Rape phosphatide concentrate in the technologies of surfactants production by the Actinobacteria

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Keywords:

Gordonia
Rhodococcus
Biosurfactants
Phosphatid
Concentrate

Article history:

Received 01.07.2014
Received in revised form 03.08.2014
Accepted 02.09.2014

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Abstract

Introduction. Due to the fact that the production of microbial surfactants is limited by the low yield of end products and high cost of processes, the actual task is to optimize and reduce the cost of the technology of biosurfactants synthesis. One of the solutions of this problem is to use the industrial wastes, including rape phosphatide concentrate (PC).

Materials and methods. Hexadecane and rape phosphatide concentrate (2%) were used as a carbon source in a nutrient medium for the cultivation of bacteria. Lipids were extracted from a cell mass and supernatant by the mixture of chloroform-methanol 2:1. The qualitative analysis of metabolites was performed by a thin layer chromatography.

Results and discussion. The peculiarities of synthesis of biosurfactants by strains *G. rubripertincta* UCM Ac-122 and *R. erythropolis* Au-1 during the growth on the nutrient media with rape phosphatide concentrate as a carbon source was studied. Quantity of biomass was 9.4 – 10.1 g/l, exopolymers – 8.9-9.5 g/l and the content of cell-bound trehalose lipids was 1.37 – 2.26 g/l; whereas the content of exogenous trehalose lipids – metabolites of *R. erythropolis* Au-1 was 2.95 g/l. It was found that the addition of trehalose lipids (0.01 g/l) to the nutrient medium caused the increase of biomass on 14.6 – 17.0 % and cell-bound lipids on 13.9 – 15.5 %.

Conclusions. Rape phosphatide concentrate is economically viable carbon source in the technologies of surfactant production by Actinobacteria. Its use promotes an increasing of exogenous surfactants strain *R. erythropolis* Au-1 in 3-fold compared with cultivation on nutrient medium with hexadecane. Trehalose lipids show a stimulating effect on growth and synthesis of biosurfactants by strains of *G. rubripertincta* UCM Ac-122 and *R. erythropolis* Au-1.

Introduction

Microbial synthesis of surfactants is a perspective direction of biotechnology. Biosurfactants attract considerable interest due to their potential advantages if compared with their synthetic analogues in many fields of environmental, food, biomedical and other industrial applications. However, several factors limit the large-scale production of these compounds, in particular, the low yield of a product and the high cost of downstream processes. Therefore, important tasks are to search new active microorganisms-producers, to increase efficiency of biosurfactant's synthesis and to make technology of biosurfactant production cheaper [1, 2].

It was established that the bacterial strains *Gordonia rubripertincta* UCM Ac-122 and *Rhodococcus erythropolis* Au-1 from the collection of microorganisms of Danylo Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine are perspective producers of biosurfactants [3, 4]. These bacteria synthesize exogenous polymers (EP) with emulsifying properties and complexes of surfactant lipids, the main component of which are trehalose lipids (TL). It is known that Actinobacteria, in particular of genus *Rhodococcus*, are able to consume actively a wide range of water-insoluble carbon sources. The microbial cell wall of these bacteria consists mainly of mycolic acids and trehalose lipids, so it is cognate with hydrophobic substances [5]. Based on these data, the cultivation of actinobacteria for accumulation of biomass and cell-bound surfactants is often conducted on nutrient media with hydrophobic carbon sources, particularly, with hexadecane (HD) [6], but the use of this substrate is impractical from the economical point of view.

The use of the alternative substrates such as agro-based wastes is one of the attractive strategies for economical biosurfactant production [7]. In particular, it was shown that the cultivation of *Acinetobacter* sp. IMV B-7005 was carried out using a mixture of molasses and fumarate [8], *Nocardia vaccinii* K-8 – glycerol [9], bacteria of the genus *Pseudomonas* – overheated sunflower oil [1] and so on. However, the results of the studies do not resolve all the issues of the optimization of biosurfactants technology.

Thus the aim of our work was to study the synthesis of biosurfactants by strains *G. rubripertincta* UCM Ac-122 and *R. erythropolis* Au-1, using phosphatide concentrate (PC), which is a by-product of rapeseed oil and diesel fuel production.

