UDC 579.663

POST-HARVEST TREATMENT OF VEGETABLES WITH EXOMETABOLITES OF Nocardia vaccinii IMV B-7405, Acinetobacter calcoaceticus IMV B-7241 AND Rhodococcus erythropolis IMV AC-5017 TO EXTEND THEIR SHELF LIFE

T. P. Pirog B. S. Geichenko A. O. Zvarych

National University of Food Technologies, Kyiv, Ukraine

E-mail: tapirog@nuft.edu.ua

Received 26.09.2019 Revised 02.12.2019 Accepted 20.12.2019

The aim of the work was to study the possibility of *Nocardia vaccinii* IMV B-7405, *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 supernatants usage with various concentrations of surfactants for post-harvest treatment of vegetables.

N. vaccinii IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 were grown on waste sunflower oil and ethanol. For vegetables treatment, supernatants of the culture broth with surfactant concentration of 0.01-0.5 g/l were used. The concentration of surfactants was determined by the gravimetric method after extraction with Folch mixture. The total number of heterotrophic bacteria and fungi on the surface of vegetables was determined by the Koch method on meat-peptone agar and wort agar, respectively.

It was shown that treatment of broccoli, Brussels sprouts, sweet pepper and tomatoes with *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 supernatants was accompanied by 6-17 and 8-50 times decrease of bacteria and fungi number on their surface, respectively, compared with that on the surface of vegetables washed with tap water. The possibility of double use of the same supernatant for various batches of vegetables washing was established. Non-treated and water-washed vegetables rotted faster than those treated with surfactant-containing supernatants.

N. vaccinii IMV B-7405, *R. erythropolis* IMV Ac-5017 and *A. calcoaceticus* IMV B-7241 exometabolites used for treating vegetables to extend their shelf life have the following advantages in comparison with known microbial surfactants: they exhibit high antimicrobial activity when the surfactant concentration was several times lower and in the form of supernatant, which lets you exclude the expensive stage of isolation and purification of the target product from the technological process. In addition, surfactant-containing supernatants are highly effective in their repeated use.

Key words: storage of vegetables, microbial spoilage, surfactants.

Every year, the number of publications on the prospects of the practical use of non-toxic biodegradable surfactants (SAS) of microbial origin in various industries, agriculture, medicine, environmental protection is increasing [1-6].

One of the new trends in the use of microbial surfactants in agro-industrial complex is the post-harvest treatment of fruits and vegetables to extend their shelf life [1, 7-9], which is due to the antimicrobial and

anti-adhesive properties of these microbial synthesis products. According to [10], depending on the region, the loss of fruit and vegetable yields in the world is between 15 and 50%, the main cause of such significant losses is microbial spoilage.

Nowadays physical and chemical methods are used for the treatment of fruits and vegetables during transportation and storage, but their side effects, high energy costs and ecological incompatibility [10] have led scientists to find safe alternative methods, in particular biological ones [9-12]. Thus, the natural biopolymer chitosan or its combinations with aromatic oils, organic acids, and metal nanoparticles are used to protect fruits and vegetables during storage [10-11]. In [12] it is noted that bacteriocins nisin, enterocin AS-48, bovicin HC5, enterocin 416K1, pediocin and bificin C6165 are promising for the treatment of both fruits and fruit concentrates, juices, salads to prevent their microbial deterioration; moreover, nisin and pediocin are allowed as a dietary supplements in many countries. Among microbial surfactants, rhamnolipids are permitted for use in the food industry [13].

Earlier [14] we showed that the treatment of vegetables (squashes, cucumbers, tomatoes) with N. vaccinii IMV B-7405 surfactant solutions was more effective than washing with water. In our studies [14], surfactant solutions extracted from the supernatant of the culture liquid with organic solvents were used, which are described in the literature [7-9] as preparations; and such surfactant solutions were used for washing only once, and then were poured out.

In [15–16], we found that surfactants synthesized by Acinetobacter calcoaceticus IMV B-7241 and Rhodococcus erythropolis IMV Ac-5017 were characterized by high antimicrobial and anti-adhesive activity; and antimicrobial activity was shown not only by surfactant solutions, but also by the corresponding supernatants. It should be noted that after surfactant extraction from supernatants with the Folch mixture, the following lost their surfactant properties, and the aqueous phase remaining after surfactants removal was not characterized by antimicrobial activity. These data may indicate that the major exometabolites with antimicrobial properties in the supernatants of these strains are the surfactants themselves.

