Screening of microorganisms producing biosurfactants from renewable substrates

Liliya Biktasheva[1*], *Alexander* Gordeev[1], *Anastasia* Kirichenko[1], *Natalia* Danilova[1] and *Svetlana* Selivanovskaya[1]

[1]Kazan (Volga Region) Federal University, 18, Kremlevkskaya, Kazan, 42008, Russian Federation

Abstract. Biosurfactants are one of the promising biotechnological products applied in agriculture. Their use, however, is currently far from economically viable, due to the expensive feedstock for the growth of microorganisms. The solution to this problem can be to reduce the cost of production by using organic waste as a nutrient substrate. In this study, oil-containing wastes were considered as substrates - waste frying sunflower oil and petroleum-contaminated soil. At the first stage of research, we screened native waste microorganisms capable of synthesizing biosurfactants. As a result of the study, strains with the ability to form biosurfactants were isolated. Six strains (A, B, C, D, E, F) were isolated from waste frying sunflower oil, two strains (A1, B1) were isolated from petroleum-contaminated soil. The highest yield of biosurfactants is typical for strains A and A1 - 0.429 and 0.502 mg $ml^{-1,}$ while the best ratio of biosurfactant mass to cell biomass is typical for strains A1 and E - 0.9 and 0.6. The most effective producer of biosurfactants turned out to be strain E with an emulsifying activity of E24 equal to 80% and a surface tension of the culture liquid of 27.1 mN m^{-1}.

1 Introduction

Currently, biosurfactants are attracting more and more attention due to their promising qualities and possible breadth of application. The ability of biosurfactants to change the surface tension of water, the presence among them of substances with fungicidal properties, high biodegradability and safety for the environment allows them to be used in agriculture as adjuvants and fungicides. It is known that microorganisms of the genus *Bacillus* form biosurfactants that can suppress the growth of both pathogenic fungi and bacteria [1]. Thus, *Bacillus mojavensis* is an endophyte that synthesizes biosurfactants that enhance bacterial immunity and inhibit the growth of fungi of the *Fusarium* genus [2]. Bacteria of the genus Pseudomonas are capable of destroying fungal mycelium using rhamnolipids due to their amphiphilic nature, integrating into plasma membranes [3]. The emulsifying ability of biosurfactants and their ability to reduce the surface tension between water and oil, remaining in a wide range of pH and salinity, allow us to explore the possibility of their use in the field of increasing oil production of high-viscosity oils instead of chemical surfactants [4].

* Corresponding author: biktasheval@mail.ru

The successful use of biosurfactants largely depends on the economic balance between the costs of their production and the benefits of using them. In this connection, attention is drawn to the search for cheap raw materials for their production, which can be organic waste [5].

In addition, the use of organic waste as a raw material for biotechnological production makes it possible to contribute to solving the problem of waste disposal and recycling. These wastes can include both agricultural waste (rapeseed cake, sunflower cake, potato peels), food waste (vegetable oils, alcohol and dairy waste) and industrial waste (oil industry waste, sewage sludge, grease trap waste) [6]. The use of these wastes to obtain biosurfactants allows them to be safely disposed of, while some of them are difficult to process by other methods.

A variety of microorganisms produce biosurfactants of various chemical compositions. The nature and amount of biosurfactant produced depends both on the site where the microorganism was isolated and on the nutrient substrates available for their growth [7]. There are many microorganisms isolated from contaminated soils, sewage and sewage, industrial and agro-industrial waste. These microorganisms have the ability to grow on substrates that are considered potentially harmful to other non-producing microorganisms [8]. Thus, Chebbi et al. (2017) found a strain of *Pseudomonas aeruginosa* from motor oil-contaminated soil, Liu et al. (2016) isolated fungi of the species *Wickerhamiella domercqiae* from rice straw waste, Singh et al. (2019) isolated bacteria of the genera *Stenotrophomonas* sp. and *Brevisbacillus brevis* from textile sludge [9-11].

Depending on the type of raw material, the obtained biosurfactant may have a certain structure and, accordingly, properties [12]. For example, Mouafo et al. (2018) noted that, depending on the substrate, *Lactobacillus synthesizes* either glycolipids (on a medium with glycerol) or glycoproteins (sugarcane molasses) [13]. Haba et al. (2000) note that the surface tension of *Candida* is also dependent on nutrient substrates, with sunflower oil causing a more effective reduction in surface tension than olive oil [14].

Some authors note that oil substrates allow the synthesis of surfactants more intensively [15]. Therefore, our main goal is to consider organic waste for the production of biosurfactants as an environmentally friendly and cost-effective raw material. The first stage of this work was the screening of native microorganisms of oily waste. In this work, studies were carried out on the isolation of microorganisms capable of forming biosurfactants from waste frying sunflower oil and petroleum-contaminated soil.

