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CHARACTERIZATION, STABILITY AND ANTIMICROBIAL ACTIVITY OF BIOSURFACTANTS PRODUCED BY *CANDIDA* YEASTS ISOLATED FROM FLOWERING PLANTS

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Yeasts are known to produce surface-active compounds e.g. biosurfactants including mannosylerythritol lipids, sophorolipids and other glycolipid compounds. Sugar-rich niches including nectar and flowering plants have been suggested as potential sources for biosurfactant-producing yeasts. Previously 160 yeast strains were isolated from various flowering plants and bees Apis mellifera. Two yeast strains preliminarily identified belonging to the genus Candida were selected as the most promising biosurfactant producers. ***The aim*** *of this work was to characterize biosurfactants produced by the selected yeast strains, e.g. determine their surface properties, stability and potential antimicrobial activity against various bacterial and yeast strains.* ***Methods****: Yeast strains were identified according to phenotypic characteristics. Biosurfactants were extracted by ethyl acetate. Antimicrobial activity was determined by disc diffusion and serial dilution methods. Stability of biosurfactants was assessed by oil-spreading method under various pH, temperature, salt concentrations.* ***Results:*** *Two yeast strains 79a and 156a were identified as Candida gropengiesseri and Candida bombicola. Both yeast strains have been shown to produce a mixture of several glycolipids with Rf 0.15, 0.21, 0.31–0.35, 0.41–0.44, 0.5 and 0.62. The addition of hydrophobic carbon source (sunflower oil) to the medium resulted in 5-fold increase in biosurfactant production by both strains. The decrease in surface tension of the medium up to 36.0-36.6 mN/m as a result of 6 day cultivation of yeasts in SL medium was detected. Produced biosurfactants retained stability at elevated temperatures and high salt concentrations although lost their activity at alkaline pH. Biosurfactant extracts demonstrated weak antibacterial activity against gram-positive bacteria but lacked inhibitory effect against gram-negative bacteria and yeasts.* ***Conclusions: T****wo yeast strains C. gropengiesseri and C. bombicola isolated from flowering plants produce glycolipid biosurfactants stable at high salinity and temperature and exhibiting weak antimicrobial activity against gram-positive bacteria. The surface activity of both strains is indicative of their potential as biosurfactant producers.*

Keywords: yeasts, glycolipid biosurfactants, stability, antimicrobial activity

Biosurfactants are surface-active amphiphilic compounds produced by various groups of microorganisms. Yeasts as biosurfactants producers are attractive due to their GRAS status, e.g. lack of pathogenicity, low toxicity and potential application in food, pharmaceutical and cosmetic industries [1]. Best-known biosurfactants produced by yeasts include glycolipids mannosylerythritols (by yeasts belonging to the genus *Pseudozyma*), sophorolipids (by yeasts belonging to the genera *Candida* and *Starmerella*) and cellobiose lipids (by yeasts belonging to the genera *Pseudozyma* and *Cryptococcus*) [2]. Also complex polymeric biosurfactants of carbohydrate-

protein-lipid and carbohydrate-protein nature were produced by various yeast species [1].

Biosurfactants produced by yeasts have been shown to exhibit antibacterial, immune-modulating and anticancer activities [2]. Biosurfactant produced by *Candida lipolytica* strain was found to be effective against a broad spectrum of gram-positive bacteria and weakly inhibitory against some gram-negative bacteria and yeasts [3]. Sophorolipids have been shown to possess antimicrobial activity against gram-positive and gram-negative bacteria [4, 5] as well as anticancer activity [6], and immune-modulating properties [7]. Other glycolipid biosurfactants produced by yeasts exhibited antimicrobial activity against various yeasts as well as bacteria [3, 8].

The search for new biosurfactant producers of yeast origin continues in various ecological niches. One of the most promising biosurfactant groups are sophorolipids produced predominantly by yeasts of *Starmerella bombicola* clade that are associated with sugar-rich substrates – bees and flowers [9]. Other possible natural sources for biosurfactant-producing yeasts include hydrocarbon-contaminated soils [10], various fruits and vegetables [11].

Previously 160 yeast strains were isolated from various flowering plants and bees *Apis mellifera* and screened for biosurfactant production [12]. A high proportion of the isolates (45%) have been shown to possess surface activity by oil-spreading method. Two yeasts strains preliminarily identified belonging to the genus *Candida* were selected as the most promising biosurfactant producers. Here, we provide for the first time the characterization of yeasts – producers of glycolipid biosurfactants isolated from flowers in the territory of Ukraine.

The aim of this work was to characterize biosurfactants produced by the selected yeast strains, e.g. determine their surface properties, stability and potential antimicrobial activity against various bacterial and yeast strains.