In connection with the above, the purpose of the study was to investigate the possibility of use the *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 surfactant-containing supernatants with different concentrations of surfactants for post-harvest treatment of vegetables.

Materials and Methods

Objects of research. The strains identified by us as Nocardia vaccinii K-8, Acinetobacter calcoaceticus K-4 and Rhodococcus erythropolis EC-1 [17] isolated from oil contaminated soil samples were the main objects of study. Strains were registered at the Depository of microorganisms, Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine as *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017.

By the chemical nature, *R. erythropolis* IMV Ac-5017 surfactants are a complex of glyco- (trehalose mono- and dimycolates), neutral and phospholipids. Glyco- (trehalose mono- and dimycolates, trehalose mono- and diacelates) and aminolipids are contained in the surfactants of *A. calcoaceticus* IMV B-7241. *N. vaccinii* IMV B-7405 synthesizes a complex of neutral, glyco- and aminolipids. Neutral lipids are represented by mycolic and n-alkanoic acids; glycolipids are represented trehalose diacelates and trehalose mycolates [18].

Cultivation of surfactant producers. N. vaccinii IMV B-7405 was grown in the liquid mineral medium of the following composition (g/l): NaNO₃ — 0,5; MgSO₄·7H₂O — 0,1; CaCl₂·2H₂O — 0,1; KH₂PO₄ — 0,1; FeSO₄·7H₂O — 0,001; distilled water — up to 1 l, pH 6.8–7.0. Yeast autolysate was added to the medium — 0.5% (v/v). Sunflower oil after potato frying was used as a carbon source (McDonald's fast food restaurant, Kyiv) at a concentration of 2% (v/v).

The cultivation of *R. erythropolis* IMB Ac-5017 was carried out in the medium of the following composition (g/l): NaNO₃ — 1,3; MgSO₄·7H₂O — 0,1; NaCl — 0,1; Na₂HPO₄ — 0,16; KH₂PO₄ — 0,14; CaCl₂ — 0,1; FeSO₄·7H₂O — 0,001; distilled water — up to 1 l, pH 6.8-7.0. Ethanol in a concentration of 2% (v/v) was used as the substrate.

For the cultivation of A. calcoaceticus IMV B-7241 the following medium was used (g/l): $(NH_2)_2CO - 0,35$, NaCl - 1,0, Na₂HPO₄·12H₂O - 0,6, KH₂PO₄ - 0,14, MgSO₄·7H₂O - 0,1; CaCl₂ - 0,1; distilled water - up to 1 l, pH 6.8-7.0. Yeast autolysate was added to the medium - 0.5% (v/v) and trace element solution - 0.1% (v/v) containing (g/100 ml): ZnSO₄·7H₂O - 1,1; MnSO₄·H₂O -0,6; FeSO₄·7H₂O - 0,1; CuSO₄·5H₂O -0,004; CoSO₄·7H₂O - 0,03; H₃BO₃ - 0,006; KI - 0,0001; EDTA (Trilon B) - 0.5. Ethanol in a concentration of 2% (v/v) was used as a carbon and energy source.

The cultures of exponential growth phase grown on media of the above composition with 0.5% of the corresponding substrate were used as the inoculum. The amount of inoculum $(10^4-10^5 \text{ cells/ml})$ was 10% of the volume of

medium. The cultivation of the strains was carried out in 750 ml flasks with 100 ml of medium on a rocker (320 rpm) at 30 °C for 120 h.

Determination of surfactants concentration. The amount of surfactants synthesized was determined by gravimetric method after extraction from culture liquid supernatant with Folch mixture (chloroform and methanol, 2: 1) as described in our previous work [18].

Obtaining of surfactant-containing supernatants. Supernatants obtained by centrifugation of the post-fermentation culture liquid for 25 min (5 000 g) were used as the preparations for vegetable treatment. The supernatants were sterilized for 30 min at 112 °C. Supernatants with a surfactants concentration of 0.01-0.5 g/l were used for the processing of vegetables. To achieve the desired concentration, the supernatants were diluted with sterile tap water.

Selection and processing of vegetables. Green tomatoes (Malachite casket), broccoli (Spring), Brussels sprouts (Long Island), and sweet peppers (Kolobok) were grown in open soil, without pesticides (Gvozdiv, Kyiv region, GPS 50°14'53.5"N 30°28'41.3"E). Ripe vegetables with no visible damage and infections were selected.

