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THE EFFECT OF PRO- AND EUKARYOTIC INDUCTORS ON THE SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF *ACINETOBACTER CALCOACETICUS* IMV B-7241 SURFACTANTS

By now, the mixed cultivation («co-cultivation») of antimicrobial compound producers with other microorganisms or the introduction of biological inductors in different physiological states (live and inactivated cells, as well as the corresponding supernatants) into the culture medium is a simple, cheap, and effective way to increase the synthesis of practically important microbial metabolites and regulate their biological activity. In most studies, researchers use bacterial strains of various species as inductors, however, in recent years, there have been an increasing number of publications reporting the use of eukaryotic inductors, in response to which there's observed an increase in the synthesis of antimicrobial compounds by the bacterial producers. In addition, the effectiveness of biological inductors depends on the conditions of their cultivation and physiological state. ***Aim****. To study the effect of the methods of preparation and physiological state of biological inductors (gram-negative bacteria Enterobacter cloacae C-8 and yeast Saccharomyces cerevisiae BTM-1) on the activity of biosynthetic enzymes and antimicrobial activity of Acinetobacter calcoaceticus IMV B-7241 surfactants.* ***Methods****. Purified glycerol and crude glycerol in equimolar carbon concentration were used as a substrate for cultivation of A. calcoaceticus IMV B-7241. The microbial inductors were grown on both agar and liquid media with glucose as a carbon source. Live or inactivated cells of S. cerevisiae BTM-1 or E. cloacae C-8, as well as the corresponding supernatant, were added to the medium in an amount of 2.5—10% (v/v). The extracellular surfactants were obtained from the supernatant of the culture liquid by extraction with a mixture of chloroform and methanol (2:1). The antimicrobial activity of surfactants against bacterial (Bacillus subtilis BT-2, Escherichia coli IEM-1, Staphylococcus aureus BMS-1, Pseudomonas sp. MI-2)*

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and yeast (Candida albicans D-6 and Candida tropicalis PE-2) test cultures was determined by the indicator of the minimum inhibitory concentration. The activity of enzymes for the biosynthesis of surface-active glyco- (phosphoenolpyruvate carboxylase, phosphoenolpyruvate synthetase, phosphoenolpyruvate carboxykinase, trehalose phosphate synthase) and aminolipids ($NADP^+$-dependent glutamate dehydrogenase) was analyzed in cell-free extracts obtained after sonication of cells. ***Results.*** *The introduction into the culture medium of A. calcoaceticus of IMV B-7241 with glycerol of various purification degrees, both pro- and eukaryotic inductors in different physiological states was accompanied by the synthesis of surfactants, the antimicrobial activity of which against the test cultures was higher by one to two orders of magnitude compared to preparations obtained without inductors. It was found that E. cloacae C-8 cells grown in liquid medium were slightly more effective as inductors than those grown in agar medium: the minimum inhibitory concentrations against bacterial and yeast cultures of surfactants synthesised in their presence were 1—6 and 2.5—8 μg/mL, respectively. When live S. cerevisiae BTM-1 or E. cloacae C-8 cells were used as inductors, the production of microbial surfactants with higher antimicrobial activity than those synthesized in the presence of inactivated cells or supernatants was observed: the minimum inhibitory concentrations against the test cultures were in the range of 0.85—16, 2—20 and 1.5—22 μg/mL, respectively. The higher antimicrobial activity of surfactants synthesized in the presence of a pro- or eukaryotic inductor in the medium with purified glycerol may be caused by an increase of aminolipids in their composition, as evidenced by a 1.6—2.1-fold increase in $NADP^+$-dependent glutamate dehydrogenase activity in A. calcoaceticus IMV B-7241 cells compared to the values of cultivation without inductors. The same level of activity of this enzyme during the cultivation of the IMV B-7241 strain in the medium with crude glycerol in the presence of inductors and without them may indicate the synthesis under such conditions of other, than aminolipids, metabolites with antimicrobial activity.* ***Conclusions.*** *As a result of this study, it was established that the antimicrobial activity of A. calcoaceticus IMV B-7241 surfactants can be increased by introducing pro- and eukaryotic inductors in the form of live or inactivated cells, as well as the corresponding supernatants into the medium with glycerol of different degrees of purification.*

Keywords: *yeast and bacterial inductors, surfactants, antimicrobial activity.*

In our previous work (Pirog et al., 2023c), we noted an increasing number of publications in recent years regarding the co-cultivation of producers of antimicrobial compounds with competitive microorganisms (biological inductors). This co-cultivation has shown an increase in the antimicrobial activity and/or synthesis of the final product and even the synthesis of new metabolites that are not typical for the producer's monoculture. This method of cultivation is a simple, cost-effective, and highly efficient way to increase the synthesis of practically important microbial metabolites and regulate their biological activity.