Materials and methods

Bacterial strains *G. rubripertincta* UCM Ac-122 and *R. erythropolis* Au-1 from the Ukrainian collection of microorganisms of D. Zabolotny Institute of Microbiology and Virology were used. Cultivation of bacteria was carried out using the following nutrient medium (g/l): $NaNO_3$ – 3.0; yeast extract – 1.0; K_2HPO_4 – 2.0; KH_2PO_4 – 2.0; $MgSO_4 \times 7H_2O$ – 0.5; sodium citrate – 1.0 (pH 6.8-7.0). As carbon sources were used hexadecane or rape phosphatide concentrate (trademark "Majola") of the following composition (%): oil – 72.91; not fatty additives – 13.22; phosphatides – 12.39; moisture – 1.48. Microorganisms were cultivated in 750 ml Erlenmeyer flasks with 150 ml medium on the rotary shaker (220 rpm) at 28-30 °C during 5 days.

Cells were separated by centrifugation at 6000 rpm for 15 min. The biomass was determined by gravimetric method. The surface tension of CLS was determined by Du-Nui method [10] with tensiometer KRÜSS K6 ("KRÜSS" GmbH, Germany).

For determining the emulsifying activity 10 ml of the culture liquid supernatant was mixed with 10 ml of Vaseline oil during 2 minutes and transferred into a measuring tube.

The emulsification index (E_{24}) was determined after 24 h as the ratio of the height of emulsion layer to the total height of the liquid in the tube [10].

Lipids were extracted from cell mass and CLS with the mixture of chloroform-isopropanol (2:1). This extract was evaporated under vacuum to constant weight.

Exopolymers were precipitated from CLS by acidification with 10% hydrochloric acid to pH 3, then the mixture was kept at 4˚C for 12 h. The precipitate was separated by centrifugation (6000 rpm, 15 min.), washed, filtered and dried to constant weight at a temperature of 60˚C.

Qualitative analysis of lipids was performed by thin layer chromatography (TLC) on plates Sorbifil PTSH-AF-A-UF (CJSC «Sorbpolymer», Russia). Mobile phase [11]: chloroform-methanol-water 65:15:2. Lipids were preliminary identified by spraying plates with specific reagents:

a. 5 % alcohol solution of phosphorus molybdenum acid (total lipids);

b. 4-methoxybenzaldehyde reagent (glycolipids);

c. 5% alcoholic solution of ninhydrin (peptide lipids).

The influence of biosurfactants on cultivation of Actinobacteria was studied by adding their solution at a concentration of 0.01 g/l to the culture medium.

Results and discussion

The growth parameters of strains *G. rubripertincta* UCM Ac-122 and *R. erythropolis* Au-1 on the nutrient media with phosphatide concentrate and hexadecane were studied. The data from the experiments are presented in the Table 1.

Table 1

The growth parameters of the strains *G. rubripertincta* UCM Ac-122 and *R. erythropolis* Au-1 on the nutrient media with phosphatide concentrate and hexadecane

Bacterial strains	Carbon sources	Surface tension, mN/m	Emulsifying activity E_{24}, %	Exopoly-mers, g/l	Biomass, g/l	Biosurfac-tants, g/l
R. erythropolis Au-1	HD	31,5±0,5	7,6±0,5	5,1±0,3	13,3±0,7	3,36±0,20
	PC	48,5±0,5	60,6±2,3	9,5±0,6	10,1±0,6	1,37±0,13
G. rubripertincta UCM Ac-122	HD	39,7±0,2	55,7±2,8	5,8±0,4	10,4±0,5	3,16±0,16
	PC	41,5±0,4	54,9±1,8	8,9±0,7	9,38±0,5	2,26±0,11

HD – hexadecane.

PC – phosphatide concentrate.

The increase of exopolymers content in 1.9 times, reduction of the biomass content on 23.9 %, and the cell-bound lipids – in 2.45 times was shown when the PC was used compared to HD for cultivation of *R. erythropolis* Au-1. The CLS, obtained in the nutrient medium with PC, had higher emulsifying activity (60.6%) then CLS obtained using HD (7.6 %) (Table 1). Also, it was shown that the cultivation of *G. rubripertincta* UCM Ac-122 on the nutrient medium with PC (compared with the use of HD) caused the increase of exopolymers content on 54% and reduction of the biomass accumulation and cell-associated lipids (biosurfactants), respectively, on 9.8 % and 28.5 %. The emulsifying activity and the surface tension of the CLS with PC remained practically unchanged (Table 1).