Vegetables were divided into three groups: the first group (control) was not undergo any treatment, the second one was treated with sterile tap water, and the third one with surfactant-containing supernatants with different surfactant concentrations. For treatment with water or supernatant, the vegetables were placed in a 500 ml graduated cylindrical beaker, 250 ml of water (supernatant) were added, kept for 5 min, and then water (supernatant) was poured out [19]. In one variant, the surfactant-containing supernatant was not poured out after washing the vegetables, but used again to process a new batch of not yet washed vegetables, then again it was not poured out, and used a third time to wash the next batch of vegetables. When presenting the material, such variants of consecutive application of the same supernatant three times for washing three different batches of vegetables will be referred to as once, double and thrice use of the preparation.

Untreated and washed with water and supernatant, the vegetables were placed in Petri dishes and left for observation at room temperature. Microbiological analysis was performed before vegetables storage.

Microbiological analysis. For microbiological control, several vegetables from each

group were collected with sterile tweezers, homogenized for 3 min on a T 10 basic ULTRA-TURRAX device, after which 1 g of homogenate was introduced into a test tube with 9 ml of sterile tap water and stirred. The number of microorganisms (colony forming units, CFU/ml) was determined by Koch[,] method on meat-peptone agar (MPA) for the detection of heterotrophic bacteria, and on wort-agar (WA) for the detection of fungi. Petri dishes with MPA were incubated for 24 h at 37 °C, with WA — for 48 h at 30 °C.

Vegetables quality assessment. Assessment of the quality of vegetables was carried out visually during the shelf life. The end of the experiment was considered the day when all the fruits were marked with spoilage signs: rot, discoloration and consistency change, cracks and wrinkles.

Statistical data processing. All experiments were performed in 3 replicates, the number of parallel determinations in the experiments was 3-5. Statistical processing of the experimental data was performed as described previously [18]. Mean indices differences were considered significant at the significance level P < 0.05.

Results and Discussion

Broccoli is a cruciferous vegetable characterized by high nutritional value, primarily due to high content of antioxidant compounds, vitamins and anticarcinogenic substances [20]. Peculiarities of broccoli harvesting are that ripe inflorescences before transportation and sale are cut off, so this product is especially vulnerable to microbial deterioration as well as phytopathogenic microorganisms and saprophytic microbiota that can get into plant tissues through the cut. Nowadays, the widespread method of postharvest broccoli treatment is washing with an aqueous solution of sodium hypochlorite (50-150 mg/ml), which, although it has an antimicrobial effect, is dangerous to the environment and humans [21].

In this regard, other variants for the treatment of this vegetable are investigated: washing with water acidified due to electrolysis, washing with calcium chloride solution, UV irradiation, ozone treatment, the use of edible films [20, 22].

Table 1 shows the data about the effect of *R. erythropolis* IMV Ac-5017 supernatant with different surfactants concentration on the total number of microorganisms on the surface of broccoli.

Processing variant	Cell number, 10 ² CFU/ml		
	Bacteria	Fungi	
Without treatment	$350{\pm}28$	$1880{\pm}120$	
Water	$260{\pm}19{*}$	1200±80**	
Surfactants (0.01 g / l)	63±8*	108±11**	
Surfactants (0.05 g / l)	$15{\pm}4{*}$	15±4* 22±4**	
Surfactants (0.25 g / l)	$30{\pm}6{*}$	43±7**	

 Table 1. The influence of the treatment method and surfactants concentration in R. erythropolis

 IMV Ac-5017 supernatant on the total number of heterotrophic bacteria and fungi on the surface of broccoli

Notes: * — P < 0.05 — regarding control (number of heterotrophic bacteria cells on the surface of untreated vegetables); ** — P < 0.05 — regarding control (number of fungal cells on the surface of untreated vegetables).

These data indicate that in the case of broccoli treatment with *R. erythropolis* IMV Ac-5017 supernatant with a surfactant concentration of 0.01-0.25 g/l, the number of bacteria and fungi on their surface decreased by two to three orders of magnitude compared to those established for untreated and waterwashed vegetables. The maximal decrease in the number of microorganisms was observed for use of supernatant with 0.05 g/l surfactant concentration for broccoli washing.

Note that we could not find any publication in the literature regarding the using microbial surfactants for broccoli treatment to extend their shelf life, although other treatments are available. Thus, in the case of ultraviolet radiation action (6 kJ/m^2) on freshly cut inflorescences, the total bacteria number on the vegetable surface was reduced 16-fold [21], while the treatment with *R. erythropolis* IMV Ac-5017 supernatant with 0.05 g/l surfactants concentration — 23 times, which is more effective than the action of UV rays and traditional disinfectant — sodium hypochlorite solution [21].