Our experimental study has shown that the presence of live and inactivated cells of *Bacillus subtilis* BT-2, as a biological inductor, in the culture medium of *Acinetobacter calcoaceticus* IMV B-7241 increased not only the antimicrobial but also the anti-adhesive activity of the synthesized microbial surfactants (Pirog et al., 2023c). However, in that study, the biological inductor was grown on meat-peptone agar. Nevertheless, this method of preparing the inductor is unsuitable for scaling up the process of surfactant biosynthesis.

Moreover, the literature indicates that the positive outcomes of co-cultivation (or the presence of a biological inductor in the medium) often depend on the nature of the competitive microorganisms (bacteria, fungi, or yeast) and their physiological state (Wang et al., 2013; Luti et al., 2018; Song et al., 2020).

It should be noted that the majority of publications in this field focus on co-cultivation of bacteria (Pirog & Ivanov, 2023b; Qiao et al., 2022), yet in recent years, there have appeared more and more papers devoted to the cultivation of microorganisms with eukaryotes, in particular yeast (Wang et al., 2022), and the usage of yeast inductors as a factor in the regulation of the synthesis of secondary metabolites by prokaryotes (Luti et al., 2018).

Considering the above, the **aim** of this study was to investigate the effect of the preparation method and physiological state of biological inductors

(gram-negative bacteria *Enterobacter cloacae* C-8 and yeast *Saccharomyces cerevisiae* BTM-1) on the activity of biosynthetic enzymes and antimicrobial activity of *A. calcoaceticus surfactants* IMV B-7241.

Materials and Methods. The main object of this research was a strain of oil-oxidising bacteria, *Acinetobacter calcoaceticus*, isolated from an oil-contaminated soil sample and registered in the Depository of Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under IMV number B-7241.

The strains of bacteria (*Bacillus subtilis* BT-2, *Escherichia coli* IEM-1, *Staphylococcus aureus* BMS-1, *Pseudomonas* sp. MI-2) and yeast (*Candida albicans* D-6, *Candida tropicalis* PE-2) from the collection of live cultures of the Department of Biotechnology and Microbiology of the National University of Food Technologies were used as test cultures for determining the antimicrobial activity of surfactants.

The cultivation of *A. calcoaceticus* IMB B-7241 was carried out as described in our previous work (Pirog et al., 2023c). The following carbon sources were used (%, v/v): purified glycerol — 3, crude glycerol — 5. The concentrations of glycerol of different quality were equimolar by carbon.

The inductors were prepared in two variants. In the first variant, yeast *S. cerevisiae* BTM-1 and bacteria *E. cloacae* C-8 were grown on a shaker at 320 rpm for 24 h in a liquid mineral medium of the same composition as for the cultivation of surfactant producer, but glucose (0.5%) was used as a carbon source. After 24 h of cultivation, the culture liquid was centrifuged in a sterile Eppendorf microtube (10000 g, 10 min). The resulting supernatant was used as an inductor and added at the amount of 2.5 mL per 100 mL of surfactant producer culture medium. The biomass was resuspended in sterile tap water to an amount equal to that taken for centrifugation. The resuspended biomass (live inductor cells) was added at the amount of 2.5 mL of suspension per 100 mL of culture medium. A part of the resuspended biomass was autoclaved at 131 °C for 1 h to obtain inactivated cells (10 mL of suspension per 100 mL of culture medium).

In the second variant of preparing the *E. cloacae* C-8 inductor, it was cultivated on meat-peptone agar (MPA) for 24 hours. Subsequently, the cells were suspended in 100 mL of sterile tap water, and 2.5 mL of this suspension was added per 100 mL of surfactant producer culture medium. The inactivated cells (sterilized in an autoclave at 131 °C for 1 hour) were added at a rate of 10 mL of suspension per 100 mL of culture medium.

Cultivation of *A. calcoaceticus* IMV B-7241 in the presence of supernatant, live and inactivated *S. cerevisiae* BTM-1 or *E. cloacae* C-8 cells and without inductors was carried out in 750 mL flasks with 100 mL of the medium on a shaker (320 rpm) at 30 °C for 7 days.