2 Materials and methods

2.1 Enrichment and isolation of pure strain

Soil artificially contaminated with used waste frying sunflower oil and petroleum-contaminated soil were used as inoculum for enrichment culture in mineral media (MMA). The microorganisms were enriched in a 250 ml Erlenmeyer flask containing 100 ml of a sterilized mineral medium to which glycerol was added. The flasks were incubated for 5 days at 28°C and 120 rpm, and then the mixed culture was transferred to Petri dishes. Eight single colonies were selected and seeded onto new Petri dishes.

2.2 Production and Extraction of biosurfactant.

Isolates were cultivated in glycerol nitrate medium at 28 °C and 120 rpm for 5 days. The concentration of crude glycerol in the medium was 40 g L^{-1}. The medium except crude

glycerol contained (g L^{-1}): $NaNO_3$ (4.0), K_2HPO_4•$3H_2O$ (4.0), KH_2PO_4 (3.0), $MgSO_4$•$7H_2O$ (0.5), KCl (0.5), NaCl (0.5), $CaCl_2$•$2H_2O$ (0.2).

The cells were removed from the culture by centrifugation at 5000 rpm for 20 minutes, the resulting cell pellet was dried in an oven at 105°C overnight. Dry cell biomass was determined gravimetrically. For the extraction of biosurfactant, the cell-free supernatant was acidified to pH 2 using 6N HCl, then stored at 4°C overnight and centrifuged at 5000 rpm and 4°C for 20 min. The precipitate was purified by dissolving in a $CHCl_3$:CH_3OH (2:1, v/v) mixture followed by rotary evaporating under vacuum. The crude biosurfactant was quantified gravimetrically.

2.3 Biosurfactant Efficacy Evaluation

Evaluation of the effectiveness of the biosurfactant was carried out on a cell-free supernatant obtained at the previous stages. The surface tension was measured using a K20 tensiometer (KRUSS, Germany) using the Du Nouy ring method at room temperature. The emulsification test was done as follows: 5 mL of cell-free supernatant and 5 mL of petroleum were added in test tubes followed by rapid vigorous vortexing for 2 min. The emulsifying layer was evaluated after 24 hours. By adopting the formula given below the E24% was calculated.

$$E24\% = (\text{Height of the emulsified layer/total height of liquid column}) \times 100\% \quad (1)$$

3 Results and Discussions

In the course of the work, screening of microorganisms of organic waste was carried out, for which waste frying sunflower oil and petroleum-contaminated soil were used. Six different strains (A, B, C, D, E, F) were isolated from waste frying sunflower oil, and 2 strains were isolated from petroleum-contaminated soil (A1, B1). The isolated strains were cultivated on a medium with glycerol under equal conditions to select the most effective producers of biosurfactants.

At the first stage of the work, an assessment was made of the mass of isolated biosurfactants, as well as its ratio with the total cell biomass (Fig. 1).

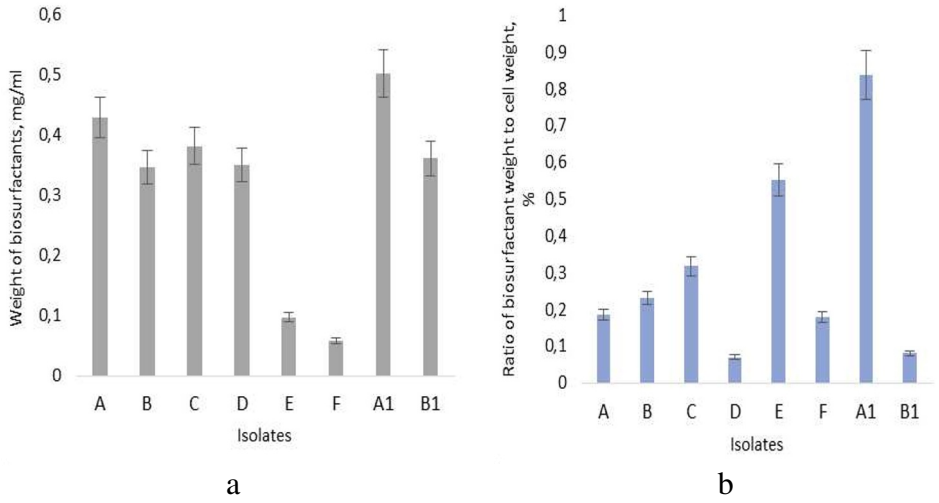
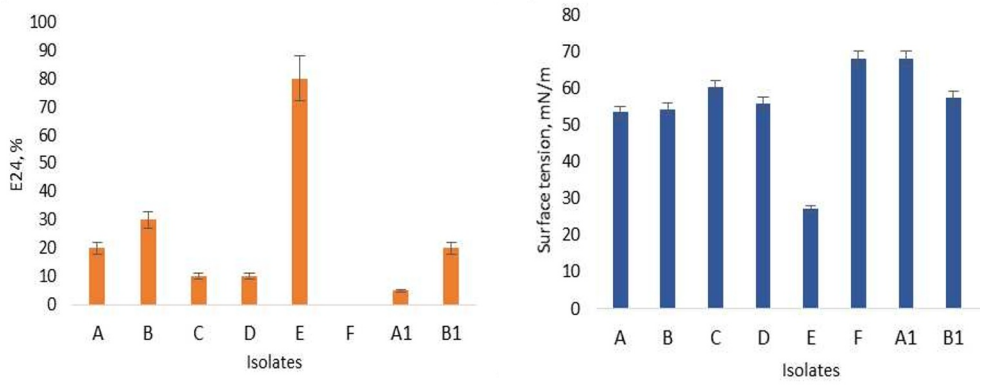