Brussels sprouts are another vegetable (except broccoli) with a fine tissue structure and a developed surface that makes them susceptible to microbial spoilage.

Subsequent experiments showed that the treatment of Brussels sprouts with A. calcoaceticus IMV B-7241 supernatant with a surfactant concentration of 0.25 and 0.5 g/l was accompanied by a 8-fold decrease in bacteria number, and fungi number — by a 6-9-fold decrease compared with untreated vegetables. It should be noted that, unlike R. erythropolis IMV Ac-5017 supernatant, a decrease of surfactant concentration in A. calcoaceticus IMV B-7241 supernatant to 0.01-0.05 g/l did not increase its efficiency. Vegetables treated with exometabolites of A. calcoaceticus IMV B-7241 did not show any visible signs of deterioration for 21 days, while the untreated ones showed the first signs of rot after 10-12 days of storage.

The next step explored the possibility of three consecutive use of the surfactantcontaining supernatant of A. calcoaceticus IMV B-7241 for the treatment of three different batches of Brussels sprouts to reduce the bacteria number on their surface. In these studies, the number of fungi was not determined, since Brussels sprouts are insensitive to post-harvest spoilage caused by fungi [https://www.ethylenecontrol.com]. Regardless of the surfactant concentration (0.25 and 0.5 g/l), after once and double use of the supernatant, the bacteria number on the surface of the vegetables was found to be practically the same (63–75 CFU/ml) and almost 8 times lower than after washing with water (Table 2). In the case of the supernatant being used three times, its effectiveness as an antimicrobial agent was slightly reduced; however, the bacteria treatment on the surface of the vegetables was almost 4 times lower than after washing with water (127–130 and 500 CFU/ml, respectively).

Further experiments showed the possibility of repeated use of the *A. calcoaceticus* IMV B-7241 supernatant not only for the treatment of Brussels sprouts, but also for sweet peppers (Table 2 and Fig. 1).

Thus, the number of bacteria on the surface of peppers after one and two times of supernatant use with a surfactant concentration of 0.5 g/l was 110-132 CFU/ml and was 5-6 times lower than the number of bacteria on the surface of washed with water vegetables (Table 2). Two-fold reduction of the surfactant concentration in the supernatant (up to 0.25 g/l) was accompanied by some decrease in its efficiency. However, regardless

Surfactants concentration in the supernatant, g/l	The multiplicity of supernatant use	The number of bacterial cells ($10^2 \mathrm{CFU}/\mathrm{ml}$) on the surface of		
		Brussels sprouts	Sweet peppers	
0.25	once	63±8*	$170 \pm 15 **$	
	twice	$75{\pm}9{*}$	$190{\pm}17{**}$	
	thrice	127±12*	$230{\pm}19{**}$	
0.5	once	65±7*	$110{\pm}11{**}$	
	twice	72±8*	$132{\pm}12{**}$	
	thrice	130±12*	200±17**	
Control (single wash with water)		$500{\pm}35$	$640{\pm}40$	

Table 2. The number of heterotrophic bacteria on Brussels sprouts and sweet pepper surface depending	
on the multiplicity of A. calcoaceticus IMB B-7241 supernatant use	

Notes: * — P < 0.05 — regarding control (number of bacterial cells on the surface washed with water brussels sprouts); ** — P < 0.05 — regarding control (number of bacterial cells on the surface washed with water pepper).

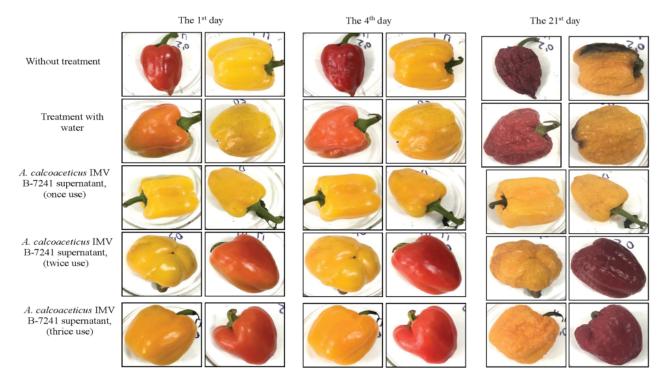


Fig. 1. The influence of different processing methods on the storage of sweet peppers

of the surfactant concentration in the supernatant, even after its threefold use, the bacteria number on the surface of the peppers was 3 times lower than after washing the vegetables with water (Table 2). It should be noted that the number of fungi on the surface of sweet pepper in all variants of treatment was 4-5 times lower than bacteria number (data in Table 2 are not shown). Visual observation of peppers during their storage after treatment with *A. calcoaceticus* IMV B-7241 supernatant showed that even on the 21^{st} day no signs of their spoilage were detected, unlike untreated and water-washed vegetables (Fig. 1).