Materials and methods. *Yeast strains used in the study.* Two yeast strains *Candida* spp. 79a and 156a were previously isolated from flowers of *Tulipa gesneriana* and *Viburnum* sp. [12].

To assess the antibacterial activity of biosurfactant extracts produced by yeasts the following test strains of gram-positive and gram-negative bacteria were used: *Escherichia coli* UCM B-906 (ATCC 25922), *Pseudomonas aeruginosa* UCM B-907 (ATCC 27853), *Proteus vulgaris* UCM B-905 (ATCC 6896), *Klebsiella pneumoniae* UCM B-920 (ATCC 10031), *Bordetella bronchiseptica* UCM B-222 (ATCC 4617), *Stapylococcus aureus* UCM B-4001 (ATCC 6538P), *Bacillus subtilis* UCM B-901 (ATCC 6633), *Bacillus cereus* UCM B-908 (ATCC 11778), *Micrococcus luteus* UCM Ac-634 (ATCC 10240), *Bacillus pumilus* UCM B-913 (NCTC 8241). To assess potential antifungal activity the following yeast strains were used: *Rhodotorula mucilaginosa* UCM Y-1406, *Debaryomyces hansenii var. fabryii* UCM Y-2531 (ATCC 20278), *Metschnikowia lunata* UCM Y-47 (ATCC 22033), *Pichia guillermondii* UCM Y-34 (ATCC 46036), *Saccharomyces cerevisiae* UCM Y-2474 (ATCC 9763), *Debaryomyces occidentalis var. occidentalis* UCM Y-24 (ATCC 2322), *Candida tropicalis* UCM Y-2502 (ATCC 750), *Rhodosporidium diobovatum* UCM Y-43 (ATCC 22265), *Candida albicans* UCM Y-1918 (ATCC 885-653),

Candida boidinii UCM Y-1572 (ATCC 18810)*, Lodderomyces elongisporus* UCM Y-2500 (ATCC 11503), *Pichia membranifaciens* UCM Y-1588 (ATCC 26288). All the test strains were obtained from Ukrainian Collection of Microorganisms.

Culture media. Biosurfactant production by yeasts was assessed in the medium SL containing 100 g/L glucose, 1.5 g/L yeast extract, 4 g/L NH_4Cl, 1 g/L KH_2PO_4, 0.1 g/L NaCl, 0.5 g/L $MgSO_4$x7H2O in distilled water [10]. Antimicrobial activity was determined in Mueller-Hinton medium (HiMedia) and Saburaud medium containing 40g/L glucose, 10 g/L peptone, 20 g/l agar, pH 5.5.

Phenotypic identification of yeast isolates. Identification of yeast strains was carried out based on the morphological and physiological characteristics according to the Kurtzman et al [13]. 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) characteristics were described. Differentiation between ascomyceteous and basidiomyceteous yeasts was based on Dizazonium blue reaction. Fermentation of sugars was carried out in Dunbar tubes containing 2% corresponding carbon source at 26-28^{0}C for 3-4 weeks. The assimilation of 39 different carbon sources including carbohydrates, polyols, organic acids was performed in broth YNB medium (Yeast Nitrogen Base) for 3 weeks at 26-28°C. Assimilation of nitrogen sources (potassium nitrate and sodium nitrite) was done in broth YCB medium (Yeast Carbon Base) for 3 weeks at 26-28°C. Yeast ability to grow in vitamin-free medium, at 37^{0}C, on 50% glucose agar and medium containing 10 % NaCl and 5 % glucose and tolerance to antibiotic cyclohexymide at concentrations 0.1% and 0.01% was assessed.

Biosurfactant production. The incubation of biosurfactant-producing yeasts was done for 6 days at 25^{0}C on the orbitary shaker at 210 rpm in broth medium SL. Food grade sunflower oil at concentration 5% (v/v) was added to the medium as the hydrophobic carbon source. Biosurfactants were extracted from the culture medium thrice with the equal volume of ethyl acetate, organic layer was collected and dried using rotor evaporator [14]. The obtained extract was washed thrice with hexane to remove the remaining hydrophobic substances, dried and weighted to determine the biosurfactant yield.

Thin layer chromatography. 2 ml of culture medium were extracted with 2 ml ethyl acetate and extracts were applied onto TLC ALUGRAM SIL G/UV_{254} plates (MACHEREY-NAGEL, Germany) The developing system: chloroform/methanol/water=65/15/2 (v/v/v). The visualizing reagent α-naphthol/sulfuric acid was applied to TLC plates. Sprayed plates were heated at 125^{0}C for 5 min. Pink-colored spots (glycolipid-positive) were observed. Retention factor Rf was determined as a ratio of the distance traveled by the compound to the distance traveled by the solvent.