The effect of the use of rape PC on the quality of the cell-associated lipids was studied. The data are presented in Fig.1. It was shown that the *R. erythropolis* Au-1 strain produced glycolipids: trehalose-mono-mykolates, trehalose-di-mykolates, trehalose esters and fatty acids when uses both carbon sources. When the strain was cultivated on the nutrient medium with PC, there was the increase of amount of phosphatidic acids and fatty alcohols, also small amounts of peptide lipids were present. The strain *G. rubripertincta* UCM Ac-122 produced glycolipids, peptide lipids and neutral lipids when using HD and PC (Fig.1).

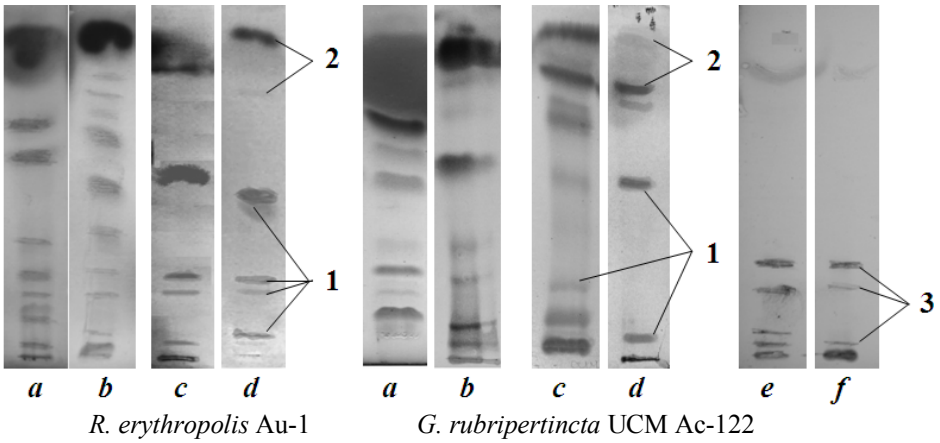

Fig.1. Thin layer chromatograms of cell-associated total lipids (a, b), glycolipids (c, d) and peptide lipids (d, e) of bacterial strains *R. erythropolis* Au-1 and *G. rubripertincta* UCM Ac-122. The carbon source – hexadecane (a, c, e) and phosphatide concentrate (b, d, f):
1 – trehalose lipids; 2 – neutral lipids; 3 – peptide lipids.

It is known that the strain *G. rubripertincta* UCM Ac-122 synthesizes cell-bound biosurfactants [4], so lipids were extracted from the cell mass. But the bacteria of genus *Rhodococcus* also produce exogenous biosurfactants [6]. Based on these data, we studied the presence of exogenous surfactants synthesized by *R. erythropolis* Au-1. It was showed that the strain produces exogenous lipids, but their amount changes significantly depending on the of carbon source – 2.95 g/l when rape PC was used and 0.98 g/l when HD was used (Fig. 2). It was established that the total amount of surface-active lipids was unchanged, but the concentration of exogenous biosurfactants was increased.

We used the thin layer chromatography for the control of composition of cell-bound and extracellular lipids of strain *R. erythropolis* Au-1. It was shown that this strain during growth in nutrient medium with hexadecane as carbon source synthesized small amounts of exogenous lipids; but, the use of phosphatide concentrate can significantly increase the production of those surfactants.

The investigated Actinobacteria are considered as promising producers of both biosurfactants and exopolymers. The data on the production of EP by the strains *R. erythropolis* Au-1 and *G. rubripertincta* UCM Ac-122, the yield of which lies within the range 8.9-9.5 g/l depending on the bacterial strain and carbon source are presented in Table 1. The emusification properties of the selected EP with Vaseline oil were studied (Fig. 3).