In [14], we found that the maximum reduction of bacteria and fungi on the surface of tomatoes was achieved with N. vaccinii IMV B-7405 surfactants use at a concentration of

Table 3. The effect of the multiplicity of the use of N. vaccinii IMV B-7405 supernatant for the treatment of sweet peppers and tomatoes on the number of microorganisms on the surface of vegetables

The multiplicity of supernatant use	The number of bacterial cells $(10^2 {\rm CFU/ml})$ on the surface of		The number of fungi cells (10^2CFU/ml) on the surface of	
	pepper	tomatoes	pepper	tomatoes
once	$59{\pm}8{*}$	$13 \pm 3 * *$	$10{\pm}3{\#}$	$2{\pm}1{\#}{\#}$
twice	$83\pm9*$	18±4**	$12{\pm}3\#$	$2{\pm}1{\#}{\#}$
thrice	$190{\pm}17{*}$	32±6**	$45{\pm}7{\#}$	$3{\pm}1{\#}{\#}$
Control (single wash with water)	$520 {\pm} 40$	$160{\pm}15$	80±9	17±4

Notes: * — P < 0.05 — regarding control (number of bacterial cells on the surface washed with water pepper); ** — P < 0.05 — regarding control (number of bacterial cells on the surface of water-washed tomatoes); # — P < 0.05 — regarding control (the number of fungal cells on the surface washed with water pepper); ## — P < 0.05 — regarding control (number of fungal cells on the surface of water-washed tomatoes); the surfactant concentration in the supernatant is 0.5 g/l.

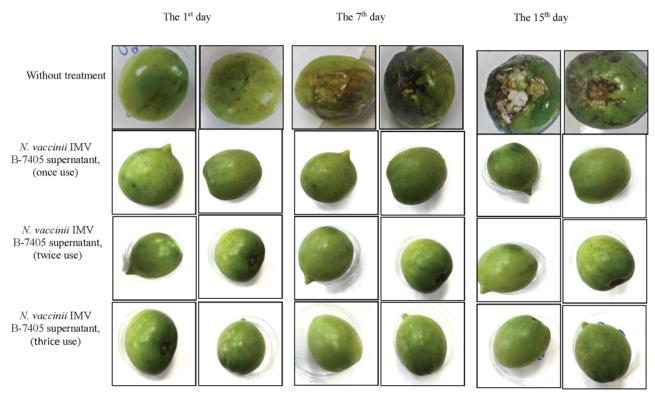


Fig. 2. The influence of different treatment methods on the storage of tomatoes

0.5 g/l. In these studies, surfactant solutions extracted from the culture broth supernatant with Folch mixture (chloroform and methanol, 2: 1) were used as surfactant preparations. In the next stage, the possibility of thrice use for the processing of sweet pepper and tomatoes of the *N. vaccinii* IMV B-7405 supernatant with a surfactant concentration of 0.5 g/l was investigated (Table 3, Fig. 2). The results obtained showed the high efficiency of such

surfactant preparation for the treatment of these vegetables. Thus, after using the supernatant twice, the number of bacteria on the surface of pepper and tomatoes was 6.3 and 8.9, respectively, and the fungi — 6.7 and 8.5times lower than after washing vegetables with water. In the case of the supernatant was used three times, the number of microorganisms on the surface of the vegetables was slightly higher than after one and two times of application, but lower than after washing with water (Table 3).

Visual observation of the tomatoes during their storage after treatment with *N. vaccinii* IMV B-7405 supernatant showed that even on the 15^{th} day no signs of their rotting were detected, unlike untreated vegetables (Fig. 2).

Analysis of the literature showed that publications on the use of surfactants of microbial origin for post-harvest treatment of fruits and vegetables can be divided into three groups.