Surface tension determination. Yeast cultivation was carried out for 6 days in SL medium as described previously. The surface tension of the culture medium was measured using tensiometer Lauda TD 1C (Germany).

Oil-spreading method. 50 ml of distilled water have been poured to a large Petri dish (15cm diameter) and 20µl of crude oil have been added to the water surface. 10µl of cell-free culture medium have been added to the surface of

oil. The diameter of clear zone formed on the surface was measured [15]. The experiment was done in triplicate.

Stability study of biosurfactants. The stability of the produced biosurfactants was assessed in terms of salinity, pH and temperature according to Techaoei et al. with some modifications [16]. The culture medium after 6 days cultivation in SL medium was centrifuged at 5000g for 10 min. The cell-free broth was heated for 1 hour at 50^0C, 70^0C, 100^0C and 120^0C and then cooled to room temperature. pH value of the cell-free broth was adjusted to the following values: 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 using 1 N NaOH or 1 N HCl. The effect of salinity on biosurfactant stability was tested by the addition of sodium chloride at concentrations 2, 4, 6, 8, 10 %. Oil-spreading activity of the cell-free broth was determined 1 hour following such treatments.

Antimicrobial assays. Biosurfactant extracts were dissolved in 96^0 ethanol and paper discs were supplemented with biosurfactant extracts at concentrations 100 and 200 μg per disc. Paper disc supplemented with ethanol were served as control. Disc diffusion method against bacteria strains was performed on Muller-Hinton agar plates [17]. Briefly, suspension of bacterial culture grown overnight were spread on the plates and 30mm paper discs supplemented with glycolipid extracts were placed above with the sterile tweezers. Plates were incubated at 37^0C for 24-48 h and the diameter of the zone of inhibition was measured. Disc diffusion method against yeast strains was performed as described previously with the following modifications: yeast incubation was performed on Sabouraud agar plates at 28^0C for 48-72 h.

Antimicrobial activity of biosurfactant extracts was also determined by serial dilution method against bacterial tests strains on Mueller-Hinton agar plates and against yeast test strains in Sabouraud agar plates supplemented with the studied extracts at concentrations 5-500 mg/L. Additionaly antibacterial activity of biosurfactant extracts against gram-positive bacterial strains was assessed in 96-well microplates containing Mueller-Hinton broth supplemented with the studied extracts at concentrations 100, 200, 400, 600, 800, 1000 mg/L. Microplates were incubated at 37^0C for 24-48 h. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the biosurfactant extract that completely inhibited the visible growth. All the tests were performed in triplicate.

Data are displayed as means ± standard deviations of triplicate experiments.

Results. Two selected yeast strains previously tentatively identified as *Candida* sp. were subjected to a number of morphological and physiological tests. The colonies of the both strains on malt agar after 3 days at 25^0 C were white and cream, smooth with entire margin, butyrous. In YPD broth after 3 days at 25^0 C yeast cells were round, ellipsoidal, elongated, cylindrical, size (1.3-2.9)x(2.3-5.0) μm, occurred mostly single or in small chains (Fig. 1). Budding was multilateral. Sediment and surface ring were observed in broth YPD. No spore formation was detected. In Dalmau plates on potatoe and Gorodkowa agar no filament formation was detected for yeast strain 79a while short poorly branched filaments were formed by the strain 156a (Fig. 1, B). Diazonium B reaction was negative for both strains.

Fermentation of glucose, sucrose and raffinose was observed, maltose, lactose, tregalose and galactose were not fermented. D-glucose, raffinose, L-sorbose, sucrose, xylitol, D-sorbitol, D-mannitol, glycerol, ethanol, succinate, citrate, D-gluconate, D-glucono-1,5-lactone were assimilated, slow assimilation was observed for maltose, D-galactose, inulin. No assimilation of melizitose, melibiose, cellobiose, D-xylose, lactose, D-arabinose, L-arabinose, D-ribose, trehalose, L-rhamnose, erythritol, inozitol, ribitol, galactitol, N-acetyl-D-glucoseamine, DL-lactate, hexadecane, salicin, soluble starch and D–glucuronate was detected. Potassium nitrate and sodium nitrite were not assimilated as nitrogen sources. No growth at 37^0 C was observed. Both yeast strains grew on 50% glucose agar and were sensitive to 0.01% cycloheximide. Starch-like compounds were not produced. Based on their phenotypic characteristics yeast strain was identified as *Candida gropengiesseri*, yeast strain 156a as *Candida bombicola.*

