UDC 759.873.088.5:661.185

ANTIMICROBIAL ACTIVITY OF EXOCELLULAR METABOLITES *ACINETOBACTER CALCOACETICUS* IMV V-7241, *RHODOCOCCUS ERYTHROPOLIS* IMV Ac-5017, *NOCARDIA VACCINII* K-8 ON PHYTOPATHOGENIC BACTERIA

Pokora K.A., Chebotarova K.V.

Abstract. *We investigated the effect of exocellular metabolites, including surface-active substances (surfactants) of Rhodococcus erythropolis IMV Ac-5017, Acinetobacter calcoaceticus IMV B-7241 and Nocardia vaccinii K-8 on some phytopathogenic bacteria. It was shown that after 2 h treatment with surfactant preparations (0.15 – 0.4 mg/mL of strains IMV Ac-5017 and IMV B-7241 survival of genus pathogenic bacteria (10^5 – 10^7 in mL of Pseudomonas and Xanthomonas was 0 – 33%. In the presence of a surfactant preparations (0.085 – 0.85 mg/mL), and other exocellular metabolites of N. vaccinii K-8 number of cells of studied phytopathogenic bacteria reduced by 95 – 100 %. The results show the possibility of use microbial surfactants for the development of environmentally friendly products to control the number of pathogenic bacteria.*

Key words*: surface-active substances, phytopathogenic bacteria*

Introduction. Annual crop losses from pests in Ukraine are about 50 %. Much of those losses caused by bacterial diseases of plants and fruits, so in terms of agricultural production problem against pathogenic microorganisms is particularly acute [1]. Leading role in protecting plants today are chemical methods (toxic pesticides), which pollute the environment and agricultural products. The alternative is to develop and implement environmentally safe biological agents such as microbial surface-active substances (SAS), which have numerous advantages over chemical analogues: low toxicity, biodegradability, stability in extreme conditions, various biological activities (antimicrobial, fungicidal, antitumor, and antiviral) [5-8].

In previous studies from the oil-contaminated soil samples were selected oil-oxidizing bacteria, identified as *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* K-8. The ability of these strains to synthesize the exocellular surfactant during growth on hydrophilic and hydrophobic substrates was determined. The surfactant of strain IMB Ac-5017 is a complex of glyco-, phospho- and neutral lipids, and the surfactants of strains IMB B-7241 and K-8 – complex of glyco-, amino- and neutral lipids. Glycolipids of all strains are presented by trehalose mycolates. The aim of this work was to study the influence of extracellular metabolites of *A. calcoaceticus* IMB B-7241, *R. erythropolis* IMB Ac-5017 and *N. vaccinii* K-8 on some phytopathogenic bacteria.

Methods of research. *R. erythropolis* IMB Ac-5017 was grown in liquid nutrient medium, g/L: $NaNO_3$ – 1.3; $MgSO_4 \times 7H_2O$ – 0.1; NaCl – 1.0; Na_2HPO_4 – 0.6; KH_2PO_4 – 0.14; $FeSO_4 \times 7H_2O$ – 0.01, pH 6.8 – 7.0, fried sunflower oil (2 vol. %) was used as a carbon and energy source. For the cultivation of *A. calcoaceticus* IMB B-7241 was used a nutrient medium of following composition, g/L: $(NH_2)_2CO$ – 0.35; $MgSO_4 \times 7H_2O$ - 0,1; NaCl – 1.0; Na_2HPO_4 – 0.6; KH_2PO_4 – 0.14; pH 6.8–7.0, yeast autolyzate and trace elements solution were added at concentrations 0.5 and 0.1%, respectively. Ethanol (2 vol. %) was used as a source of carbon and energy. Strain *N. vaccinii* K-8 was grown on synthetic nutrient medium of the flowing composition, g/L: $NaNO_3$ – 0.5; $MgSO_4 \times 7H_2O$ – 0.1; $CaCl \times 2H_2O$ – 0.1;

KH_2PO_4 – 0.1; $FeSO_4 \times 7H_2O$ – 0.1, yeast autolyzate and glycerol – 0.5 and 1.5 vol. %, respectively. Cultivation of bacteria was carried out in 750 mL flasks with 100 mL of nutrient medium on a shaker (320 r/min) at 28 – 30 ° C during 120 h. Inoculum was grown in appropriate medium to mid-exponential growth phase. The volume of inoculum was 5–10 % of the volume of nutrient medium.