Articles of the first group [23-27] are devoted to the application for the postharvest biocontrol of phytopathogens of biological products number based on the microorganisms- antagonists of certain pathogens that affect certain fruits and vegetables. The influence of microbial surfactants in this case is indirect and is considered as one of the possible mechanisms of antagonism, in particular, for preparations based on the biomass of bacteria of Bacillus and Pseudomonas genus [23-27]. Thus, in [23] it was found that the effect of preinfected with pathogen *Penicilium digitatum* mandarin treatment with a suspension of Bacillus subtilis ABS-S14 endospores, a solution of lipopeptides isolated from the supernatant (10 g/l) and solutions of purified individual surfactants (fengicin and iturin in a concentration of 1 g/l) was almost the same (inhibition of infection development by 60-70% after 5-7 days). The authors of [24] have shown that in the case of spraying of phytopathogenic fungi-affected oranges, apples, grapes and drupaceous fruits with Bacillus amylolique faciens BUZ-14 supernatant, a faster inhibition of infection compared to the treatment with vegetative or spore cells suspension was observed. The fact is interesting that the antifungal activity of the supernatant against various phytopathogenic fungi depended on the duration of strain BUZ-14 cultivation. The authors explain this phenomenon by the fact that during the cultivation of B. amyloliquefaciens BUZ-14 there is a qualitative and quantitative change in the ratio of components in the synthesized antimicrobial complex.

In the publications of the second group [7-8, 28], the compositions containing purified microbial surfactants (mainly rhamno- and sophorolipids) and other components were used as preparations for post-harvest treatment of fruits and

vegetables. Thus, treatment of cherry tomatoes with rhamnolipids solution (0.5 g/l)and a suspension of yeast Rhodotorula glutinis ($1 \cdot 10^8$ cells/ml) allowed the infection rate of these vegetables reducing with the agent Alternaria alternata by 60% [8]. The authors argue that a solution containing only rhamnolipids proved to be ineffective. In [7] it was reported that in the treatment of tomatoes and cucumbers with germicidal (bactericidal) composition (2.5% of Candidabombicola ATCC 22214 sophorolipid in combination with sodium silicate, sodium carbonate and polyethylene glycol) there were not visible signs of microbial spoilage the vegetables for 7 days. It should be noted that the first germicidal composition based on microbial sophorolipids was patented in 1998 [28]. In addition to surfactants (instead of sophorolipids, it may be sodium lauryl sulfate, or a mixture of microbial and chemical surfactants) it contained a mixture of organic acids (citric, glycolic, lactic, malic, tartaric). This composition provided 100% inhibition of *Escherichia coli* bacteria, as well as representatives of the genera Salmonella and Shigella on the surface of fruits and vegetables [28].

The third group of publications [9, 29-30], to which our research also relates [14], concerns the use of only surfactant solutions for the processing of fruit and vegetable products, without any other ancillary components. Thus, in the patent [29] it is proposed, to prolong the shelf life of citrus fruits, apples, pears, apricots, to spray them with a solution of purified sophorolipids with a concentration of 3 g/l (producer is Wickerhamiella domercqiae Y2A). The authors of [9] found that among three studied microbial surfactants (producers are B. subtilis 10T, B. subtilis 3285 and Pseudomonas sp.), only Pseudomonas sp. rhamnolipid proved to be effective against Aspergillus oryzae MTCC 1846, Fusarium solani MTCC 350 and Curvularia sp., which cause damage to lemons, potatoes and tomatoes, respectively. In the case of treatment of pre-infected with these pathogens fruits and vegetables with solutions of rhamnolipid at a concentration of 1 g/l, no signs of microbial spoilage during 15 days of storage at room temperature were observed, while the first signs of decay of untreated fruits were appeared as early as in 6–7 days [9]. In [30] found that previously infected with Botrytis cinerea grape, tomato and strawberry fruits treatment with a

solution of lipopeptide (8 g/l) synthesized by *Bacillus methylotrophicus* XT1 CECT 8661, was accompanied by an inhibition of infection by 70-100% in 6 days.