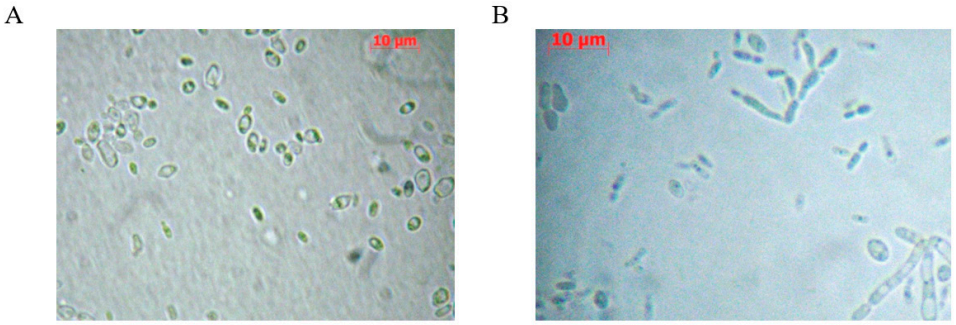

Fig. 1. Cell morphology of yeast strains *C. gropengiesseri* 79a (A) and *C. bombicola* 156a (B), 3 days cultivation on malt agar

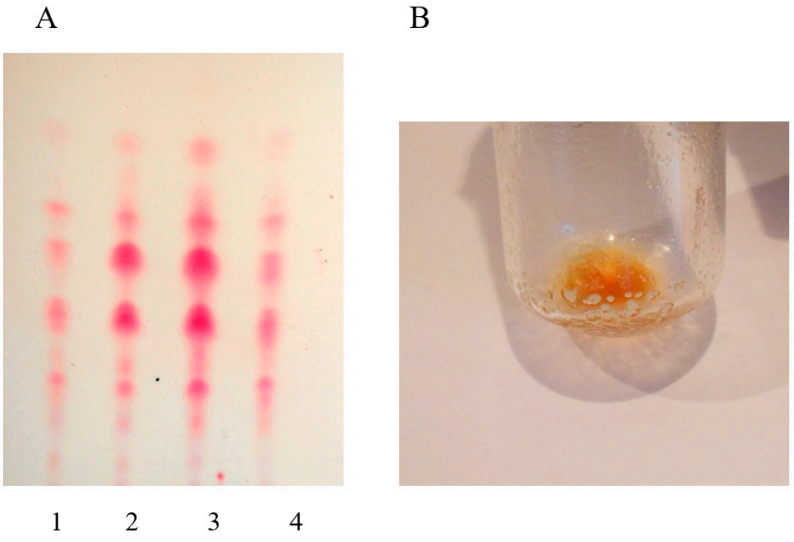

Fig. 2. Characterization of glycolipid biosurfactants produced by yeasts: A – TLC plate with glycolipid extracts from culture medium of yeast strains *C. gropengiesseri* 79a (1-2) and *C. bombicola* 156a (3-4) after 6 days cultivation in oil-free SL medium SL (1-4) and SL medium supplemented with 5% sunflower oil (2-3), B – glycolipid extract from the culture medium of the yeast strain *Candida gropengiesseri* 79a

Yeast strains *C. gropengiesseri* 79a and *C. bombicola* 156a synthesized a mixture of at least 6 forms of glycolipid biosurfactants with Rf 0.15, 0.21, 0.31–0.35, 0.41–0.44, 0.5 and 0.62 (Fig. 2, A), glycolipid extract appeared as yellow-brownish viscous material (Fig. 2, B).

The addition of 5% food-grade sunflower oil to SL medium resulted in the intensification of visualization of glycolipid fractions on TLC plate (Fig. 2, A) and increased the concentration of biosurfactants more than 5-fold after 6 days cultivation in SL medium – from 2.03–2.87 g/L in oil-free medium to 10.65–16.11 g/L in oil-supplemented medium (Table 1). Surface tension of the SL medium as a result of 6 days cultivation of both yeast strains was decreased from 73.65 to 36.0–36.6 mN/m in oil-free medium and from 63,02 to 32,3–33,5 mN/m in medium supplemented with 5% sunflower oil (Table 1).