Preparation of cell-free extracts and determination of the activity of surfactant biosynthesis enzymes: phosphoenolpyruvate (PEP) synthetase (EC 2.7.9.2), FEP carboxykinase (EC 4.1.1.49), glutamate dehydrogenase (EC 1.4.1.4), FEP carboxylase (EC 4.1.1.31), trehalose phosphate synthase (EC 2.4.1.15) were performed as described previously (Pirog et al., 2023c).

Extracellular surfactants were isolated from the culture supernatant by extraction with a mixture of chloroform and methanol (2:1) as described in (Pirog et al., 2020). To obtain the supernatant, the culture liquid was centrifuged at 5000 g for 20 min.

The antimicrobial activity of surfactants was analyzed by the minimum inhibitory concentration (MIC) as described in (Pirog et al., 2020).

All experiments were performed in 3 repeats, the number of parallel determinations in the experiments was 3—5. Statistical processing of the experimental data was performed as described previously (Pirog et al., 2020). Differences in mean values were considered reliable at the significance level of $p<0.05$.

Results. Table 1 presents the values of minimal inhibitory concentrations of the surfactants pro-

duced by *A. calcoaceticus* IMV B-7241 in the presence of grown under various conditions prokaryotic inductors in different physiological states. Regardless of the type of culture medium of *E. cloacae* C-8 (agar or liquid medium), surfactants synthesized in the presence of live or inactivated cells of the biological inductor demonstrated antimicrobial activity one to two orders of magnitude higher compared to preparations obtained without an inductor. However, the inductor grown in a liquid medium exhibited slightly higher efficacy than after cultivation on meat-peptone agar: the MICs for bacterial and yeast cultures of surfactants were 1—6 and 2.5—8 μg/mL, respectively.

In addition, the surfactants synthesized in the presence of live *E. cloacae* C-8 cells in the medium with both purified and crude glycerol were characterized by slightly higher antimicrobial activity compared to the preparations obtained using inactivated cells or their supernatant. It should be noted that the effectiveness of inactivated cells and supernatant as inductors was practically identical.

The data showing the effect of the eukaryotic inductor in various physiological states on the antimicrobial activity of *A. calcoaceticus* surfactants IMV B-7241 are shown in Table 2.

The introduction of live *S. cerevisiae* BTM-1 cells into the medium with purified or crude glycerol was accompanied by the synthesis of surfactants, the minimum inhibitory concentrations of which against the tested cultures were 5—37 and 13—50 times lower, respectively, than those established for surfactants synthesized without an inductor. The less effective of the used eukaryotic inductors were inactivated cells, in the presence of which in the medium with glycerol of various degrees of purification, the synthesis of surfactants was found, the MICs of which were only 1.7—5.4 times lower than the minimum inhibitory concentrations of the preparations obtained without an inductor. When the supernatant of *S. cerevisiae* BTM-1 was added to the medium with both substrates, their MICs were 2.7—18.7 times lower in comparison with the values determined for surfactants obtained without an inductor.

In Table 3, the activity of key enzymes involved in the biosynthesis of surface-active glyco- (PEP synthetase, PEP carboxykinase, trehalose phos-

Table 1. **The effect of preparation method and physiological state of prokaryotic inductor (*Enterobacter cloacae* C-8) on the antimicrobial activity of *Acinetobacter calcoaceticus* IMV B-7241 surfactants**

Glycerol as a substrate for surfactant biosynthesis	Inductor	Medium for inductor cultivation	Minimum inhibitory concentrations (μg/mL) against					
			Escherichia coli IEM-1	*Bacillus subtilis* BT-2	*Staphylococcus aureus* BMS-1	*Pseudomonas* sp. MI-2	*Candida albicans* D-6	*Candida tropicalis* PE-2
Purified	Control (without inductor)		14	28	14	28	28	28
	Live cells	MPA	5	5	2.5	5	2.5	2.5
		Liquid with glucose	2	2	2	1	1	1
	Inactivated cells	MPA	8	8	8	8	4	4
		Liquid with glucose	6	6	6	3	2	2
	Supernatant	Liquid with glucose	3	3	3	6	3	3
Crude	Control (without inductor)		20	40	20	40	40	40
	Live cells	Liquid with glucose	8	16	8	16	16	16
	Inactivated cells	Liquid with glucose	10	20	10	20	20	20
	Supernatant	Liquid with glucose	11	22	11	22	18	18

When determining the minimum inhibitory concentrations, the error did not exceed 5%.

phate synthase) and aminolipids ($NADP^+$-dependent glutamate dehydrogenase) is shown depending on the physiological state of the prokaryotic inductor in the culture medium of *A. calcoaceticus* IMV B-7241.