The data and results obtained by us earlier show that both surfactant solutions [14] and surfactant-containing *N. vaccinii* IMV B-7405, *R. erythropolis* IMV Ac-5017 and *A. calcoaceticus* IMV B-7241 supernatants used for treatment vegetables to extend their shelf life have the following advantages over microbial surfactants described in the literature [9, 29, 30]: exhibit high antimicrobial activity at much lower surfactant concentrations (0.01-0.5 g/l) and in the form of a supernatant, which lets us

REFERENCES

- 1. *Harjot P. K., Bhairav P., Sukhvir K.* A review on application of biosurfactants produced from unconventional inexpensive wastes in food and agriculture industry. *World J. Pharm. Res.* 2015, 4(8), 827–842.
- 2. Mnif I., Ghribi D. Glycolipid biosurfactants: main properties and potential applications in agriculture and food industry. J. Sci. Food Agric. 2016, 96(13), 4310–4320. https/doi. org/10.1002/jsfa.7759
- Vecino X., Cruz J. M., Moldes A. B., Rodrigues L. R. Biosurfactants in cosmetic formulations: trends and challenges. Crit. Rev. Biotechnol. 2017, 37(7), 911–923. https/doi.org/10.1080 /07388551.2016.1269053
- 4. De Graeve M., De Maeseneire S.L., Roelants S. L. K. W., Soetaert W. Starmerella bombicola, an industrially relevant, yet fundamentally underexplored yeast. FEMS Yeast Res. 2018, 18(7). https/doi.org/10.1093/femsyr/foy072
- 5. Jimoh A. A., Lin J. Biosurfactant: A new frontier for greener technology and environmental sustainability. *Ecotoxicol. Environ. Saf.* 2019, 184:109607. https/doi. org/10.1016/j.ecoenv.2019.109607.
- Naughton P. J., Marchant R., Naughton V., Banat I. M. Microbial biosurfactants: current trends and applications in agricultural and biomedical industries. J. Appl. Microbiol. 2019, 127(1), 12–28. https/doi.org/10.1111/ jam.14243.
- Dengle-Pulate R., Joshi J., Bhagwat S., Prabhune A. Application of sophorolipids synthesized using lauryl alcohol as a germicide and fruit-vegetable wash. World J. Pharm. Res. 2015, 3(7), 1630–1643.
- 8. Yan F., Xu S., Chen Y., Zheng X. Effect of rhamnolipids on *Rhodotorula glutinis* biocontrol of *Alternaria alternata* infection in

to exclude the expensive stage of extraction and purification of the final product from the technological process. In addition, surfactantcontaining supernatants are highly effective when reused. It should be noted that at present there is no such information in the literature.

The research was carried out within the framework of the project of the Ministry of Education and Science of Ukraine "Complex microbial preparations of multifunctional purpose: from regulation of biosynthesis and properties to perspectives of practical use" (2019–2021), State registration number GDR: 0119U002575.

cherry tomato fruit. *Postharvest Biol. Technol.* 2014, 97, 32–35. https://doi.org/10.1016/j. postharvbio.2014.05.017.

- 9. Sharma V., Garg M., Devismita T., Thakur P., Henkel M., Kumar G. Preservation of microbial spoilage of food by biosurfactantbased coating. Asian J. Pharm. Clin. Res. 2018, 11(2), 98-101.
- 10. Romanazzi G., Feliziani E., Baños S. B., Sivakumar D. Shelf life extension of fresh fruit and vegetables by chitosan treatment. Crit. Rev. Food Sci. Nutr. 2017, 57(3). 579–601.
- 11. Romanazzi G., Feliziani E., Sivakumar D. Chitosan, a biopolymer with thrice action on postharvest decay of fruit and vegetables: eliciting, antimicrobial and film-forming properties. Front. Microbiol. 2018, 9:2745. https/doi.org/10.3389/fmicb.2018.02745.
- 12. Barbosa A. A. T., Mantovani H. C., Jain S. Bacteriocins from lactic acid bacteria and their potential in the preservation of fruit products. Crit. Rev. Biotechnol. 2017, 37(7), 852-864. https/doi.org/10.1080/07388551 .2016.1262323.
- Sinumvayo J. P., Ishimwe N. Agriculture and food applications of rhamnolipids and its production by Pseudomonas aeruginosa. J. Chem. Eng. Process. Technol. 2015, 6(2), 223. https/doi.org/10.4172/2157-7048.1000223.
- 14. Pirog T., Beregova K., Geichenko B., Stabnikov V. Application of surface-active substances produced by Nocardia vaccinii IMV B-7405 for the treatment of vegetables. Ukrainian food journal. 2019, 8(1), 99–109. https/doi. org/10.24263/2304-974X-2019-8-1-11.
- 15. Pirog T. P., Konon A. D., Sofilkanich A. P., Iutinskaia G. A. Effect of surface-active substances of Acinetobacter calcoaceticus IMV B-7241, Rhodococcus erythropolis IMV

Ac-5017, and Nocardia vaccinii K-8 on phytopathogenic bacteria. Appl. Biochem. Microbiol. 2013, 49(4), 360-367. https/doi. org/10.1134/S000368381304011X.

