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NOCARDIA VACCINII К-8 AND *ACENITOBACTER CALCOACETICUS* IMV B-7241 – POTENTIAL DESTRUCTORS OF AROMATIC COMPOUNDS

Up to 13 million lives could be saved each year by reducing environmental risks, according to the World Health Organization's first country-by-country analysis of the impact of environmental factors on human health [6, p. 1937]. Environmental pollution is a great concern to all countries around the world. China has made great efforts in this area and plans to invest $175 billion in environmental protection between 2006 and 2010, according to the National Development and Reform Commission of China [4, p. 2].

Aromatic hydrocarbons are serious pollutants, being present mainly in industrial wastewater from chemical, petrochemical, pharmaceutical, textile, and steel industries. Due to their ubiquitous occurrence, recalcitrance, bioaccumulation potential and carcinogenic activity, the aromatic compounds have gathered significant environmental concern [1, p. 14]. They are widely distributed environmental contaminants that have detrimental biological effects, toxicity, mutagenecity and carcinogenicity [1, p. 14; 3, p. 1590]. Although aromatic hydrocarbons may undergo adsorption, volatilization, photolysis and chemical degradation, microbial degradation is the major degradation process. Bioremediation is the tool to transform the compounds to less hazardous (nonhazardous) forms with less input of chemicals, energy and time [2, p. 315; 6, p. 1939]. Besides microbiological methods are economically advantageous, do not

require large capital investments and operating costs, and local sewage treatment plants take low areas and very easy to maintain [5, p. 623].

In the previous work the oil-oxidizing bacteria identified as *Nocardia vaccinii* K-8 and *Acenitobacter calcoaceticus* K-4 were isolated from the oil-polluted samples of soil. The ability of the strain to synthesize the metabolites with surface-active and emulsifying activity (biosurfactants) during their cultivation on different hydrophobic (*n*-hexadecane, liquid paraffin) and hydrophilic (glucose, ethanol) substrates was determinded. The strain K-4 was deposited in the Depositary of microorganisms of the Institute of Microbiology and Virology of National Academy of Sciences of Ukraine at the number of IMV Ac-7241.

In scientific publications [4, p. 5] repeatedly stated that in the microbial communities of ecosystems contaminated by aromatic xenobiotics, common bacteria genus are *Nocardia* and *Acenitobacter*. Previously it was shown that the bacteria *N. vaccinii* K-8 and *A. calcoaceticus* IMV Ac-7241 intensified the processes of oil degradation in contaminated sites. Since crude oil always contains aromatic hydrocarbons (10–50%), we assumed that the studied strains may be potential destructors of aromatic compounds. In this regard, the aim of our work was to study the ability of strains IMV B-7241 and K-8 to grow on nutrient media containing substrates of aromatic nature as a carbon and energy source.

A. calcoaceticus IMV B-7241 was cultivated on the 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; the yeast autolysate – 0.5 vol. % and trace elements solution – 0.1 vol. % were also added.

N. vacinii K-8 strain was grown on the synthetic nutrient medium containing (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 – 0.5 vol. %.

Phenol, 4-chlorphenol, hexachlorobenzene, naphthalene, benzoic, sulfanilic and N-phenilantranilic acids, benzene, toluene (0.3–0.5 %) were used as soul carbon and energy sources.

The inoculum – 24-hour culture cultivated on the meat infusion agar (MIA). The cultivation of bacteria took place in the 750 ml Erlenmeyer flasks with 100 ml of medium on rotor shaker (320 rpm) at 28–30 ºC during 120 h. The adaptation of bacteria to aromatic compounds was carried out by gradual increase of their concentration from 0.1 to 0.25 % in nutrient medium followed by bacteria inoculating on mineral medium with 0.3–0.5% of the substrate.

The quantity of synthesized surfactant was evaluated by such indexes: conditional surfactant concentration (CSC*) and emulsification index (E_{24}, %) of the cultural liquid. The number of viable cells was determined by the Koch method on MIA, biomass – by the optical density of cultural liquid, followed by recalculation to absolutely dry biomass by calibration graph.

It was determined that *N. vaccinii* K-8 and *A. calcoaceticus* IMV B-7241 intensively grew on phenol, hexachlorobenzene, naphthalene, N-phenilantranilic and benzoic acid, slightly worse on toluene and benzene and died on 4-chlorphenol and sulfanilic acid. The utilization of aromatic compounds accompanied by the formation of extracellular metabolites with surface-active and emulsifying properties (Table 1 and 2). Thus, during *A. calcoaceticus* IMV B-7241 cultivation on phenol (0.5%) the highest conditional surfactant concentration (CSC*) and emulsification index (E_{24}%) were 3.6 and 70% respectively (while on ethanol CSC* – 1.0 and E_{24} – 43%). The maximum indexes of surfactant synthesis by *N. vaccinii* K-8 were observed as a result of strain growth on media with naphthalene (0.5%): CSC* – 2.6 and E_{24} – 70%, while on glycerol (0.5%) – 2.0 and 60%, respectively.

It was shown that the increased concentrations of biomass (30–40 % of control) have been observed in the case of three consecutive inoculating of strains K-4 and IMV B-7241 on medium containing aromatic compounds (0.3–0.5%).

It was determined that *N. vaccinii* K-8 and *A. calcoaceticus* IMV B-7241 grew intensively on media with all studied substrates of aromatic nature after pre-

adaptation in liquid mineral nutrient medium. The process of cultivation was accompanied by changes of cultural characteristics and 2–3-fold biomass increase.

Table 1

Surfactant synthesis during *Acenitobacter calcoaceticus* IMB B-7241 cultivating on aromatic compounds

Aromatic compound	Concentration, %	Biomass, g/L	Final pH	CSC*	E_{24}, %
Phenol	0.3	0.2±0.010	8.7±0.6	3.2±0.20	65±4.2
	0.5	0.22±0.009	8.3±0.5	3.6±0.30	75±4.8
Benzoic acid	0.3	0.10±0.005	6.8±0.4	2.1±0.10	55±3.6
	0.5	0.19±0.009	6.5±0.5	2.8±0.10	52±4.1
Ethanol (control)	0.3	0.46±0.029	6.4±0.2	0.8±0.05	40±2.6
	0.5	0.55±0.033	6.0±0.3	1.0±0.06	43±2.0

Table 2

Indexes of surfactant synthesis by *Nocardia vaccinii* K-8 on aromatic compounds

Aromatic compound	Concentration, %	Biomass, g/L	Final pH	CSC*	E_{24}, %
Phenol	0.3	0.20±0.010	8.4±0.6	1.8±0.05	50±2.7
	0.5	0.24±0.008	8.1±0.3	2.5±0.08	70±5.1
Naphthalene	0.3	0.20±0.011	8.4±0.4	2.4±0.10	50±2.5
	0.5	0.21±0.010	8.5±0.5	2.6±0.12	70±3.5
Ethanol (control)	0.3	0.21±0.010	6.8±0.6	1.4±0.12	50±1.8
	0.5	0.22±0.015	7.0±0.8	2.0±0.12	60±2.1

So, *N. vaccinii* K-8 and *A. calcoaceticus* IMV B-7241 are able to assimilate aromatic compounds, as well as to synthesize practically valuable surfactants.

That’s why these strains are promising for use in the remediation of water and soil polluted with crude oil and aromatic xenobiotics.

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