Fig. 1. Weight of isolated biosurfactants (a) and ratio of biosurfactants to cell weight (b)

It can be noted that the highest production of biosurfactants was noted for strain A1 − 0.502 mg ml^{-1}, as well as strain A − 0.429 mg ml^{-1} (Fig. 1a). This yield of the product is comparable to the other research, so Nayarisseri et al. (2018) obtain the yield of the biosurfactant of the *Bacillus subtilis* strain within 0.324 g l^{-1} [16]. The lowest yield was noted for strains F and E – 0.09 and 0.06 mg ml^{-1}, respectively. The low yield of biosurfactants of these strains may indicate their inability to produce biosurfactants, but additional selection of the medium for their cultivation may also be required.

The ratio of the mass of obtained biosurfactants to cell biomass (g biosurfactant/g biomass) was also calculated (Fig. 1b). The highest yield was noted for strains A1 and E, 0.9 and 0.6, respectively. The lowest yield was noted for strains D and B1, 0.07 and 0.08, respectively. It can be noted that, according to the research data, the biosurfactant yield for most of the strains we isolated is effective. For example, Vedaraman et al. (2011) describes the yield of *Bacillus subtilis* surfactin in terms of biomass − 0.23 [8].

The ability to change the surface tension of water and the emulsifying ability are indicators of the effectiveness of biosurfactants, which in turn depends on the amount of biosurfactants produced and their chemical nature. For which, at the next stage, the emulsifying activity and changes in surface tension in the cell-free culture fluid were evaluated (Fig. 2 a-b).



a b

Fig. 2. Emulsifying activity (a) and surface tension of culture liquid (b)

It was noted that according to the results of the E24 test, strain E has the highest emulsifying activity − 83% (Fig. 2a). Strains F and A1 show a minimum emulsifying activity of 0 and 5%. It is known that the biosurfactant is considered effective at E24 more than 50%, which suggests that the E strain is effective [17].

An assessment of the surface tension in the culture liquid showed (Fig. 2b) that the most effective biosurfactant is the one synthesized by strain E – 27.1 mN m^{-1}. Strains F and A1 show the least efficiency − 67.9 and 68.1 mN m^{-1}, the values of which are close to those of the control sample − distilled water (72 mN m^{-1}). The remaining strains show approximately equal surface tension in the range from 53.5 to 60.3 mN m^{-1}. According to the other research data, it can be argued that strain E effectively reduces surface tension relative to other effective strains [18]. Numerous works also confirm the successful use of oily wastes, such as waste frying sunflower oil and used motor oil, as substrates for obtaining biosurfactants [5].

4 Conclusion

In the course of this study, microorganisms with the ability to synthesize biosurfactants were isolated from oil-containing organic waste. Six strains were isolated from waste frying sunflower oil, two strains were isolated from petroleum-contaminated soil. The highest yield of biosurfactants is typical for strains A and A1 − 0.429 and 0.502 mg ml^{-1}, while the best ratio of biosurfactant mass to cell biomass is typical for strains A1 and E − 0.9 and 0.6. Strain E, isolated from waste frying sunflower oil, turned out to be the most effective producer of biosurfactants; its emulsifying activity is 80%, and the surface tension of the culture liquid is 27.1 mN m^{-1}. Obviously, this strain deserves additional attention as the most promising, since even at low yields, and, accordingly, at a low concentration of the target component in the culture medium, it showed the highest performance. Strain E will be further investigated to improve the yield of biosurfactants on various media and under various cultivation conditions. However, other strains also require additional research and cultivation on other substrates, including organic waste.