Table 1

Biosurfactant production by yeast strains *C. gropengiesseri* 79a and *C. bombicola* 156a

Yeast strain	Oil-free SL medium		SL medium supplemented with 5% sunflower oil	
	Biosurfactant concentration, g/l	Surface tension, mN/m	Biosurfactant concentration, g/l	Surface tension, mN/m
C.gropengiesseri 79a	2.03±0.11	36.6±0.82	10.65±1.22	32.3±1.61
C.bombicola 156a	2.87±0.25	36.0 ±0.26	16.11±1.98	33.5 ±1.67

Biosurfactants produced by the selected yeast strains were shown to retain their full stability at high temperatures up to 100^0C and some loss of surface activity was observed at 120^0C (Fig. 3, A). Biosurfactants were also stable at high sodium chloride concentrations (2–10%) (Fig. 3, B), however a rapid loss of stability was detected at pH increase. Only 25% and 43.9% of oil-spreading activity was retained by yeast strains *C. gropengiesseri* 79a and *C. bombicola* 156a correspondingly at pH 12.

Biosurfactants produced by yeasts have been shown to possess antimicrobial activity against various bacteria, yeasts, molds [2]. However extracts of glycolipids produced by yeast strains *C. gropengiesseri* 79a and *C. bombicola* 156a did not exhibit any inhibitory effect against a number of gram-positive and gram-negative bacteria as well as yeasts when testing by disc diffusion method. Weak inhibitory effect was observed only against one gram-positive strain *B.cereus* UCM B-908 with diameter of zone 8,33mm (data not shown). When testing antimicrobial activity by serial dilution method extracts of biosurfactants produced by both strains did not inhibit the growth of any test strains of yeasts and gram-negative bacteria at concentrations 5-500 mg/L.

Weak inhibitory effect of biosurfactant extracts was observed on agar medium against gram-positive bacteria strains *S. aureus* UCM B-4001, *B. subtilis* UCM B-901, *M. luteus* UCM Ac-634, *B. cereus* UCM B-908 at concentration 500 mg/L. When assessing antibacterial activity of the biosurfactant extracts in Mueller-Hilton broth their activity was found to be more pronounced, the most sensitive bacterial strain being *M. luteus* UCM Ac-634, MIC of both tested biosurfactant extracts was 100 mg/L. Biosurfactant produced by yeast strain

C. bombicola 156a exhibited higher antimicrobial activity against gram-positive bacterial strains *B. pumilus* B-913, *S. aureus* B-4001, *B. cereus* B-908 (MIC – 200–400 mg/L) (Table 3).

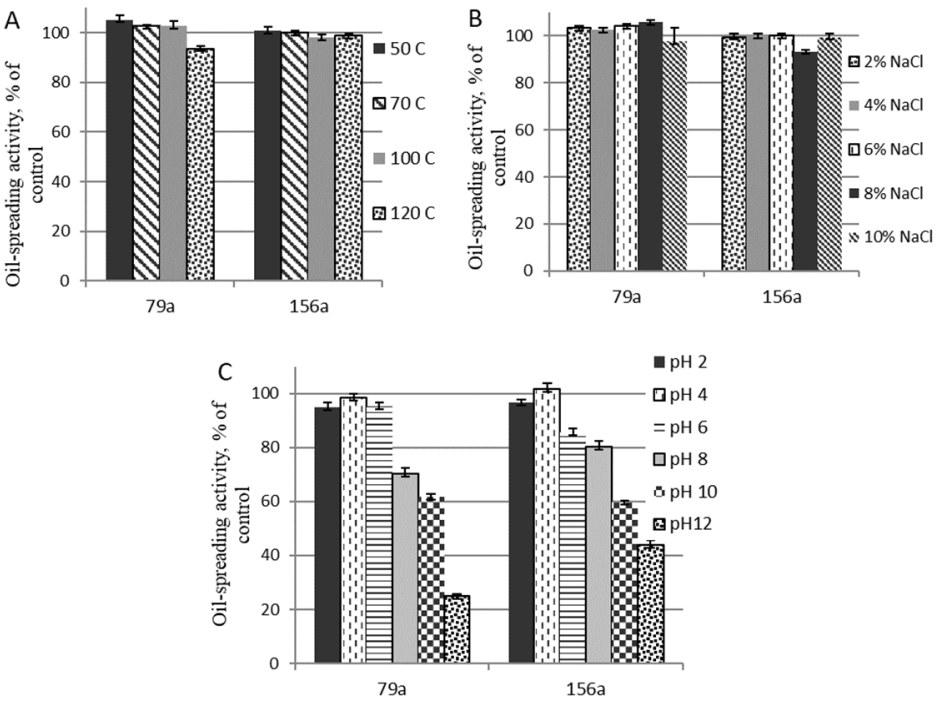

Fig. 3. Effect of temperature (A), sodium chloride (B) and pH (C) on oil-spreading activity of yeast strains *C. gropengiesseri* 79a and *C. bombicola* 156a. Data are displayed as % of the control oil-spreading activity (that of the untreated cell-free broth after 6 days cultivation)