In the cells of the surfactant producer cultivated on purified glycerol in the presence of all types of inductors (live or inactivated cells of *E. cloacae* C-8, or the corresponding supernatant), an increase of 1.6 to 2-fold in $NADP^+$-dependent

Table 2. **Antimicrobial activity of surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 in the presence of eukaryotic inductor (*Saccharomyces cerevisiae* BTM-1)**

Glycerol as a substrate for surfactant biosynthesis	Inductor	Minimum inhibitory concentrations (μg/mL) against					
		Escherichia coli IEM-1	*Bacillus subtilis* BT-2	*Staphylococcus aureus* BMS-1	*Pseudomonas* sp. MI-2	*Candida albicans* D-6	*Candida tropicalis* PE-2
Purified	Control (without inductor)	16	32	16	32	32	32
	Live cells	3.4	1.7	0.85	3.4	3.4	0.85
	Inactivated cells	9.6	9.6	4.8	19.2	19.2	19.2
	Supernatant	5.8	5.8	2.9	11.6	11.6	11.6
Crude	Control (without inductor)	28	56	28	56	56	56
	Live cells	1.1	2.2	2.2	4.4	1.1	1.1
	Inactivated cells	5.2	10.4	5.2	10.4	10.4	10.4
	Supernatant	1.5	6.0	3.0	6.0	6.0	3.0

When determining the minimum inhibitory concentrations, the error did not exceed 5%; *S. cerevisiae* BTM-1 yeast was grown in a liquid medium with glucose.

Table 3. **Effect of prokaryotic inductors in the culture medium of *Acinetobacter calcoaceticus* IMV B-7241 on the activity of key enzymes of surfactant biosynthesis**

Carbon source	Inductor (*Enterobacter cloacae* C-8)	Activity (nmol min^{-1} mg^{-1} of protein)			
		$NADP^+$-dependent glutamate dehydrogenase	PEP-synthase	PEP-carboxylase	Trehalose phosphate synthase
Purified glycerol	Control (without inductor)	322±16	21061±1053	643±32	22±1
	Live cells	643±32	4508±225	693±34	19±1
	Inactivated cells	516±25	3981±199	612±30	18±1
	Supernatant	523±26	5152±257	603±30	19±1
Crude glycerol	Control (without inductor)	357±17	19897±994	714±35	45±2
	Live cells	357±17	9286±464	791±39	44±2
	Inactivated cells	357±17	8787±439	714±35	35±2
	Supernatant	357±17	7965±398	757±37	34±2

The bacterial strain *Enterobacter cloacae* C-8 was grown in a liquid medium with glucose.

glutamate dehydrogenase activity and a decrease of 4 to 5-fold in PEP synthetase activity compared to those in the medium without inductors were observed. The activity of the other two enzymes involved in the biosynthesis of glycolipids surfactant under these cultivation conditions in *A. calcoaceticus* IMV B-7241 cells practically did not differ from the activity in the cells of the surfactant producer cultivated without inductors.

We observed slightly different patterns during the cultivation of *A. calcoaceticus* IMV B-7241 on crude glycerol (Table 3). The activity of NADP+-glutamate dehydrogenase in the cells of the surfactant producer remained at the same level regardless of the presence and type of prokaryotic inductor in the culture medium. Similarly, the presence of an inductor in the medium with crude glycerol had practically no effect on the activity of PEP-carboxykinase and trehalose phosphate synthase in *A. calcoaceticus* IMB B-7241 cells. At the same time, the introduction of both live and inactivated *E. cloacae* C-8 cells and the corresponding supernatant into the medium with crude glycerol was accompanied by a 2.5-fold decrease in PEP-synthetase activity in the cells of the surfactant producer (Table 3).

Table 4 shows data on the effect of eukaryotic inductors in the culture medium of *A. calcoaceticus* IMV B-7241 on the activity of key enzymes involved in the biosynthesis of surfactants. In the presence of all types of yeast inductors in the medium with purified glycerol, the activity of glycolipid biosynthesis enzymes (PEP-synthetase, PEP-carboxykinase, trehalose phosphate synthase) in the surfactant-producing cells remained practically unchanged, while the $NADP^+$-dependent glutamate dehydrogenase activity increased by 1.6—2.1 times compared to the cultivation of the IMV B-7241 strain without inductors. Meanwhile, after introducing all types of eukaryotic inductors into the medium containing crude glycerol, the change in the activity profile of all investigated enzymes in *A. calcoaceticus* IMV B-7241 cells (except for trehalose phosphate synthase) was similar to that observed in the presence of prokaryotic inductors (Table 3).

