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BIOSYNTHESIS AND CHARACTERISTICS OF METABOLITES OF *Rhodococcus erythropolis* AU-1 STRAIN

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ABSTRACT

Practically important metabolites of *Rhodococcus erythropolis* Au-1 strain were obtained: trehalose lipids, exopolysaccharide and cellular polymer – polyhydroxyalkanoate. The structures of the products were established by the methods of TLC, IR and UV-Vis spectroscopy. The purification of trehalose lipids from crude extract was carried out using silica gel column chromatography, one of the trehalose lipids fractions was identified as a trehalose dimycolate. The presence of (NH) group was detected in the exopolysaccharide. For the first time, a biodegradable polymer was isolated from bacteria cells of the strain *R. erythropolis* Au-1, it was identified as the polyhydroxyalkanoate. The physico-chemical properties of the metabolites of the strain *R. erythropolis* Au-1 were established: the surface tension of trehalose lipids fractions was 30,5-43,2 mN/m; the emulsification activity of the exopolysaccharide solutions (1-10 g/L) with vaseline oil was 42-58%. The differential thermal analysis was used to define starting temperatures of degradation of trehalose dimycolate (130 °C), exopolysaccharide (180 °C) and polyhydroxyalkanoate (164 °C, melting point 42oC). The obtained results expand the possibilities of practical application of trehalose lipids, exopolysaccharide and polyhydroxyalkanoate of the strain *R. erythropolis* Au-1.

Keywords: *Rhodococcus erythropolis*, trehalose lipids, exopolysaccharides, polyhydroxyalkanoates, thermal analysis, UV-Vis and IR spectroscopy

INTRODUCTION

The use of synthetic products is an acute problem due to their ecotoxicity and low biodegradability. Therefore, the successful development of modern technologies involves the introduction of new eco-friendly substances, which include biosurfactants and biopolymers of microbial origin (**Fernandes *et al*, 2020; Johnson *et al*., 2020**).

Actinobacteria of the genus *Rhodococcus* have practical potential, due to their ability to synthesize biosurfactants and biopolymers – exopolysaccharides (EPS) and cellular polyhydroxyalkanoates (PHA). Biosurfactants synthesized by *Rhodococcus* are glycolipids, specifically trehalose lipids (figure 1 a). These compounds combine high efficiency (foaming, emulsifying capacity, reduction of surface tension, solubilization, resilience to pH and temperature etc), biological activity and environmental safety. Generally they are less toxic and more biodegradable than synthetic surfactants (**Jahan *et al*, 2020; Mulligan *et al*., 2014**). The biosurfactants increase permeability of cell membranes, so enhance the effect of various biological active compounds (**Sotirova *et al*., 2012; Koretska *et al*., 2020; Yaremkevych *et al*., 2020**). Therefore, much attention is paid to the use of biosurfactants in various industries, mainly in petroleum, food, cosmetics, pharmaceuticals, bioremediation and agriculture (**Ceresa et al., 2021; Markande *et al*., 2021; da Silva *et al*., 2021**).

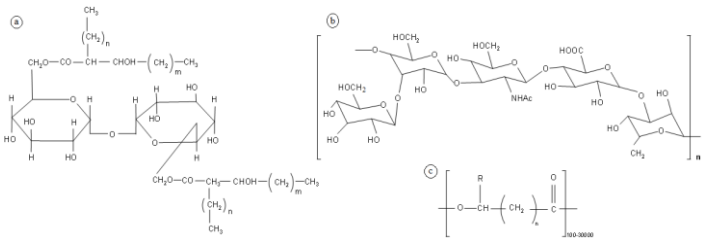

Figure 1 Structural formula of (a) trehalose-6,6'-dimycolate: m + n = 27-31 (**Rapp *et al*., 1979**); (b) exopolysaccharide of *R. erythropolis* strain PR4: -Ac are fatty acids (**Urai *et al*., 2007**); (c) polyhydroxyalkanoate: n=1, 2, 3, R – CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, C_9H_{19}, $C_{10}H_{21}$, $C_{11}H_{23}$, $C_{12}H_{25}$, $C_{13}H_{27}$ (**Ojumu *et al*., 2004**).

Exopolysaccharides are high molecular weight extracellular polymers consisting of units of sugar moieties and can be attached to a carrier lipid, also be associated with proteins, lipids, metal ions etc. (**Rana & Upadhyay, 2020**). At the same time exopolysaccharides of *Rhodococcus erythropolis* are composed of D-galactose, D-mannose, D-glucose and D-glucuronic and consist fatty acid residues (**Urai *et al*., 2007),** figure 1 b. microorganisms synthesize EPS to protect against extreme conditions in extreme conditions [**Deming & Young, 2017; Kazak *et al*., 2010**]. Exopolysaccharides have industrial applications in food, cosmetics, pharmaceutical, petroleum industries due to their properties, such as emulsifying, antioxidant, anti-biofilm, antiviral activities etc. (**Madhuri & Prabhakar, 2014**; **Rana & Upadhyay, 2020**).