Table 3

Antibacterial activity of biosurfactant extracts against gram-positive bacteria in broth medium

Yeast strain – producer of biosurfactant	MIC of biosurfactant extract, mg/L				
	M. luteus Ac-634	*B. pumilus B-913*	*S. aureus B-4001*	*B. cereus B-908*	*B. subtilis B-901*
C. gropengiesseri 79a	100	800	800	400	1000
C. bombicola 156a	100	200	400	200	1000

Discussion. Based on the phenotypic tests two selected yeasts producing biosurfactants were identified as *C. gropengiesseri* 79a and *C. bombicola* 156a. Both these species belong to the so called *S. bombicola* clade which members are associated with flowers and insects and many are known to produce glycolipid biosurphactants sophorolipids [9]. It is suggested that produced biosurfactants serve as carbon storage and help to cope with excess carbon in the medium [18]. New species belonging to *S. bombicola* clade are being constantly discovered and their isolation source is usually flowers or flower-associated insects [19, 20]. Biosurfactant-producing yeasts were also

isolated from tiger lily wild flowers [21], fleabane flowers [22]. Previously 160 yeast strains were isolated by us from flowering plants and bees with high proportion of those demonstrating surface activity [12]. Based on such data we hypothesize that the surfaces of and the insides of flowers can serve as the specific niche for biosurfactant-producing yeasts.

The studied yeast strains produced a mixture of at least 6 forms of glycolipids with Rf 0.15, 0.21, 0.31–0.35, 0.41–0.44, 0.5 and 0.62. Yeasts usually produce glycolipid biosurfactants as a mixture of several compounds, as could be seen for sophorolipids with Rf 0.13, 0.18 and 0.56 synthesized by *Rhodotorula babjevae* [23], with Rf 0.3, 0.34, 0.56 and 0.6 by *C. bombicola* [24], with Rf 0.08, 0.43, 0.47, 0.53, 0.57 and 0.61 by *Lachancea thermotolerans* [25] and Rf 0.08, 0.13, 0.18, 0.27, 0.33, 0.39, 0.43, 0.47, 0.57, 0.60 by *Torulopsis bombicola* [26]. Mannosylerythrytol lipids are produced by yeasts of genera *Pseudozyma* and *Candida* usually as one or several known forms MEL-A, MEL-B, MEL-C and Mel-D with Rf 0.52–0.77 [27]. The Rf values of glycolipid extracts produced by *C. gropengiesseri* 79a and *C. bombicola* 156a are indicative of their belonging to sophorolipids.

The efficient biosurfactants possess the ability to decrease the surface tension between phases, usually between water and air, from 72 to 35 mN/m [28]. 6 days cultivation of yeast *C. gropengiesseri* 79a and *C. bombicola* 156a resulted in the decrease of surface tension of SL medium from 73.65 to 36.0–36.6 mN/m which is indicative of promising biosurfactant producers on par with biosurfactants produced by *R. babjevae* [23], *Wickerhahomyces anomalus* [29], *S. bombicola* [30]. Biosurfactant concentration after 6 day cultivation in SL medium without hydrophobic carbon source was 2.03–2.87 g/L and the addition of sunflower oil to the medium resulted in the more than 5-fold increase in the yield. It is a well-known fact that the addition of hydrophobic carbon source to the medium results in the increase of biosurfactant production by yeasts as yeasts do not need to synthesize de novo lipid moiety of biosurfactant [1, 30]. There is a great variation in the reported production of glycolipid biosurfactants by various yeast strains: from less than 0.5 g/L of sophorolipids by various strains of *S. bombicola* clade [9] and cellobiose lipids by various *Pseudozyma* strains [31] to 19 g/L sophorolipid by *R. babjevae* [23]. The optimization of cultivation conditions and processing could result in the stark increase of biosurfactant yields – up to 129 g/L of manossylerythritol lipids by *Pseudozyma hubeiensis* [32] or up to 623 g/l of sophorolipids by *C.bombicola* [33].

The cell-free culture medium of both yeast strains *C. gropengiesseri* 79a and *C. bombicola* 156a retained surface activity at up to 120^0C and in the presence of 2-10% NaCl. Yeast glycolipid biosurfactants are known to be stable at high salinity and temperature [1]. Interestingly the loss in oil-spreading activity of both studied strains was detected with the increase in pH. Mostly glycolipid biosurfactants produced by yeasts are stable at wide range of pH [23, 34]. As a matter of fact only few biosurfactants produced by yeasts lose their activity at alkaline pH which include sophorolipids [35, 36].