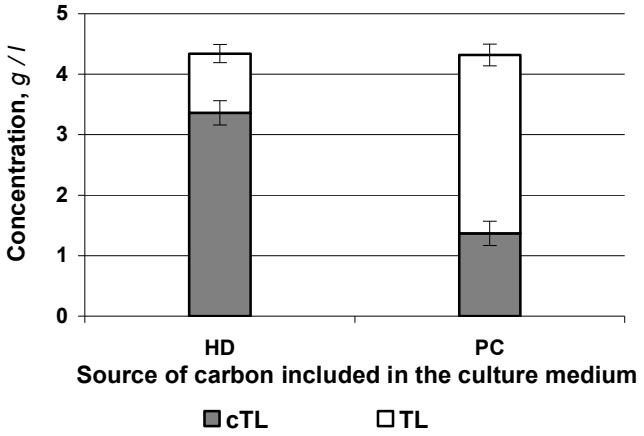

Fig. 2. Effect of carbon sources on the synthesis of biosurfactants by *R. erythropolis* Au-1
TL – exogenous lipids, the main component of which is trehalose lipids.
cTL – cell-associated lipids, the main component of which is trehalose lipids.
HD – hexadecane, PC – phosphatide concentrate.

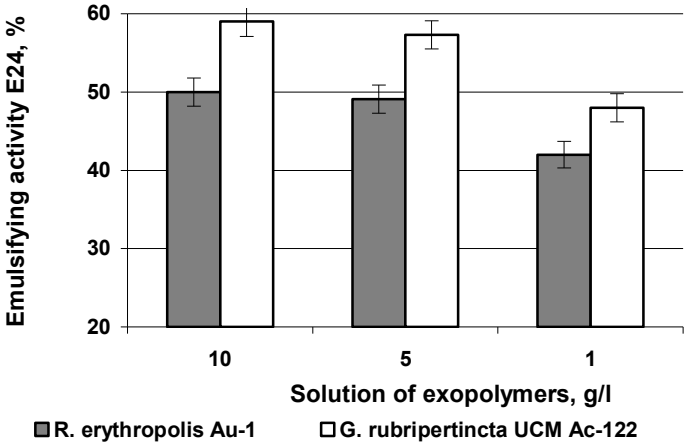

Fig. 3. Emulsification indices of the EP solutions of various concentrations with Vaseline oil

It was confirmed that EP is the main emulsification factor of the culture liquid supernatant. Thus, the EP solutions (10 g/l and 5 g/l) have high emulsification activity, and the further reduction of concentration resulted in decrease of the emulsification index E_{24} to 42-48% depending on a producer.

Thus, CLS of Actinobacteria can be considered as effective emulsifiers. The stability of the emulsions (28 days) of vaseline oil with CLS of the studied microorganisms, obtained via cultivation on the nutrient medium with PC were studied as well. The data are given on the Fig. 4.

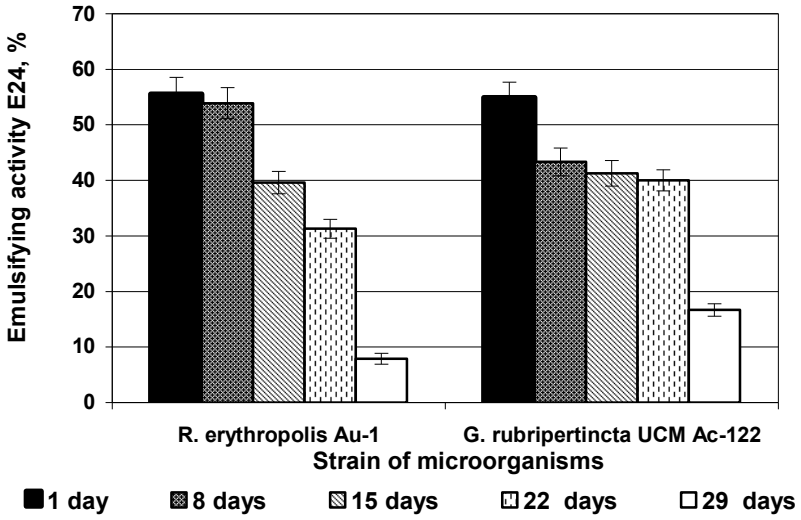

Fig. 4. The stability in time of the emulsions of Vaseline of with CLS of the studied Actinobacteria