Polyhydroxyalkanoates are natural polyesters accumulated in bacterial cells and served as both energy source and carbon storage, especially when bacteria grow on unbalanced media (**Nisha *et al*., 2012; Sudesh *et al*., 2000**). PHA consists of 100 - 30,000 monomeric units of 3-, 4-, 5 hydroxyalkanoic acids (**Ojumu *et al*., 2004**) (figure 1 c). Due to their biodegradability, biocompatibility, chemical-diversity PHA are widely used in agriculture and medicine (**Kalia *et al*., 2021; Zhang *et al*., 2018**). PHA also are considered promising eco-friendly substitutes for plastics (**Raza *et al*., 2018; Shah *et al*., 2008**).

Thus, the aim of the present study is obtaining and investigation of metabolites of *R. erythropolis* Au-1 strain, promising for the replacement of synthetic materials.

MATERIALS AND METHODS

Materials

The objects of the study were the metabolites of the bacterial strain *Rhodococcus erythropolis* Au-1 (Ukrainian collection of microorganisms of D. Zabolotny

Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (*Rhodococcus erythropolis* UCM Ac-603).

Cultivation of *R. erythropolis* Au-1

The cultivation of bacteria was performed in Erlenmeyer flasks (750 ml) with a working volume 150 ml on a rotary shaker (220 rpm) at 30 °C in liquid nutrient medium with the following composition (g/L): $NaNO_3$ – 3.0; K_2HPO_4 – 2.0; KH_2PO_4 – 2.0; $MgSO_4 \times 7H_2O$ – 0.5; $Na_3C_6H_5O_7 \times 2H_2O$ – 1.0; yeast extract – 1.0; carbon source – 20.0; distilled water – up to 1 L Cultivation time – 5 days. Hexadecane (for the biosynthesis of trehalose lipid surfactants) or glycerol (for the biosynthesis of PHA) were used as carbon sources. The inoculum (24h culture) was taken in the amount 10 % of the medium's volume, the cell titer – 5×10^8 CFU/mL.

Isolation and purification of trehalose lipid surfactants

The bacterial cells were separated from the cultural liquid by centrifugation at 6000 rpm for 15 min. Trehalose lipids surfactants were extracted from cells with Folch mixture (chloroform-methanol 2:1) (**Folch *et al.*, 1957**). The trehalose lipids fractions were separated via chromatography method (**Smyth *et al.*, 2010**) on a column of 220×15 mm with silica gel 60 (0.06-0.20 mm, ROSS, Belgium). A sample of trehalose lipid surfactants (0.1 g) was dissolved in 15 mL of Folch mixture and was placed on a silica gel surface. The column was washed with hexane for removing of residual hexadecane, non-polar lipids and pigments. Next, the fractions were eluted with solvent systems with the gradually increasing polarity: chloroform, chloroform: methanol (90:10, 50:10, 40:10, 30:10, 20:10, 10:10), methanol. The composition of each fraction was monitored by thin layer chromatography method (TLC).
The plates "Merck" (25 Cromatofolhas AL TLC 20 × 20 cm Silicagel 60, Germany) were used for TLC. Qualitative analysis was performed with a solvent system – chloroform-methanol-water 65: 15: 2. Identification of glycolipids as part of surfactants was performed by the plates visualization with 4-methoxy-benzaldehyde spray reagent (**Kretschmer & Wagner, 1983; Smyth *et al.*, 2010**). The green spots on the plate indicated the glycolipids presence.

Determination of physicochemical properties

The surface tension of biosurfactants solutions was determined by Du Nui method with a platinum ring (**Butt *et al.*, 2003**) on Krüss K6 tensiometer (Krüss GmbH, Germany). The emulsification activity was measured using the method described **Rahman *et al.* (2003)**. About 2 mL of vaseline oil and 2 mL of cell-free medium (supernatant) were inoculated to a test tube and homogenized by vortexing at high speed for 2 min. After 24 h, the emulsification activity was determined as the percentage of height of the emulsified layer (mm) divided by the total height of the liquid column (mm).

Isolation and identification of polyhydroxyalkanoate

Bacterial cells (after extraction of cell-bound biosurfactants) were disintegrated with ethanol (100 rpm, 2 h, 35 °C). Centrifuged (15 min, 6000 rpm), the precipitate was transferred to a Soxhlet extractor, where it was extracted with chloroform for 5 cycles of extraction. The solvent was evaporated in vacuum. The polymer was purified via dissolving in chloroform and precipitated with cold ethanol. The resulting polyhydroxyalkanoate precipitate was decanted and dried to constant weight (**Semeniuk et al., 2020a, b**). The preliminary identification of PHA was via TLC with a solvent system: ethyl acetate-benzene 1:1, visualization – in iodine chamber (5-10 min); the appearance of yellow-brown spots with $R_f = 0.8$ indicated the PHA presence (**Senthilkumar *et al.*, 2016**).

Isolation of exopolysaccharide

The exopolysaccharide was precipitated from the culture liquid supernatant (CLS) with two volumes of 96% ethanol and kept at 4 °C for 12 h. (**Williams & Wimpenny, 1977**). The precipitate was separated by centrifugation (5000 rpm, 30 min) and purified by reprecipitation from distilled water twice. The obtained EPS was dried at 80 ˚C, the mass was determined gravimetrically.