Acknowledgments

The work has been performed with the financial support of Russian Science Foundation, grant No. 23-24-00611.

References

1. Crouzet J, Arguelles-Arias A, Dhondt-Cordelier S, Cordelier S, Prsic J, Hoff G, Mazeyrat-Gourbeyre F, Baillieul F, Clement C, Ongena M and Dorey S 2020 Biosurfactants in Plant Protection Against Diseases: Rhamnolipids and Lipopeptides Case Study. Front. Bioeng. Biotechnol. 8 1014
2. Bacon C W and Hinton D M 2011 Bacillus mojavensis: Its Endophytic Nature, the Surfactins, and Their Role in the Plant Response to Infection by Fusarium verticillioides Bacteria in Agrobiology: Plant Growth Responses (Berlin: Springer) 370
3. Aranda F J, Espuny M J, Marques A, Teruel J A, Manresa A and Ortiz A 2007 Thermodynamics of the interaction of a dirhamnolipid biosurfactant secreted by Pseudomonas aeruginosa with phospholipid membranes. Langmuir 23 2700–2705
4. Geetha S J, Banat I M and Joshi S J 2018 Biosurfactants: Production and Potential applications in Microbial Enhanced Oil Recovery (MEOR). Biocatal. Agric. Biotechnol. 14 23-32
5. Makkar R, Cameotra S and Banat I 2011 Advances in utilization of renewable substrates for biosurfactant production. AMB Express 1
6. Makkar R S and Cameotra S S 2002 An update on the use of unconventional substrates for biosurfactant production and their new applications. Appl. Microbiol. Biotechnol. 58 428–434
7. Kaur K, Sangwan S and Kaur H 2017 Biosurfactant production by yeasts isolated from hydrocarbon polluted environments. Environ. Monit. Assess. 189 603
8. Vedaraman N and Venkatesh N M 2011 Production of surfactin by Bacillus subtilis MTCC 2423 from waste frying oils. Braz. J. Chem. Eng. 28 175-180
9. Chebbi A, Hentati D, Zaghden H, Baccar N, Rezgui F, Sayadi S and Chamkha M 2017 Polycyclic aromatic hydrocarbon degradation and biosurfactant production by a newly isolated Pseudomonas sp. strain from used motor oil-contaminated soil. Int. Biodeter. Biodegrad. 122 128–140

10. Liu X G, Ma X J, Yao R S, Pan C Y and He H B 2016 Sophorolipids production from rice straw via SO3 micro-thermal explosion by Wickerhamiella domercqiae var. sophorolipid CGMCC 1576. AMB Express 6 13568-016-0227-7
11. Singh R, Singh S K and Rathore D 2019 Analysis of biosurfactants produced by bacteria growing on textile sludge and their toxicity evaluation for environmental application. J. Dispers. Sci. Technol. 14
12. Zhou H, Chen J, Yang Z, Qin B, Li Y and Kong X 2015 Biosurfactant production and characterization of Bacillus sp. ZG0427 isolated from oil-contaminated soil. Ann. Microbiol. 65 2255–2264
13. Mouafo T H, Mbawala A and Ndjouenkeu R 2018 Effect of Different Carbon Sources on Biosurfactants' Production by Three Strains of Lactobacillus spp. Biomed. Res. Int. 13 5034783
14. Haba E, Espuny M J, Busquets M and Manresa A 2000 Screening and production of rhamnolipids Pseudomonas aeruginosa 47T2 NCIB 40044 from waste frying oils. J. Appl. Microbiol. 88 87–379
15. Vijaya B, Jayalakshmi N R and Manjunath K 2013 Enumeration of biosurfactant producing microorganisms from oil contaminated soil in and around Bangalore (India). Int. J. Curr. Sci. 5 86–94
16. Nayarisseri A, Singh P and Singh S 2018 Screening, isolation and characterization of biosurfactant producing Bacillus subtilis strain ANSKLAB03. Bioinformation 14
17. Oliveira EM, Sales V H G, Andrade M S, Zilli J E, Borges W L and Souza T M 2021 Isolation and Characterization of Biosurfactant-Producing Bacteria from Amapaense Amazon Soils. Int. J. Microbiol. 11
18. Rufino R, Luna J, Galba C T and Sarubbo L 2014 A characterization and properties of the biosurfactant produced by Candida lipolytica UCP 0988. Electron. J. Biotechnol. 17 34–38