The following preparations were used in experiments: preparation 1 – supernatant of cultural liquid, preparation 2 – solution of surfactant extracted from the supernatant (preparation 1) with the mixture of methanol and chloroform (2:1); preparation 3 –water phase remaining after surfactant extraction.

Pseudomonas syringae UKM B-1027, *Pseudomonas corrugate* 9070, *Pseudomonas savantanoi* pv. *glicinea* 8571, *Pseudomonas syringae* pv. *coronafaciens* – UKM B-1154, *Pseudomonas syringae* pv. *atrofaciens* UKM B-1015, *Xantomonas translucens* 7696, *Xantomonas vesicatoria* 7790, *Pectobacterium carotovorum* UKM B-1095, *Xanthomonas campestris* pv. *campestris* UKM B-1049 (pathogens of cereals and legumes), were used at test cultures and kindly provided by the department of phytopathogenic bacteria of the Institute of Microbiology and Virology National Academy of Sciences of Ukraine.

Determination of antimicrobial action was carried out in culture suspension. Suspensions of test cultures were prepared in test tubes (1.5 mL), then 1.5 mL of preparation was added and kept within 1 and 2 h at the temperature optimal for growth of test culture. The quantity of living cells was determined by the Koch method after the exposition. Survival of test cultures was determined as the ratio of the quantity of living cells in the samples treated with preparations to the number of cells in the original suspension and expressed as a percentage [2].

Results and discussion. It was determined in the case of the addition of the preparation 2, synthesized by *A. calcoaceticus* IMV B-7241 (0.3 mg/mL) and *R. erythropolis* IMV Ac-5017 (0.8 mg/mL), in cells suspension of all investigated test cultures survival was 10 %. Under the influence of preparation 1 of strains IMV Ac-5017 and IMV B-7241 was observed the stimulation growth of bacteria that can be explained by the presence of other biologically active substances (not surfactants), such as phytohormones, in supernatant [2].

Regardless of the degree of purification of preparations 1 – 3 of *N. vaccinii* K-8 (1.7 mg/mL) (table) the quantity of pathogenic bacteria decreased by 98–100 %. It should be noted that the drug 3 of strain K-8 was the most effective among all. We assume that the antimicrobial substances, which don't have surface-active properties, are left in the water phase after extraction of surfactant.

At the next step we examined the influence of the lower concentrations (up to 0.042 mg/mL) preparation 2 of *N. vaccinii* K-8 on survival of phytopathogenic bacteria. From literature it is known that representatives of the genus *Pseudomonas* produce ramnolipid SAS, effective against gram-positive and gram-negative bacteria in very low concentrations (16–256 μg/mL). Lactic acid bacteria *Lactococcus lactis* 53 and *Streptococcus thermophilus* A, SAS which synthesize with antimicrobial activity against human pathogens (*Staphylococcus epidermidis, Enterococcus faecalis*) in concentrations of 25–100 μg /mL [3].

Previously it was shown that SAS of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 (1.5–0.15 mg/mL) exhibit antimicrobial action against *Esherihia coli* and *Bacillius subtilis*. It was shown that in some cases, lower concentrations of surfactant of lactic acid bacteria (up to 1.5 mg/mL) were significantly more effective than the concentration of 10–100 μg /mL [3]. Futher it was determined that various concentrations (0.021–0.85 mg/mL) of preparations of exocellular metabolites of strain K-8 were effective against *X. vesicatoria* 7790 and *P. corrugate* 9070. There was no correlation between the concentration of surfactant

and cell survival. Obviously, as the dilution is changing not only on the concentration of active components and their "availability" for the culture of the test chamber. From the literature it is known [4], that microbial surfactants inherent antimicrobial activity of in very low concentrations, so further our research will be aimed at determining the minimum inhibitory concentrations of surfactants [4].

Table 1.