Infrared spectroscopy

Infrared absorption spectra of the samples of trehalose-6,6'-dimycolate, exopolysaccharide, and polyhydroxyalkanoate, were analyzed on a Thermo Scientific Nicolet iZ10 Fourier spectrometer (USA) using diamond windows. The spectra were recorded in the wavenumber range within 4000 – 525 cm^{-1}. The literature data (**Gordon & Ford, 1973**) was used for spectrum interpretation.

Ultraviolet - Visible Spectroscopy

UV-Vis spectra was registered as described in (**Panda *et al.*, 2008**). The purified and dried cellular polymer (20 mg) was transferred into a test tube, and 10 ml of concentrated H_2SO_4 was added. The solution was heated on a water bath for 10 minutes. After cooling and thorough agitation, the absorption spectrum of the obtained solution was registered. Concentrated H_2SO_4 was used as a solvent for comparison.
The absorption spectra of the samples in the UV-visible region were analyzed at 200-800 nm in quartz cuvettes (1 mm) on a UV mini-1240 spectrophotometer (Shimadzu, Japan).

Thermal analysis

Thermal analysis was performed on a Q–1500D derivatograph of the Paulik-Paulik-Erdey system in the temperature range of 20– 1000 °C with a free air access to the oven. The heating rate was 5°C/min. The average weight of the samples was 80 mg, the reference substance was aluminum oxide (**Khovanets' *et al.*, 2017**).

RESULTS AND DISCUSSION

Characteristics of trehalose-6,6-dimycolate

Trehalose lipid surfactants were obtained via extraction from cells mass of the *R. erythropolis* Au-1 strain in the amount of 3.4 g\L. These surfactants were found to contain non-polar lipids and polar lipids (**Koretska *et al.*, 2014; Kretschmer & Wagner, 1983**). Polar lipids have better surface activity, and trehalose lipids make up a significant mass fraction among them. The structure of trehalose-6,6'-dimycolate is shown in fig. 1.
In order to isolate trehalose-6,6'-dimycolate, the extract of trehalose lipid surfactants of *R. erythropolis* Au-1 strain was divided into fractions using the adsorption chromatography method on a column with silica gel (Figure 2).

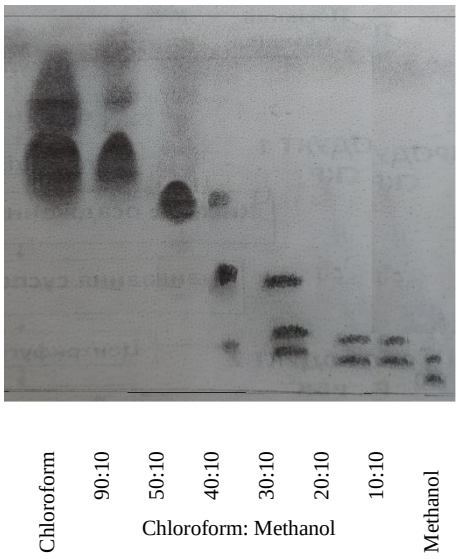

Figure 2 TLC of trehalose lipid surfactants fractions of *R. erythropolis* Au-1 strain; solvent system – chloroform- methanol-water, 65:15:2, visualization with 4-methoxy-benzaldehyde.

The R_f of obtained fractions and surface tension of their aqueous solutions (0.5 g/L) were determined (Table 1).

Table 1 Composition and characteristics of surfactant fractions of *R. erythropolis* Au-1

Fraction №	Eluent		Surface tension, mH/m	R_f
1	Chloroform		52.0±0,4	0.90; 0.78; 0.67
2	Chloroform: Methanol	90:10	49.5±0,4	0.90; 0.78; 0.67
3		50:10	43.2±0,4	0.50
4		40:10	37.5±0,5	0.50; 0.30; 0.12
5		30:10	32.0±0,5	0.30; 0.14; 0.09
6		20:10	31.7±0,4	0.14; 0.09
7		10:10	30.5±0,3	0.14; 0.09
8	Methanol		30.6	0.09; 0.04

Legend: surface tension was determined for lipid solutions (0.5 g/L); mobile phase: chloroform- methanol- water, 65:15:2.

It was found that fractions (№ 1, 2) contain nonpolar lipids (**Kretschmer & Wagner, 1983**) and are characterized by insignificant surface activity (49.5-52.0 mN/m). In the fraction №3 the main component is trehaloso-6,6'-dimycolate, the surface tension of its solution is 43.2 mN/m. Trehalose diacylates were detected in fractions № 4 and 5 (surface tension 32.0 - 37.5 mN/m). Fractions 6-8 contain trehalose monomycolates, trehalose monoacylates, lipopeptids and are characterized by lower surface tension: 30.5 - 31.7 mN/m. Therefore, it was shown that the fractions of trehalose lipids are effective surfactants: the surface tension of their solutions was 30.5-43.2 mN/m.