Many glycolipid biosurfactants produced by yeasts are reported to inhibit various bacteria and fungi. We did not observe any inhibitory effect of biosurfactant extracts against a wide range of yeasts and some gram-negative bacteria although some degree of antibacterial activity against gram-

positive test bacterial strains was observed (MIC 100-1000 mg/L). Glycolipid biosurfactant produced by strain *Wickerhamomyces anomalus* was highly effective against gram-positive bacteria at concentration 2.6 g/L, being less effective against gram-negative bacteria *E.coli* and *P. aeruginosa* and also *S. aureus* [29]. *Candida sphaerica* produced biosurfactant that inhibited up to 57% growth of gram-positive bacteria and *C. albicans* at 10 g/L [34]. Mannosylerythritol lipids and cellobiose lipids produced by yeasts were shown to inhibit a number of yeasts and molds [2, 8]. Conflicting reports exist regarding antimicrobial activity of sophorolipids produced by yeasts of *S. bombicola* clade. Kim et al demonstrated antibacterial effect of sophorolipids produced by *C. bombicola* strain against gram-positive bacteria at concentrations 0.5–4 mg/L but not against *E.coli* [37]. High antimicrobial activity of sophorolipids at MIC 4.88–19.5 mg/L was demonstrated against a wide range of gram-positive and gram-negative bacteria isolated from salted hides [38]. However there are also reports about relatively high MICs of sophorolipids against various bacteria. Sleimann et al described antibacterial activity of sophorolipids against *E.coli* and *S.aureus* at doses much higher than clinically relevant (higher than 128–512 mg/L) [39], Lydon et al reported acidic sophorolipids being effective against *E. faecalis* and *P. aeruginosa* at 5 g/l [5]. Inhibition of various *E.coli* strains by sophorolipids was reported at rather high concentrations of 1–10 g/L, the antimicrobial activity of biosurfactants being dependent among other factors on the structural form of sophorolipid [4]. Antifungal activity of sophorolipids against various pathogenic fungi was reported by Yoo et al at concentrations 0.1–2 g/L [40], Sen et al at concentrations 62–1000 mg/L [23]. Several authors reported the difference in antimicrobial activity of different structural forms of sophorolipids [4, 37].

As it was shown that the studied yeast strains synthesize several forms of glycolipids it could be presumed that some forms of these biosurfactants could possess the more pronounced antibacterial activity. The optimization of cultivation conditions would increase biosurfactant synthesis by the studied strains and further research of antimicrobial activity of different forms of the produced biosurfactants could demonstrate the higher efficiency against various gram-positive bacteria.

Conclusion. This work aims to contribute to the description of new yeast strains as promising biosurfactant producers. Mostly biosurfactants of microbial origin can't compete with synthetic ones due to the production costs however in food, pharmaceutical and cosmetic industries biosurfactants produced by non-pathogenic yeasts would provide a safe and green alternative to synthetic compounds. Two yeast strains *C. gropengiesseri* and *C. bombicola* isolated from flowering plants produce glycolipid biosurfactants stable at high salinity and temperature and exhibiting weak antimicrobial activity against gram-positive bacteria. The surface activity of both strains is indicative of their potential as biosurfactant producers.

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ХАРАКТЕРИСТИКА, СТАБІЛЬНІСТЬ ТА АНТИМІКРОБНА АКТИВНІСТЬ БІОСУРФАКТАНТІВ, ЩО ПРОДУКУЮТЬСЯ ДРІЖДЖАМИ *CANDIDA,* ІЗОЛЬОВАНИМИ З КВІТКОВИХ РОСЛИН