Specifically, the activity of $NADP^+$-dependent glutamate dehydrogenase and PEP-carboxykinase remained unchanged upon the addition of inductors, while the activity of PEP-synthetase and trehalose phosphate synthase decreased by 1.7—3.3 and 2—3.7 times, respectively.

Table 4. **The activity of key enzymes in surfactant biosynthesis of *Acinetobacter calcoaceticus* IMV B-7241 depending on the presence of eukaryotic inductors in the culture medium in different physiological states**

Carbon source	Inductor (*Saccharomyces cerevisiae* BTM-1)	Activity (nmol min^{-1} mg^{-1} of protein)			
		$NADP^+$-dependent glutamate dehydrogenase	PEP-synthase	PEP-carboxylase	Trehalose phosphate synthase
Purified glycerol	Control (without inductor)	345±17	17103±855	345±17	17±1
	Live cells	714±35	16034±801	414±20	14±1
	Inactivated cells	557±27	15906±795	357±17	17±1
	Supernatant	570±28	16520±826	441±22	13±1
Crude glycerol	Control (without inductor)	625±31	12345±617	625±31	108±5
	Live cells	667±33	3678±183	667±33	38±2
	Inactivated cells	667±33	7324±366	667±33	29±1
	Supernatant	667±33	3750±187	667±33	54±3

Discussion. The first report on the co-cultivation of microorganisms dated back to 1989, when Sonnenbichler et al. observed an increase in the variety of synthesized secondary metabolites through the co-cultivation of *Heterobasidion annosum* and *Gloeophyllum abietinum* fungi. Referencing publications from 1978, the review by (Li et al., 2023) highlighted the increased synthesis of levorin and amphotericin B upon the co-cultivation of the producers of these polyene antibiotics with *Candida* yeast. Therefore, initially, the literature reported the possibility of increasing the synthesis of secondary metabolites as a result of the co-cultivation of microorganisms, and later - the synthesis of new biologically active compounds that are not typical for monocultures. From the late 1990s to the present, the number of published articles devoted to the co-cultivation of microorganisms as a factor in regulating the synthesis and activity of secondary metabolites has increased by more than two orders of magnitude (Selegato & Castro-Gamboa, 2023).

However, despite the large number of publications on this topic, many questions remain unanswered. The first of these relates to the choice of a co-culture agent or biological inductor. Li et al. in their review (Li et al., 2023) attempted to summarize the available literature information on this matter. So, most often, both the producer and competitive microorganisms (or inductors) are inhabitants of the same habitats. In various studies, researchers have used pathogenic microorganisms for co-cultivation with secondary metabolite producers. Authors of the work (Li et al., 2023) referenced individual studies where partners to marine microorganism producers of biologically active compounds include bacteria containing mycolic acids, phototrophic organisms, or microorganisms containing gene clusters for synthesizing halogen peroxidases.

However, there are numerous other publications where microorganisms used as competitive, were not included in the classification provided in the review (Li et al., 2023). For instance, some researchers (Luti et al., 2018; Song et al., 2020; Luti & Yonis, 2013; Mahmoud et al., 2015; Fouad & Khalid, 2016; Sharma et al., 2017; Wang et al., 2013; Ramchandran et al., 2020) observed an increased synthesis when the culture medium of the producers of antimicrobial metabolites included yeast *S. cerevisiae*. Notably, in articles (Luti et al., 2018; Song et al., 2020; Luti & Yonis, 2013; Mahmoud et al., 2015; Fouad & Khalid, 2016; Sharma et al., 2017; Wang et al., 2013; Ramchandran et al., 2020), authors investigated the effect on secondary metabolite synthesis not only of saccharomycetes yeast as inductors but also of bacteria and fungi. However, they did not justify or explain the choice of biological inductors but simply stated the fact of their use. Moreover, some studies used only live cells of prokaryotes and eukaryotes (Luti et al., 2018; Sharma et al., 2017), or only inactivated ones (Ramchandran et al., 2020), or exclusively supernatants (Fouad & Khalid, 2016; Shi et al., 2017) as inductors.