The fraction №3 (eluent chloroform: methanol 50:10) was identified by IR spectroscopy (Fig. 3 a). It was found the presence of the functional groups: 3500 – 3200 cm^{-1} (hydrogen-bonded (OH) groups); 2960 – 2800 cm^{-1} (stretching vibrations of (CH) groups); 1200 – 1000 cm^{-1} (stretching vibrations of (C–O–C) ether groups); a weak signal at 1700 m^{-1} may indicate a low content of (C=O) carbonyl groups.

The results obtained from the IR spectrum as well as TLC method (R_f=0.5) that reported in (**Kretschmer & Wagner, 1983**) confirm that trehalose-6,6-dimycolate is produced.

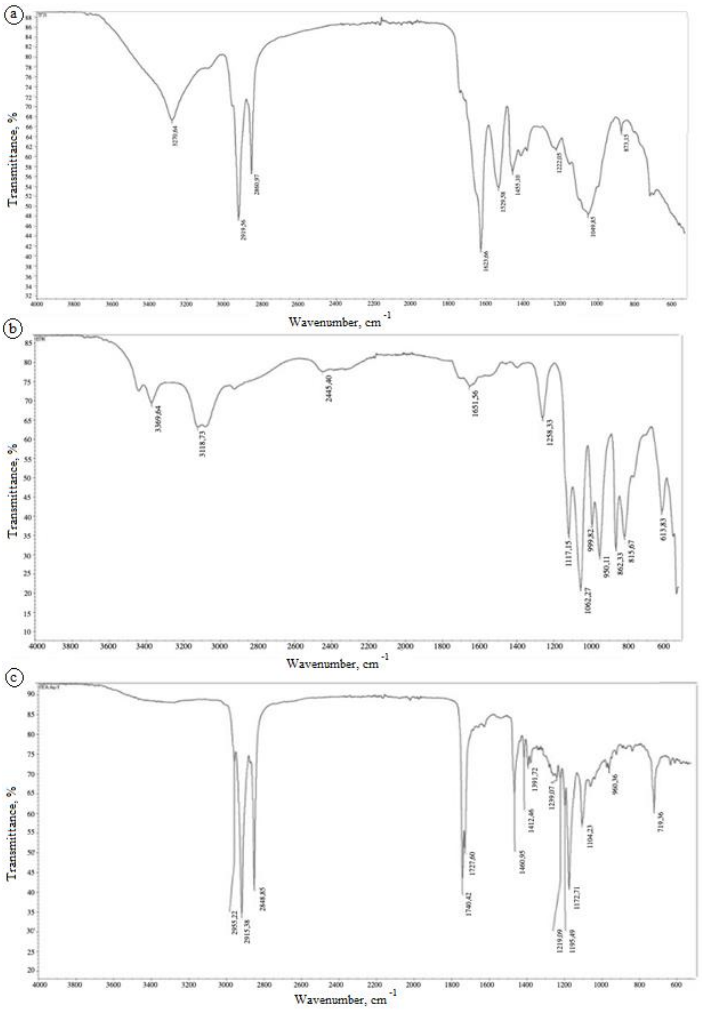

Figure 3 IR spectrums of metabolites of *R. erythropolis* Au-1: (a) trehalose-6,6'-dimycolate (fraction № 3 of trehalose lipid surfactants), (b) exopolysaccharide, (c) the cellular biopolymer

Characteristics of exopolysaccharide

An extracellular polymer of polysaccharide nature was isolated from culture liquid supernatant of *R. erythropolis* Au-1 strain in the amount of 5.0 g\L. The emulsifying activity of exopolysaccharide solutions with vaseline oil has been found. E_{24} of EPS solutions at 1 g/L, 5 g/L, and 10 g/L are 42%, 51%, and 58%, respectively. Thus, the extracellular polymer is an effective emulsifier. The exopolysaccharide of *R. erythropolis* Au-1 strain was identified by IR spectroscopy (Fig. 3 b). The EPS spectrum contains a band: at 3610 cm^{-1}, which indicates stretching vibrations of the (OH) group in the (CH_2 – OH) radical, the 3360 cm^{-1} band indicates such vibrations of the glucoside ring. In the spectrum, peaks of stretching vibrations of (CH) groups are observed within 2900 – 2800 cm^{-1}. A stretching vibration band of (C=O) carbonyl group is shown at 1700 cm^{-1}. The 1117 cm^{-1} peak refers to stretching vibrations of (C–O–C) ether group of a glycosidic bridge. The 1052 cm^{-1} band demonstrates stretching vibrations of (C–O–C) ether group of a glycosidic ring. Vibrations within 900 – 650 cm^{-1} point to stretching vibrations of (NH) group. Data of the IR spectra of the *R. erythropolis* Au-1 exopolysaccharide are consistent with the results obtained by **Urai *et al.* (2007)** for the exopolysaccharide of *R. erythropolis* PR4 strain (Fig. 1 b).

Characteristics of polyhydroxyalkanoate

For the first time, a light cream-colored biopolymer capable of forming a film was extracted from bacterial cells of the *R. erythropolis* Au-1. Its yield was 10% of the dry mass of cells. Fig. 4 shows the UV-Vis analysis of the sample of a cellular polymer after it is treated with H_2SO_4. This acid provides cleavage of PHA into crotonic acid with the absorption maximum at 235 nm (**Panda *et al.*, 2008**).