Antimicrobial activity of exocellular metabolites of N. vaccinii K-8 against some phytopathogenic bacteria

Test culture*	Preparation	Cells survival after, %	
		1 h	2 h
P. syringae pv. *coronafaciens* UKM B-1154	1	3.8±0.19	3.5±0.17
	2	0.22±0.01	0
	3	0	0
P. syringae pv. *atrofaciens* UKM B-1015	1	1.14±0.05	0.35±0.01
	2	0.42±0.02	0.14±0.01
	3	0	0
X. vesicatoria 7790	1	22.2±1.11	20.8±1.04
	2	0.37±0.01	2.08±0.10
	3	0.08±0.01	0.03±0.01
P.carotovorum UKM B-1095	1	0.08±0.01	0.39±0.01
	2	0.17±0.01	3.2±0.16
	3	0.08±0.01	2.03±0.11
X. campestris pv. *campestris* UKM B-1049	1	8.8±0.44	4.5±0.22
	2	0.1±0.01	0.6±0.01
	3	0	0

* The initial quantity of *P. syringae* pv. *coronafaciens* cells UKM B-1154 – (48 h) was 9×10^4 CFU/mL, *P. syringae* pv. *atrofaciens* UKM B-1015 (48 h) – 2.8×10^5 CFU/mL, *X. vesicatoria* 7790 (48 h) – 7.4×10^5 CFU/mL, *P. carotovorum* UKM B-1095 (48 h) – 2.3×10^6 CFU/mL, *X. campestris* pv. *campestris* UKM B-1049 (96 h) – 4.6×10^6 CFU/mL.

Conclusions.

So, as a result of our study it was found that exocellular metabolites of strains IMV B-7241, IMV Ac-5017 and K-8 inherent antimicrobial activity against some pathogenic microorganisms.

Therefore, these preparations can be used as an environmentally safe antimicrobial products, which inherent high efficiency against number of pathogenic bacteria resistant to existing traditional agents to fight bacterioses of agricultural crops.

Acknowledgements. We would like to thank the scientific supervisor Doctor of Biological Sciences Pirog T.P. and member of National Academy of Sciences of Ukraine, Doctor of Biological Sciences – Iutinska H. A.

References.

1. *Xu X.M.*, *Jeffries P.*, *Pautasso M.*, *Jeger M. J.* Combined use of biocontrol agents to manage plant diseases in theory and practice // Phytopathology. – 2011. – V. 101. № 9. – P. 1024–1031.
2. *Pirog, T.P., Konon A.D., Sofilkanych A.P., Skochko A.B.* Antimicrobial effect of surfactants *Acinetobacter calcoaceticus* K-4 and *Rhodococcus erythropolis* EK-1 // Мікробіологічний журнал. – 2011. – Т. 74, № 3. – P.14 – 20.
3. *Gudiña E.J., Teixeira J.A., Rodrigues L.R.* Isolation and functional characterization of a biosurfactant produced by *Lactobacillus paracasei. //* Colloids Surf. B. Biointerfaces. – 2010. – Vol. 76, N 1. – P. 298 – 304.
4. *Raaijmakers J.M., Bruijn I., Nybroe O., Ongena M.* Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics // FEMS Microbiol. Rev. – 2010. – Vol. 34. – P. 1037–1062.
5. *Kalyani R., Bishwambhar M, Suneetha V.* Recent potential usage of surfactant from microbial origin in pharmaceutical and biomedical arena: a perspective // International research journal of pharmacy. – 2011. –Vol. 2. № 8. – P. 11–15.
6. *Kulakovskaya, T.V., Golubev, W.I., Tomashevskaya, M.A., Kulakovskaya E.V., Shashkov A.S., Grachev A.A., Chizhov A.S., Nifantiev N.E.* Production of antifungal cellobiose lipids by *Trichosporon porosum* // Mycopathologia. – 2010. – Vol. 169. № 2. – P. 117–123.
7. *Varnier A.L., Renault J.H. and other* Bacterial rhamnolipids are novel MAMPs conferring resistance to *Botrytis cinerea* in grapevine // Plant. Cell Environ. – 2009. – Vol. 32. № 2. – P. 178–193.
8. *Grover M., Nain L., Singh S.B., Saxena A.K.* Molecular and biochemical approaches for characterization of antifungal trait of a potent biocontrol agent *Bacillus subtilis* RP24 // Curr. Microbiol. – 2010. – Vol. 60. № 2. – P. 99–106.

Author certificate:
***Pokora Khrystyna Andreevna**, student of National University of Food Technologies, e-mail:khrystya91@ukr.net*
***Chebotarova Ksenia Volodimirovna**, student of National University of Food Technologies, e-mail: katrielen@mail.ru*