For example, the researchers in (Sharma et al., 2017) found that in the presence of live cells of *Bacillus cereus* or *S. cerevisiae*, the synthesis of valinomycin by *Streptomyces lavendulae* ACR-DA1 increased by 62 and 34%, respectively. Additionally, in (Luti et al., 2018), it was demonstrated that the addition of live cells of *S. cerevisiae* to the culture medium of *Serratia marcescens* increased prodigiosin synthesis up to 170 mg/mL, which was 70% higher than when the producer was cultivated without an inductor. The use of prokaryotic inductors (*E. coli, B. subtilis*) allowed an increase in the the concentration of the final product to 220—250 mg/mL.

Furthermore, in (Ramchandran et al., 2020), it was shown that in the presence of heat-inactivated yeast cells of *C. albicans* SC 5314 in the culture medium of the surfactants producer *B. subtilis* RLID 12.1, the concentration of synthesized lipopeptides AF3 and AF5 increased by 1.4 and 2 times, respectively, compared to the levels obtained without an inductor.

Fouad and Khalid (2016) discovered that the addition of supernatant after cultivation of *S. aureus* ATCC 43090, *Bacillus* sp. ATCC 6633, *Aspergillus niger*, or *S. cerevisiae* at concentrations of 2—3% (v/v) in the culture medium of *B. subtilis* NK16 (the bacteriocin producer) was accompanied by a 2-4-fold increase in the synthesis of the final product compared to the values without inductors.

In the studies (Song et al., 2020; Luti & Yonis, 2013; Mahmoud et al., 2015; Wang et al., 2013), the effect of prokaryotic and eukaryotic inductors in various physiological states on the synthesis of secondary metabolites was investigated.

For example, Mahmoud et al. (2015) demonstrated that the production of prodigiosin by *S. marcescens* S23 increased by 1.4—7 times when live cells of *E. coli, B. subtilis,* or *S. cerevisiae* were introduced into the culture medium. The use of yeast as an inductor resulted in the highest concentration of this metabolite (3.1 g/L). In the presence of inactivated bacterial and yeast cells, a 7-9-fold increase in pigment synthesis was observed compared to that without inductors.

In another study by Song et al. (2020), it was found that the synthesis of the antibiotic rimocidin by *Streptomyces rimosus* M527 depended on the physiological state of the inductor. When the inductor in the form of supernatant or live yeast cells was introduced into the culture medium of the antibiotic producer, the concentration of rimocidin increased by 64 and 36%, respectively, compared to conditions without the inductor. However, inactivated yeast cells as an inductor did not affect the synthesis of rimocidin.

In the papers (Luti & Yonis, 2013), it was demonstrated that the introduction of live and heat-inactivated yeast cells of *S. cerevisiae* (0.5 and 1%) into the culture medium of *Pseudomonas aeruginosa* (producer of phenazine) resulted in an increase in the antibiotic concentration by 1.6—1.89 and 2.6—3.19 times, respectively, compared to the synthesized without an inductor.

It should be noted that in the studies (Luti et al., 2018; Song et al., 2020; Luti & Yonis, 2013; Mahmoud et al., 2015; Fouad & Khalid, 2016; Sharma et al., 2017; Wang et al., 2013; Ramchandran et al., 2020), the dependence of the secondary metabolites synthesis on both the nature of the inductor (prokaryotic or eukaryotic) and their physiological state was established. In addition, the authors most often used the bacteria *E. coli* and *B. subtilis*, as well as yeast *S. cerevisiae*, as inductors. While in our previous study (Pirog et al., 2023c), the effect of *B. subtilis* BT-2 in the form of live or inactivated cells, or the corresponding supernatant on the synthesis and biological activity of microbial surfactant *A. calcoaceticus* IMV B-7241 was examined, the present study used enterobacteria *E. cloacae* C-8 and saccharomyces yeast as inductors.

Moreover, in the articles (Luti et al., 2018; Song et al., 2020; Luti & Yonis, 2013; Mahmoud et al., 2015; Fouad & Khalid, 2016; Sharma et al., 2017; Wang et al., 2013; Ramchandran et al., 2020), the researchers analyzed the effect of inductors only on the synthesis of secondary metabolites.