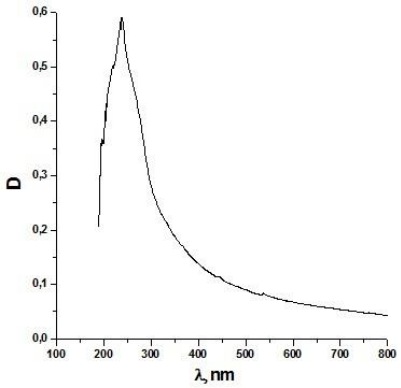

Figure 4 UV-Vis spectrum of the cellular biopolymer of *R. erythropolis* Au-1 strain.

The generated spectrum has a distinct peak at 235 nm, which affords the resulting cellular polymer to be identified as polyhydroxyalkanoate.

The TLC method was used to confirm the nature of polyhydroxyalkanoate obtained, as shown in Fig. 5.

Figure 5 TLC of the cellular biopolymer of *R. erythropolis* Au-1 strain.

On the plate, which is visualized in the iodine chamber, it is possible to notice a yellow-brown spot (Rf = 0.8), typical of PHA (**Panda *et al.*, 2008; Senthilkumar *et al.*, 2016**).

IR spectroscopy also was used to identify the cellular polymer of *R. erythropolis* Au-1 strain (Figure 3 c). It was shown the presence of following functional groups in IR spectrum: broad band at 3600 – 3100 cm^{-1} corresponds to stretching vibrations of (OH) final groups; a set of bands at 2960 – 2800 cm^{-1} refers to the symmetric and asymmetric stretching vibrations of (CH), (CH_2), (CH_3) groups; band at 1740 cm^{-1} points on stretching vibrations of (C=O) carbonyl group; band at 1238 cm^{-1} relates to the asymmetric stretching vibrations of ester groups (C–O–C); bands within 1463 – 1300 cm^{-1} correspond to bending vibrations of (CH_2) and (CH_3) groups.

Thus, the methods TLC, the UV-Vis and IR spectroscopy confirmed the isolation of biopolymer-polyhydroxyalkanoate from the cells of *R. erythropolis* Au-1 strain. The structural formula of obtained PHA is consistent with the literature (Figure 1 c).

Thermal analysis

Thermal analysis is important method used to study organic and inorganic compounds. It is used to analyze thermal stability of substances as well as to obtain visualization of the nature of processes that proceed upon heating (**Khovanets' *et al.*, 2017**). The results of the samples of obtained metabolites of *R. erythropolis* Au-1 under investigation produced by the complex thermogravimetric, differential thermogravimetric, and differential thermal analyses are given as thermograms in the figure 6.

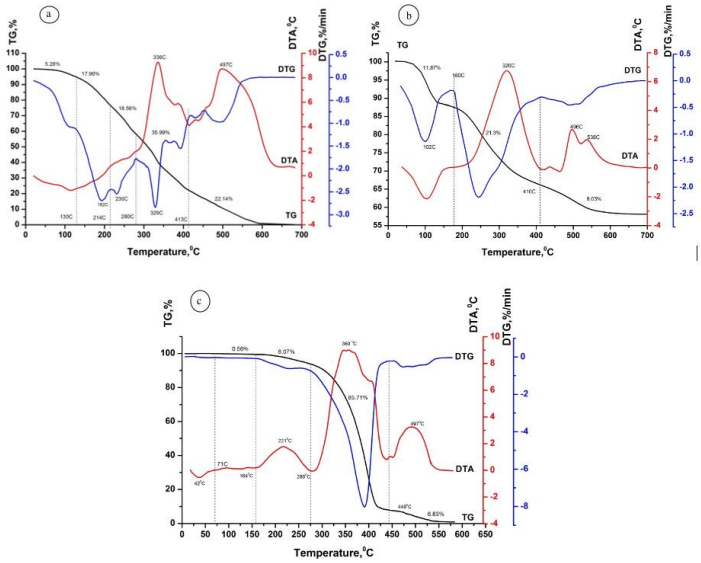

Figure 6 Thermogram of metabolites of *R. erythropolis* Au-1 strain: (a) trehalose-6,6'-dimycolate, (b) exopolysaccharide, (c) the cellular biopolymer; TG – thermogravimetric curve; DTA – differential thermal analysis curve; DTG – differential thermogravimetric curve.

Thermal analysis of trehalose dimycolate

The thermal analysis shows that thermolysis of the sample of trehalose-6,6'-dimycolate in the temperature range of 20 – 600 °C (Fig.6 a) proceed in five stages. In the temperature range of 20 – 130 °C, the first stage features the emission of solvent residues. This is followed by the sample mass loss – 5.28% – on a thermogravimetric (TG) curve and the emergence of an endothermic effect on a differential thermal analysis (DTA) curve.

