Pokora.C.A., student
Chebotarova K.V., student
Konon A.D., postgraduate student
Sofilkanych A.P., postgraduate student
Pirog T.P., Doctor of Biological Sciences, professor
National University of Food Technologies

ANTIMICROBIAL ACTIVITY OF EXOCELLULAR METABOLITES OF *ACINETOBACTER CALCOACETICUS* IMB B-7241, *RHODOCOCCUS ERYTHROPOLIS* IMB Ac-5017, *NOCARDIA VACCINII* K-8 AGAINST PHYTOPATHOGENIC BACTERIA

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. Leading role in protecting plants today are chemical methods (treatment of 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 several advantages over chemical analogues: low toxicity, biodegradability, stability in extreme conditions, various biological activities (antimicrobial, fungicidal, antitumor, and antiviral).

In previous studies from the oil-contaminated soil samples were selected oil-oxidizing bacteria, identified as *Acinetobacter calcoaceticus* IMV B-7241, *Rhodococcus erythropolis* IMV Ac-5017 and *Nocardia vaccinii* K-8. The ability of these strains to the synthesis the extracellular 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 *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017 and *Nocardia vaccinii* K-8 on some phytopathogenic bacteria.

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, additionally contributed avtolizat yeast solution and trace elements at concentrations 0.5 and 0.1% (volume fraction), respectively. Ethanol (2 vol. %) was used as a source of carbon and energy. Strain *N. vacinii* K-8 was grown on synthetic nutrient medium of the 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 autolysate 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 º 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, the 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 8511, *Pseudomonas corrugate* 9070, *Pseudomonas savantanoi* pv. *glicinea* 8571, *Pseudomonas syringae* pv. *coronafaciens* 9129, *Pseudomonas syringae* pv. *atroglaciens*, *Xantomonas translucens* 7696, *Xantomonas vesicatoria* 7790, *Pectobacterium carotovorum* 8982 (pathogens of cereals and legumes) were used as a test cultures, which were kindly provided by the department of phytopathogenic bacteria of the Institute of Microbiology and Virology of 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 [1].

It was determined in the case of the addition of the preparation 2, synthesized by *A. salcoaceticus* IMB B-7241 (0.3 mg/mL) and *R. erythropolis* IMB 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 IMB Ac-5017 and IMB 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 stage we examined the influence of the drug 2 *N. vaccinii* K-8 with lower concentration of surfactant (up to 0.042 mg/mL) on survival of phytopathogenic bacteria. From literature data it is known that representatives of the genus *Pseudomonas* produce surfactants of rhamnolipid nature, effective against gram-positive and gram-negative bacteria in very low concentrations (256–16 mg/mL). Besides, the group of lactic acid bacteria *Lactococcus lactis* 53 and *Streptococcus thermophilus* A synthesize surfactants which characterized by antimicrobial action against human pathogens (*Staphylococcus epidermidis*, *Enterococcus faecalis*) in the concentrations of 25–100 μg/ml [2, p.16].

Antimicrobial action of extracellular metabolites of *N. vacinnii* K-8 against some phytopathogenic bacteria

Test culture	Preparation	Cells survival, %	
		1 h	**2 h**
Pseudomonas syringae pv. *coronafaciens* 9129	1	3.8±0.19	3.5±0.17
	2	0.22±0.01	0
	3	0	0
Pseudomonas syringae pv.*atroglaciens* 8291	1	1.14±0.05	0.35±0.01
	2	0.42±0.02	0.14±0.006
	3	0	0
Xantomonas vesicatoria 7790	1	22.2±1.11	20.8±1.04
	2	0.37±0.01	2.08±0.1
	3	0.08±0.004	0.03±0.01
Pectobacterium carotovorum 8289	1	0.08±0.004	0.39±0.01
	2	0.17±0.008	3.2±0.16
	3	0.08±0.004	2.03±0.11
Xantomonas campestris pv. *campestris* 8003	1	8.8±0.44	4.5±0.22
	2	0.1±0.005	0.6±0.003
	3	0	0

The initial quantity of cells *P. syringae* pv. *coronafaciens* 9129 (48 h) was 9×10^4 CFU/mL, *P. syringae* pv.*atroglaciens* 8291 (48 h) – 2.8×10^5 CFU/mL, *X. vesicatoria* 7790 (48 h) – 7.4×10^5 CFU/mL, *P. carotovorum* 8289 (48 h) – 2.3×10^6 CFU/mL, *X. campestris* pv. *campestris* 8003 (96 h) – 4.6×10^6 CFU/mL.

Previously it was shown that surfactants of *A. salcoaceticus* IMB B-7241 and *R. erythropolis* IMB Ac-5017 (1.5–0.15 mg / ml) showed antimicrobial activity 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 mg/mL [2]. At the next step we determined the effect of various concentrations (0.042–1.7 mg/mL) of extracellular metabolites preparations of strain K-8 against *X. vesicatoria* 7790 and *P. corrugate* 9070. There was no correlation between the concentration of surfactant and cell survival. Obviously, as far as breeding is changing not only the concentration of active ingredients and their "accessibility" for the test cell cultures. In studies of antimicrobial action of biosurfactants they were used in very low concentrations, so further our research will be aimed at determining the minimum inhibitory concentrations of surfactants [3, p.1037].

So, the results of present work showed that extracellular metabolites of IMB B-7241, IMB Ac-5017 and K-8 strains inherent antimicrobial properties against some pathogenic microorganisms. Therefore, these preparations can be used as environmentally safe antimicrobial products, which exhibit high efficiency against a number of pathogenic bacteria resistant to existing traditional preparations.

References

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