In some of the mentioned studies (Luti & Yonis, 2013; Fouad & Khalid, 2016; Ramchandran et al., 2020), the antimicrobial activity of final products was determined, but only those synthesized in the presence of inductors. Thus, it remains unclear how the inductor affected the biological activity of the metabolites. This issue is quite important, since it is not enough to increase the concentration of synthesized antimicrobial compounds: their biological activity is to remain high. However, the activity of secondary metabolites depends on the conditions of cultivation of the producers (Pirog et al., 2019), and the presence of an inductor in the medium can negatively affect the properties of the final product. It should be noted that there are few publications that investigated the activity of secondary metabolites synthesized with and without inductors (Akone et al., 2016; Leães et al., 2016; Sung et al., 2017; Yu et al., 2017; Kimelman & Shemesh, 2019).

Therefore, in this work, similar to our previous experimental studies (Pirog et al., 2023c;

Pirog et al., 2020; Pirog & Ivanov, 2022), we focused on the study of the effect of inductors on the biological activity of surfactants.

The data presented in Tables 1 and 2 indicate that regardless of the method of inductor preparation (cultivation on agar or in liquid media), its type (*E. cloacae* C-8 or *S. cerevisiae* BTM-1), physiological state (live, inactivated cells, supernatant), and the substrate used (purified or crude glycerol), the synthesized microbial surfactants were characterized by significantly higher antimicrobial activity than the preparations obtained without inductors. These results differ from those described in studies (Akone et al., 2016; Sung et al., 2017; Yu et al., 2017), where an increase in the antimicrobial activity of secondary metabolites was observed upon the introduction of live inductor cells into the culture medium. Only in the study (Leães et al., 2016), the authors established a positive effect of inactivated cells of *S. aureus* subsp. *aureus* ATCC 25923 or *Aspergillus parasiticus* (strain number not given) on the antimicrobial activity of *Bacillus amyloliquefaciens* P11 lipopeptides against *Listeria monocytogenes* ATCC 7644 (3-3.5-fold increase compared to that obtained without inductors). Technologically, the use of inactivated cells of the inductor is simpler and more practical compared to live cells.

In our recently published review (Pirog & Ivanov, 2023a), we mentioned that currently, the mechanisms underlying the increase in antimicrobial compound synthesis in the presence of inductors remain under-researched. Researchers have identified some of these mechanisms: increased synthesis of antimicrobial compounds as a protection strategy against potential competitors; the recognition of certain proteins or receptors of inactivated inductor cells by the producer; the presence of heat-inactivated cells of the inductor affecting the expression of genes related to antimicrobial peptide synthesis; the interaction mechanism possibly involving direct contact between cells; inactivated cells of the inductor containing lysed compounds that may act as precursors for metabolite synthesis; the production of specific metabolites by fungi stimulating the transcriptional activation of silent biosynthetic gene clusters for antimicrobial compound biosynthesis.

The results of enzymatic studies (see Tables 3 and 4) showed that in the presence of all inductors in a medium with purified glycerol, the activity of $NADP^+$-dependent glutamate dehydrogenase (key enzyme of biosynthesis of aminolipids, which are characterized by higher antimicrobial activity compared to glycolipids) (Pirog et al., 2019) increased. Thus, the higher biological activity of surfactants produced by *A. calcoaceticus* IMV B-7241 on this substrate in the presence of biological inductors may be due to a higher content of aminolipids in their composition. However, introducing inductors into the medium with crude glycerol did not result in increased activity of $NADP^+$-dependent glutamate dehydrogenase. It can be assumed that under such cultivation conditions, other metabolites with antimicrobial activity, other than aminolipids, are synthesized. Our further studies will be devoted to clarifying this matter.

As a result of this study, the possibility of increasing the antimicrobial activity of surfactants produced by *A. calcoaceticus* IMV B-7241 was established by introducing pro- and eukaryotic inductors in the form of live or inactivated cells, or the corresponding supernatant into the culture medium with glycerol of different degrees of purification.

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ВПЛИВ ПРО- ТА ЕУКАРІОТИЧНИХ ІНДУКТОРІВ НА СИНТЕЗ ТА АНТИМІКРОБНУ АКТИВНІСТЬ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН ACINETOBACTER CALCOACETICUS IMB B-7241