At the second and third stages of thermolysis, thermo-oxidative destruction of aliphatic fragments of the sample occurs in the temperature ranges of 130 – 214˚C and 214 – 280˚C with the formation of volatile decomposition products. This is accompanied by sharp extrema that appear on the DTG curve with the maximum at 192 ˚C and 230 ˚C. It has been found the deviation of the DTA channel into the region of exothermic effects. The mass loss of the sample at the second and third stages of thermolysis is 17.96% and 18.56%, respectively.

At the fourth stage of thermolysis, the sample undergoes deeper destructive processes in the temperature range of 280 – 413°C. Such destructive processes are succeeded by the breakdown of fragments of a cyclic part of the molecule. The process ends up with partial combustion of residues of thermal-oxidative destruction of the aliphatic and cyclic parts of the trehalose-6,6'-dimycolate molecule. An intense mass loss of the sample such as 35.99% is accompanied by a sharp extremum on the DTG curve with the maximum at 329 °C and an extremely notable exothermic effect on the DTA curve with the maximum at 336 °C.

In the temperature range of 413 °C – 650 °C, there is combustion of carbonized residue of the sample formed at the previous stages of thermolysis. Following this, one can observe the mass loss of the sample (22.14°C) and the sharp extremum on the DTA curve with the maximum at 497 °C.

Thermal analysis of exopolysaccharide

Thermal degradation of the sample of exopolysaccharide of *R. erythropolis* Au-1 strain proceeds in three stages in the temperature range of 20 – 700 °C (Fig. 6 b). At the first stage of thermolysis, the emission of physically bound water happens in the temperature range of 20 – 180 °C. After the emission, it is possible to observe the sample mass loss (11.87 %), the distinct extremum on the DTG curve, and the endothermic effect on the DTA curve with the maximum at 103°C. At the second stage of thermolysis, there is thermal-oxidative destruction of EPS in the temperature range of 180 – 410 ˚C, which is followed by a gradual mass loss (21.3 %) of the sample and a sharply expressed exothermic effect on the DTA curve with the maximum at 320 ˚C. At the third stage of thermolysis, carbonized residue of the sample is gradually combusted. This process is in accord with a double exothermic effect noticed on the DTA curve with the maximum at temperatures 496 ˚C and 598 ˚C. The mass loss at this stage accounts for 8.03 %.

Thermal analysis of polyhydroxyalkanoate

Thermal degradation of the PHA sample of the *R. erythropolis* Au-1 strain includes five stages (Fig. 6 c). At the first stage of thermolysis, the biopolymer sample melts at 20 – 71 ˚C. There is no loss of sample weight during this process. The process proceeds with a distinct endothermic effect, which manifests itself on the DTA curve with a maximum at 42 °C. At the second stage of thermolysis, a slight mass loss of the sample at the temperature range of 71 – 164 ˚C might be because of the emission of volatile substances present in the sample. This process is accompanied by a slight mass loss of the sample, such as 0.56%. At the temperatures 164 – 280 ˚C, at the third stage of thermolysis, a more intense mass loss of the sample (6.07%) corresponds to the conduct of thermal oxidative processes in biopolymers. This can be seen by a distinct exothermic effect that is manifested on the DTA curve with the temperature maximum at 221 ˚C. Deep destructive processes, which are completed with combustion of destruction residues, happen at the temperatures 280 – 449 ˚C at the fourth stage of thermolysis. Such processes are followed by an 85.71% rapid mass loss of the sample and occurrence of a high exothermic effect on the DTA curve with the maximum at 360 ˚C. Combustion of carbonized residue of the biopolymer takes place in the temperature range of 449 – 600 ˚C at the fifth stage of thermolysis – it is observed the exothermic effect with maxima at 497 ˚C on the DTA curve.

CONCLUSIONS

Trehalose lipids (3.4 g/L), exopolysaccharide (5.0 g/L) and cellular polymer – polyhydroxyalkanoate (10% of the dry mass of cells) of actinobacteria *R. erythropolis* Au-1 strain were obtained.

The definition of products' structures was carried out using the TLC method, the IR and UV-Vis spectroscopy. It was shown that the cellular polymer belongs to polyhydroxyalkanoates; there are (NH) groups in the structure of the exopolysaccharide; one of the biosurfactants' fractions was identified as trehalose dimycolate. It was found that the surface tension of trehalose lipids fractions was 30,5-43,2 mN/m; the emulsification activity of the exopolysaccharide solutions (1-10 g/L) with vaseline oil was 42-58%. Due to differential thermal analysis, starting temperatures of degradation of trehalose dimycolate (130 ˚C), exopolysaccharide (180 ˚C) and polyhydroxyalkanoate (164 ˚C) were defined. The melting point of polyhydroxyalkanoate was obtained (42 ˚C).

Thus, the possibility of maximum use of the components of the culture fluid of the strain R. erythropolis Au-: both cells and supernatant is shown. This approach is economically and environmentally justified. The study of physico-chemical properties of the obtained products shows that in practice they can replace synthetic surfactants, emulsifiers and polymeric materials.

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REFERENCES

Butt, H. J., Graf, K., & Kappl, M. (2013). Physics and chemistry of interfaces. John Wiley & Sons.