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Резюме

Відомо, що дріжджі здатні продукувати сполуки з поверхневою активністю, тобто біосурфактанти, які включають манозилеритритол-ліпіди, софороліпіди та інші сполуки гліколіпідної природи. Місця, багаті на цукри, можуть слугувати одним з потенційних джерел для ізоляції дріжджів, що продукують біосурфактанти. В попередніх дослідженнях з квіткових рослин та бджіл *Apis mellifera* було ізольовано 160 штамів дріжджів. Два штами дріжджів, попередньо віднесені до *Candida* spp., було відібрано як найбільш перспективні продуценти біосурфактантів. **Метою** цієї роботи було охарактеризувати біосурфактанти, що продукуються відібраними штамами дріжджів, а саме: визначити їх поверхневі властивості, стабільність та потенційну антимікробну активність щодо штамів бактерій та дріжджів. **Методи**. Ідентифікацію дріжджів проводили за фенотиповими ознаками. Біосурфактанти виділяли шляхом екстракції етилацетатом. Антимікробну активність визначали з використанням диско-дифузійного методу та методу серійних розведень. Стабільність біосурфактантів досліджували за методом «розтікання нафти» за різних значень pH, температури, концентрації солі. **Результати**. Два штами дріжджів 79a та 156a були ідентифіковані як *Candida gropengiesseri* та *Candida bombicola* відповідно. Обидва штами продукували суміш принаймні декількох форм гліколіпідів з Rf 0,15, 0,21, 0,31–0,35, 0,41–0,44, 0,5 та 0,62. Додавання до середовища гідрофобного джерела вуглецю (соняшникової олії) призводило до підвищення синтезу біосурфактантів більш ніж в 5 раз. Штами *C. gropengiesseri* 79a та *C. bombicola* 156a знижували поверхневий натяг середовища SL до 36.0–36.6 мН/м за 6 діб культивування. Біосурфактанти зберігали стабільність за умов підвищеної температури та високого вмісту солі, але частково втрачали активність за умов лужного pH. Екстракти біосурфактантів проявляли слабку антимікробну активність відносно грам-позитивних бактерій, але не мали пригнічуючої дії щодо грам-негативних бактерій та дріжджів. **Висновки.** Два штами дріжджів *C. gropengiesseri* та *C. bombicola*, ізольовані з квіткових рослин, продукують гліколіпідні біосурфактанти, стабільні за високої солоності та температури та зі слабкою антимікробною активністю проти грам-позитивних бактерій. Поверхнево-активні властивості досліджених штамів вказують на їх потенціал як продуцентів біосурфактантів.

Ключові слова: дріжджі, біосурфактанти гліколіпідної природи, стабільність, антимікробна активність.

ХАРАКТЕРИСТИКА, СТАБИЛЬНОСТЬ И АНТИМИКРОБНАЯ АКТИВНОСТЬ БИОСУРФАКТАНТОВ, СИНТЕЗИРУЕМЫХ ДРОЖЖАМИ CANDIDA, ВЫДЕЛЕННЫМИ ИЗ ЦВЕТКОВЫХ РАСТЕНИЙ

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Резюме

Известно, что дрожжи могут синтезировать вещества с поверхностной активностью, то есть биосурфактанты, к которым относятся маннозилэритрит-липиды, софоролипиды и другие соединения гликолипидной природы. Места с высоким содержанием сахаров могут быть одним из потенциальных источников выделения дрожжей, продуцирующих биосурфактанты. Ранее из цветковых растений и пчел *Apis mellifera* было выделено 160 штаммов дрожжей, из которых два штамма, предварительно идентифицированных как *Candida* spp**.,** были отобраны как наиболее перспективные продуценты биосурфактантов. **Целью** данной работы было изучить биосурфактанты, продуцируемые отобранными штаммами дрожжей, а именно: определить их поверхностную активность, стабильность и потенциальную антимикробную активность в отношении бактерий и дрожжей. **Методы**. Идентификацию дрожжей проводили на основании фенотипических тестов. Биосурфактанты выделяли путем экстракции этилацетатом. Антимикробную активность определяли диско-диффузионным методом и методом серийных разведений. Стабильность биосурфактантов изучали методом «растекания нефти» при разных значениях pH, температуры, концентрации соли. **Результаты**. Два штамма дрожжей 79а и 156а были идентифицированы как *Candida gropengiesseri* и *Candida bombicola* соответственно. Оба штамма продуцировали смесь нескольких форм гликолипидов с Rf 0,15, 0,21, 0,31–0,35, 0,41–0,44, 0,5 и 0,62. Внесение в среду гидрофобного источника углерода (подсолнечного масла) приводило к повышению синтеза биосурфактантов более чем в 5 раз. Штаммы *C. gropengiesseri* 79а и *C. bombicola* 156а снижали поверхностное натяжение среды SL до 36.0–36.6 мН/м за 6 дней культивирования. Биосурфактанты сохраняли стабильность в условиях повышения температуры и высоких концентраций соли, но частично утрачивали активность при щелочном pH. Экстракты биосурфактантов проявляли слабую антимикробную активность в отношении грам-положительных бактерий, но не обладали антимикробным эффектом в отношении грам-отрицательных бактерий и дрожжей. **Выводы.** Два штамма дрожжей *C. gropengiesseri* и *C. bombicola*, выделенные из цветковых растений, синтезируют гликолипидные биосурфактанты, стабильные при высокой солености и температуре и со слабой антимикробной активностью против грам-положительных бактерий. Поверхностно-активные свойства исследованных штаммов свидетельствуют об их потенциале как продуцентов биосурфактантов.

Ключевые слова: дрожжи, биосурфактанты гликолипидной природы, стабильность, антимикробная активность.

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