На теперішній час комбіноване (спільне) культивування продуцентів антимікробних сполук з іншими мікроорганізмами або внесення у середовище культивування біологічних індукторів у різному фізіологічному стані (живі, інактивовані клітини або відповідний супернатант) є простим, дешевим та достатньо ефективним способом підвищення синтезу практично важливих мікробних метаболітів і регуляції їхньої біологічної активності. У більшості робіт дослідники використовують як індуктори штами бактерій різних фізіологічних груп, проте останніми роками з'являється все більше публікацій про еукаріотичні індуктори, у відповідь на наявність яких спостерігається підвищення синтезу антимікробних сполук бактеріями-продуцентами. При цьому ефективність біологічних індукторів залежить від умов їх вирощування та фізіологічного стану. **Мета.** Дослідити вплив способу підготовки та фізіологічного стану біологічних індукторів (грамнегативних бактерій *Enterobacter cloacae* С-8 та дріжджів *Saccharomyces cerevisiae* БТМ-1) на активність ферментів біосинтезу та антимікробну активність поверхнево-активних речовин *Acinetobacter calcoaceticus* IMB B-7241. **Методи.** Як субстрат для культивування *A. calcoaceticus* IMB B-7241 використовували очищений гліцерин та відходи виробництва біодизелю в еквімолярній за вуглецем концентрації. Вирощування індукторів здійснювали на агаризованому та рідкому середовищах з глюкозою як джерелом вуглецю. Живі або інактивовані клітини *S. cerevisiae* БТМ-1 чи *E. cloacae* С-8, або відповідний супернатант вносили у середовище в кількості 2,5—10 об.%. Позаклітинні поверхнево-активні речовини виділяли із супернатанту культуральної рідини екстракцією суміші хлороформу і метанолу (2:1). Антимікробну активність поверхнево-активних речовин щодо бактеріальних (*Bacillus subtilis* БТ-2, *Escherichia coli* ІЕМ-1, *Staphylococcus aureus* БМС-1, *Pseudomonas* sp. МІ-2) і дріжджових (*Candida albicans* Д-6, *Candida tropicalis* РЕ-2) тест-культур аналізували за показником мінімальної інгібуючої концентрації. Активність ферментів біосинтезу поверхнево-активних гліко- (фосфоенол-піруваткарбоксилаза, фосфоенолпіруватсинтетаза, фосфоенолпіруват-карбоксикіназа, трегалозофосфатсинтаза) та аміноліпідів (НАДФ$^+$-залежна глутаматдегідрогеназа) визначали у безклітинних екстрактах, які отримували обробкою клітин ультразвуком. **Результати.** Внесення у середовище культивування *A. calcoaceticus* IMB B-7241 з гліцерином різного ступеню очищення як про-, так і еукаріотичного індуктора у різному фізіологічному стані супроводжувалося синтезом поверхнево-активних речовин, антимікробна активність яких щодо досліджуваних тест-культур була на один—два порядки вищою порів-

няно з препаратами, одержаними без індукторів. Встановлено, що клітини *E. cloacae* С-8, вирощені у рідкому середовищі, виявилися дещо ефективнішими індукторами, ніж після культивування на агаризованому: мінімальні інгібуючі концентрації щодо бактеріальних і дріжджових культур, синтезованих за їх наявності поверхнево-активних речовин становили 1—6 і 2,5—8 мкг/мл відповідно. У разі використання як індукторів живих клітини *S. cerevisiae* БТМ-1 чи *E. cloacae* С-8 спостерігали утворення поверхнево-активних речовин, які характеризувалися вищою антимікробною активністю, ніж синтезовані за наявності інактивованих клітин або супернатанту: мінімальні інгібуючі концентрації щодо досліджуваних тест-культур перебували в межах 0,85—16; 2—20 і 1,5—22 мкг/мл відповідно. Вища антимікробна активність поверхнево-активних речовин, синтезованих за наявності про- чи еукаріотичного індуктора у середовищі з очищеним гліцерином може бути зумовлена підвищенням вмісту в їхньому складі аміноліпідів, про що свідчило збільшення в 1,6—2,1 рази НАДФ$^+$ — залежної глутаматдегідрогеназної активності в клітинах *A. calcoaceticus* IMB B-7241 порівняно з показниками культивування без індукторів. Однаковий рівень активності цього ферменту під час культивування штаму IMB B-7241 у середовищі з відходами виробництва біодизелю як за наявності індукторів, так і без них може свідчити про синтез за таких умов інших, відмінних від аміноліпідів, метаболітів з антимікробною активністю. **Висновки.** Отже, в результаті проведеної роботи встановлено можливість підвищення антимікробної активності поверхнево-активних речовин *A. calcoaceticus* IMB B-7241 внесенням у середовище з гліцерином різного очищення про- та еукаріотичного індукторів у вигляді живих, інактивованих клітин чи супернатанту.

Ключові слова: *дріжджові та бактеріальні індуктори, поверхнево-активні речовини, антимікробна активність.*