Ceresa, C., Fracchia, L., Fedeli, E., Porta, C., & Banat, I. M. (2021). Recent Advances in Biomedical, Therapeutic and Pharmaceutical Applications of Microbial Surfactants. Pharmaceutics, 13(4), 466. https://doi.org/10.3390/pharmaceutics13040466

Deming, J. W., & Young, J. N. (2017). The role of exopolysaccharides in microbial adaptation to cold habitats. In *Psychrophiles: from biodiversity to biotechnology* (pp. 259-284). Springer, Cham. https://doi.org/10.1007/978-3-319-57057-0_12

Fernandes, M., Alves, M., & Vicente, A. A. (2020). Factors affecting polyhydroxyalkanoates biodegradation in soil. *Polymer Degradation and Stability*, 109408. https://doi.org/10.1016/j.polymdegradstab.2020.109408

Folch, J., Lees, M., & Stanley, G. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. Journal of biological chemistry, 226(1), 497-509. https://www.ncbi.nlm.nih.gov/pubmed/13428781

Gordon, A. & Ford, R. (1973). The Chemist's Companion: A Handbook of Practical Data, Techniques, and References 1st Edition. Arnold J. Gordon, Richard A. Ford. Willey. P.560.

Jahan, R., Bodratti, A. M., Tsianou, M., & Alexandridis, P. (2020). Biosurfactants, natural alternatives to synthetic surfactants: physicochemical properties and applications. *Advances in colloid and interface science*, *275*, 102061. https://doi.org/10.1016/j.cis.2019.102061

Johnson, P., Pinfield, V. J., Starov, V., & Trybala, A. (2020). Effect of synthetic surfactants on the environment and the potential for substitution by biosurfactants. *Advances in Colloid and Interface Science*, 102340. https://doi.org/10.1016/j.cis.2020.102340

Kalia, V. C., Patel, S. K. S., Shanmugam, R., & Lee, J. K. (2021). Polyhydroxyalkanoates: trends and advances toward biotechnological applications. *Bioresource Technology*, 124737. https://doi.org/10.1016/j.biortech.2021.124737

