕРТВОЇ ДЕРЕВИНИ

Гідроліз лігноцелюлозних матеріалів до простих цукрів та подальша їх конверсія до біоетанолу є одним з найсерйозніших викликів в біопаливній промисловості. Мертва деревина в природі спершу колонізується переважно бактеріями та міцеліальними грибами, що мають активні гідролітичні ферменти. Однак дріжджі також є важливою групою мікроорганізмів, які населяють мертву деревину та можуть брати активну участь в її гідролізі. Мертва деревина може бути природним резервуаром дріжджів — перспективних продуцентів гідролітичних ферментів, у тому числі ксиланаз, целюлаз, β-глюкозидаз, або здатною до ферментації мономерів деревини — пентозних цукрів, зокрема ксилози. Отже, **метою** даної роботи було провести скринінг дріжджів, ізольованих зі зразків мертвої деревини, що мають ферменти з дією, спрямованою на гідроліз компонентів лігноцелюлози, та здатних ферментувати ксилозу, L-арабінозу та целобіозу. **Методи.** Штами дріжджів було ізольовано з 22 зразків мертвої деревини та ідентифіковано за фенотиповими ознаками згідно визначника Kurtzman та ін. Визначення гідролітичних властивостей та здатності ізольованих штамів ферментувати ксилозу, L-арабінозу та целобіозу проводили за стандартними методиками. **Результати.** 30 штамів мікроскопічних грибів було ізольовано з 22 зразків мертвої деревини, відібраних в Голосіївському лісі, м. Київ. Переважна більшість ізолятів були представниками аскоміцетових дріжджів та за фенотиповими ознаками були віднесені до родів *Candida* (8 штамів), *Debaryomyces* (5 штамів), *Kluyveromyces* (5 штамів), *Pichia* (5 штамів), *Scheffersomyces* (2 штами), *Lachancea, Hanseniaspora, Saccharomyces, Geotrichum/Galactomyces.* Також був виділений 1 штам дріжджоподібної нефотосинтезуючої водорості *Prototheca* sp. У більшості ізольованих штамів (66.6%) спостерігали зовнішньоклітинну β-глюкозидазну активність, а два штами *Candida tropicalis* демонстрували слабку пектиназну та ксиланазну активності. Жоден з ізолятів не мав зовнішньоклітинної целюлазної активності. Два штами дріжджів, віднесених за фенотиповими ознаками до виду *S. stipitis*, були здатні ферментувати ксилозу в концентрації 20—100 г/л, у тому числі і за підвищеної температури (37 °С). Оцтова кислота в концентрації 0.25—1% (v/v) повністю пригнічувала здатність цих штамів зброджувати ксилозу. За умов мікроаеробної ферментації і концентрації ксилози 40 г/л обидва штами продукували до 6 г/л етанолу через 24 години культивування. З подальшим культивуванням концентрація етанолу знижувалася, що, ймовірно, пов'язано з ре-асиміляцією етанолу клітинами дріжджів. Жоден з ізольованих штамів дріжджів не був здатним до ферментації целобіози та L-арабінози за мікроаеробних умов. **Висновки.** Дріжджова мікробіота мертвої деревини Голосіївського лісу представлена переважно аскоміцетовими дріжджами. Найчастіше серед ізольованих штамів спостерігали β-глікозидазну позаклітинну активність. Нами вперше повідомляється про ізоляцію ксилозоферментуючих дріжджів *S. stipitis* на території України. Дріжджі *Scheffersomyces stipitis* були єдиними ізолятами, здатними до ферментації ксилози та представляли 7% усіх ізольованих штамів.

Ключові слова: *мертва деревина, дріжджі, гідролітичні властивості, ферментація ксилози.*