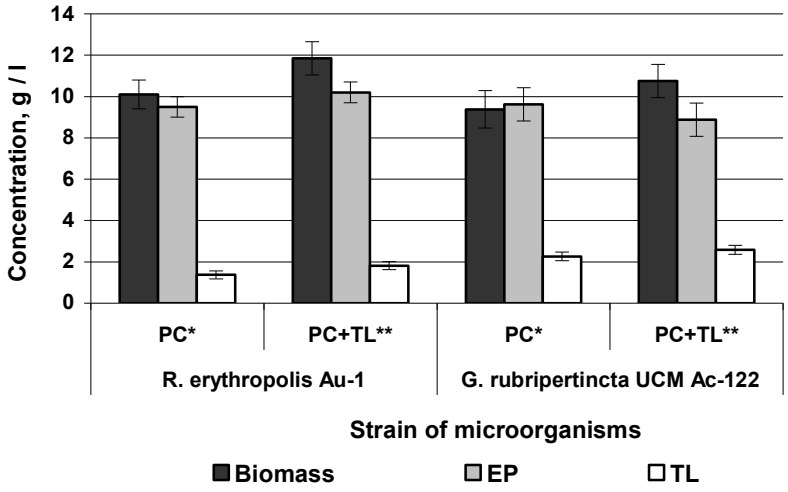

Fig.5. Effect of trehalose lipids on the growth of strains of *G. rubripertincta* UCM Ac-122 and *R. erythropolis* Au-1

PC * – carbon source phosphatide concentrate 20 g/l.
PC+TL** – carbon source phosphatide concentrate 20 g/l plus trehalose lipids (0.01 g/l).
EP – expolymers. TL – trehalose lipids.

It was revealed, the emulsification activity of the CLS of the strain *G. rubripertincta* UCM Ac-122 was rather high (around 40 %) during three weeks, while the the CLS of the strain *R. erythropolis* Au-1 was losing its properties much faster.

It was shown, that the CLS of Actinobacteria, cultivated on the nutrient medium with PC, have high emusification activity (their E_{24} is 55-57 %). Emulsions of the bacterial CLS with vaseline oil remain stable during 3 weeks.

It is known that biosurfactants can influence the permeability of cell membranes [12], enzymatic activity [13] and are able to enhance the action of other substances [14]. Based on these data, it was decided to study the effect of biosurfactants on the growth of *R. erythropolis* Au-1 and *G. rubripertincta* UCM Ac-122 (Fig. 5).

We have found that the addition of the TL solution to the culture medium during the bacteria growth had no effect on the quality of the produced biosurfactants. However, it was shown that, depending on the strain, the increase in biomass and cell-associated lipids, made respectively, 14.6 - 17.0 % and 13.9 - 15.5 % (Fig. 5). The concentration of exopolymers was practically unchanged.

Conclusions

1. It was established that bacterial strains *G. rubripertincta* UCM Ac-122 and *R. erythropoli*s Au-1 effectively consumed the rape phosphatide concentrate as a carbon source – the bacterial biomass was 9.4-10.1 g/l.
2. It was shown that biosurfactants were actively synthesized in the nutrient medium with rape phosphatide concentrate – the content of a cell-bound lipids was 1.37-2.26 g/l. Moreover, the content of exogenous trehalose lipids – metabolites of *R. erythropolis* Au-1 – has increased in 3 times if compared to the use of hexadecane.
3. The composition of biosurfactans synthesized by *G. rubripertincta* UCM Ac-122 and *R. erythropolis* Au-1 was determined, they consist of glycolipids (trehalose mycolates) phospho-, peptide and neutral lipids. The qualitative composition of biogenic surfactants was practically unchanged when using different sources of carbon.
4. It was shown, that CLS is an effective emulsifier, and the main factor which determines its emulsifying properties is EP. The yield of expolymers made 5,1-9,5 g/l depending on the strain-producer and carbon source.
5. It was established that the addition of the trehalose lipids (0.01 g/l) to a nutrient medium during cultivation of actinobacteria coused the increase of biomass on 14.6-17.0 % and cell-associated lipids – on 13.9-15.5 %, the amount of exopolymers remained unchanged if compared with the control.
6. Therefore, the rape phosphatide concentrate is a promising cost-effective carbon source for microbial surfactants technologies.

Investigations were conducted in the framework of the project STCU 5965

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