Kazak, H., Oner, E. T., & Dekker, R. F. (2010). Extremophiles as sources of exopolysaccharides. Carbohydrate Polymers: *Development, Properties and Applications*, 605-619.
Khovanets, G., Makido, O., Kochubei, V., Sezonenko, T., Medvedevskikh, Y., & Voloshynets, V. (2017). Thermal Stability of Organic-Inorganic Composites Based on Dimethacrylate-Tetraethoxysilane System. *Chemistry & Chemical Technology*, 11 (2), 158-165. https://doi.org/10.23939/chcht11.02.158
Koretska, N., Prystai, M., & Karpenko, O. (2014). Rape phosphatide concentrate in the technologies of surfactants production by the Actinobacteria. *Ukrainian food journal*, 3 (3), 429-436.
Koretska, N., Karpenko, I., Karpenko, O., Baranov, V., & Midyana, H. (2020). Trehalose lipid and rhamnolipid surfactants as plant growth regulators. *Journal of microbiology, biotechnology and food sciences*, *10*(3), 405-408. https://doi.org/10.15414/jmbfs.2020.10.3.405-408
Kretschmer A, Wagner F. (1983). Characterization of biosynthetic intermediates of trehalose dicorynomycolates from *Rhodococcus erythropolis* grown on n-alkanes. *Biochimica et Biophysica Acta*.,753, 306–313. https://doi.org/10.1016/0005-2760(83)90053-X
Madhuri, K. V. & Prabhakar, K. V. (2014). Microbial exopolysaccharides: biosynthesis and potential applications. *Oriental journal of chemistry*, *30*(3), 1401. http://dx.doi.org/10.13005/ojc/300362
Markande, A. R., Patel, D., & Varjani, S. (2021). A review on biosurfactants: properties, applications and current developments. Bioresource Technology, 124963. https://doi.org/10.1016/j.biortech.2021.124963
Mulligan, C. N., Sharma, S. K., Mudhoo, A., & Makhijani, K. (2014). 1 Green Chemistry and. Biosurfactants: Research trends and applications, 1. Boca Raton: CRC Press, Taylor&Francis Group; p. 323. https://www.academia.edu/15914943/BIOSURFACTANTS_Research_Trends_and_Applications
Nisha, J., Mudaliar, N., Senthilkumar, P., & Samrot, A. V. (2012). Influence of substrate concentration in accumulation pattern of poly (R) hydroxyalkonoate in Pseudomonas putida SU-8. African Journal of Microbiology Research, 6(15), 3623-3630. https://doi.org/10.5897/AJMR11.1509
Ojumu, T. V., Yu, J., & Solomon, B. O. (2004). Production of polyhydroxyalkanoates, a bacterial biodegradable polymers. *African journal of Biotechnology*, 3(1), 18-24.. https://doi.org/10.5897/AJB2004.000-2004
Panda B, Sharma L, Singh A, Mallick N. Thin layer chromatographic detection of poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV) in cyanobacteria. *Indian Journal of Biotechnology*. 2008; 7(2):230–234. http://nopr.niscair.res.in/handle/123456789/1833
Rahman, K. S. M., Rahman, T. J., Lakshmanaperumalsamy, P., Marchant, R., & Banat, I. M. (2003). The potential of bacterial isolates for emulsification with a range of hydrocarbons. *Acta biotechnologica*, 23(4), 335-345. https://doi.org/10.1002/abio.200390043
Rana, S., & Upadhyay, L. S. B. (2020). Microbial exopolysaccharides: Synthesis pathways, types and their commercial applications. *International journal of biological macromolecules*, *157*, 577-583. https://doi.org/10.1016/j.ijbiomac.2020.04.084
Rapp, P., Bock, H., Wray, V., & Wagner, F. (1979). Formation, isolation and characterization of trehalose dimycolates from Rhodococcus erythropolis grown on n-alkanes. *Microbiology*, 115(2), 491-503. https://doi.org/10.1099/00221287-115-2-491
Raza, Z. A., Abid, S., & Banat, I. M. (2018). Polyhydroxyalkanoates: Characteristics, production, recent developments and applications. *International Biodeterioration & Biodegradation*, 126, 45-56. https://doi.org/10.1016/j.ibiod.2017.10.001
Semeniuk, I., Kochubei, V., Skorokhoda, V., Pokynbroda, T., Midyana, H., Karpenko, E., & Melnyk, V. (2020). Biosynthesis Products of Pseudomonas sp. PS-17 Strain Metabolites. 1. Obtaining and Thermal Characteristics. *Chemistry & Chemical Technology*, 1 (14), 2020, (1), 26-31. https://doi.org/10.23939/chcht14.01.026.
Semeniuk I, Pokynbroda T, Kochubei V, Midyana H, Karpenko O, Skorokhoda V. (2020). Biosynthesis and characteristics of polyhydroxyalkanoates. 1. Polyhydroxybutyrates of *Azotobacter vinelandii* N-15. *Chem. Chem. Technol*., 14 (4): 463–467. https://doi.org/10.23939/chcht14.04.
Senthilkumar P, Dawn SS, Samrot AV, Kumar N. (2016). Production, Optimization and Characterization of Poly [R] Hydroxyalkanoate from Enterobacter sp SU16. *Indian Journal of Science and Technology.*, *9* (45). https://doi.org/10.17485/ijst/2016/v9i45/87822
Shah A, Hasan F, Hameed A, Ahmed S. (2008). Biological degradation of plastics: a comprehensive review. *Biotechnology advances*., 26: 246-65. https://doi.org/10.1016/j.biotechadv.2007.12.005
da Silva, I. F., Neves, N. A., e Silveira, B. M. P., Vespermann, K. A. C., & Valente, M. E. R. (2021). Microbial Biosurfactants for Contamination of Food Processing. *Microbial Biosurfactants*, 11-30. https://doi.org/10.1007/978-981-15-6607-3_2
Smyth, T., Perfumo, A., Marchant, R., & Banat, I. (2010). Isolation and Analysis of Low Molecular Weight Microbial Glycolipids. In *Handbook of Hydrocarbon and Lipid Microbiology*. pp. 3705-3723. Springer. https://link.springer.com/referenceworkentry/10.1007%2F978-3-540-77587-4_291
Sotirova, A., Avramova, T., Stoitsova, S., Lazarkevich, I., Lubenets, V., Karpenko, E., & Galabova, D. (2012). The importance of rhamnolipid-biosurfactant-induced changes in bacterial membrane lipids of Bacillus subtilis for the antimicrobial activity of thiosulfonates. *Current microbiology*, *65*(5), 534-541. https://link.springer.com/article/10.1007/s00284-012-0191-7
Sudesh K, Abe H, Doi Y. (2000). Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. *Prog. Polym. Sci*. , 25(10), 1503-1555. https://doi.org/10.1016/S0079-6700(00)00035-6
Urai, M., Yoshizaki, H., Anzai, H., Ogihara, J., Iwabuchi, N., Harayama, S., ... & Nakajima, M. (2007). Structural analysis of mucoidan, an acidic extracellular polysaccharide produced by a pristane-assimilating marine bacterium, Rhodococcus erythropolis PR4. *Carbohydrate research*, 342(7), 927-932. https://doi.org/10.1016/j.carres.2007.02.002
Williams AG, Wimpenny YW. (1977). Exopolysaccharide production by *Pseudomonas* NCIB 11264 grown in bat culture. *Journal of Biological Chemistry*., 102 (1), 12 – 21. https://doi.org/10.1099/00221287-102-1-13
Yaremkevych, O., Fihurka, O., Banya, A., Shvets, V., Nakonechna, A., Karpenko, O., ... & Lubenets, V. (2020). Effect of thiosulfonate-biosurfactant compositions on plants grown in oil polluted soil. *Environmental Engineering & Management Journal (EEMJ)*, *19*(11).
Zhang, J., Shishatskaya, E. I., Volova, T. G., da Silva, L. F., & Chen, G. Q. (2018). Polyhydroxyalkanoates (PHA) for therapeutic applications. *Materials Science and Engineering: C*, *86*, 144-150. https://doi.org/10.1016/j.msec.2017.12.035