- Pirog T. P., Lutsay D. A., Kliuchka L. V., Beregova K. A. Antimicrobial activity of surfactants of microbial origin. Biotechnologia Acta. 2019, 12(1), 39-57. https://doi.org/10.15407/biotech12.01.039.
- 17. Pirog T. P., Shevchuk T. A., Voloshina I. N., Grechirchak N. N. Use of clayditeimmobilized oiloxidizing microbial cells for purification of water from oil. Appl. Biochem. Microbiol. 2005, 41 (1), 51–55. https://doi. org/10.1007/s10438-005-0010-z.
- Pirog T., Sofilkanych A., Konon A., Shevchuk T., Ivanov S. Intensification of surfactants' synthesis by Rhodococcus erythropolis IMV Ac-5017, Acinetobacter calcoaceticus IMV B-7241 and Nocardia vaccinii K-8 on fried oil and glycerol containing medium. Food. Bioprod. Proces. 2013, 91 (2), 149–157. https://doi.org/10.1016/j.fbp.2013.01.001.
- Dilarri G., da Silva V.L., Pecora H. B., Montagnolli R. N., Corso C. R., Biddia E. D. Electrolytic treatment and biosurfactants applied to the conservation of Eugenia unflora fruit. Food Sci. Technol. 2016, 36 (3). http://dx.doi.org/10.1590/1678-457X.00516.
- 20. Shi J., Gao L., Zuo J., Wang Q., Wang Q., Fan L. Exogenous sodium nitroprusside treatment of broccoli florets extends shelf life, enhances antioxidant enzyme activity, and inhibits chlorophyll-degradation. Postharv. Biol. Technol. 2016, 116, 98–104. https://doi. org/10.1016/j.postharvbio.2016.01.007.
- 21. Martínez-Hernández B.G., Artés-Hernández F., Gómez P.A., Formica A.C., Arles F. Combination of electrolysed water, UV-C and superatmospheric O₂ packaging for improving fresh-cut broccoli quality. Postharv. Biol. Technol. 2013, 76, 125-134. https://doi.org/10.1016/j. ifset.2013.11.004.
- 22. Ben-Fadhel Y., Ziane N., Salmieri S., Lacroix M. Combined post-harvest treatments for improving quality and extending shelf-life of minimally processed broccoli florets (Brassica oleracea var. italica). Food Bioprocess.

Technol. 2017, 11(1), 84–95. https://doi. org/10.1007/s11947-017-1992-2.

- 23. Waewthongrak W., Pisuchpen S., Leelasuphakul W. Effect of Bacillus subtilis and chitosan applications on green mold (Penicilium digitatum Sacc.) decay in citrus fruit. Postharv. Biol. Technol. 2015, 99, 44-49. https/doi.org/10.1016/j. postharvbio.2014.07.016.
- 24. Calvo H., Marco P., Blanco D., Oria R., Venturini M. E. Potential of a new strain of Bacillus amyloliquefaciens BUZ-14 as a biocontrol agent of postharvest fruit diseases. Food Microbiol. 2017, 63, 101-110. https/doi.org/10.1016/j.fm.2016.11.004.
- 25. Zhang B., Li Y., Zhang Y., Qiao H., He J., Yuan Q., Chen X., Fan J. High-cell-density culture enhances the antimicrobial and freshness effects of Bacillus subtilis S1702 on table grapes (Vitis vinifera cv. Kyoho). Food Chem. 2019, 286, 541-549. https/doi. org/10.1016/j.foodchem.2019.02.050.
- 26. Dukare A. S., Paul S., Nambi V. E., Gupta R. K., Singh R., Sharma K., Vishwakarma R. K. Exploitation of microbial antagonists for the control of postharvest diseases of fruits: a review. Crit. Rev. Food Sci. Nutr. 2019, 59(9), 1498–1513. https/doi.org/10.1080/1040839 8.2017.1417235.
- 27. Lastochkina O., Seifikalhor M., Aliniaeifard S., Baymiev A., Pusenkova L., Garipova S., Kulabuhova D., Maksimov I. Bacillus spp.: efficient biotic strategy to control postharvest diseases of fruits and vegetables. Plants (Basel). 2019, 8(4). pii: E97. https/doi.org/10.3390/plants8040097.
- Pierce D., Heilman T. J. Germicidal composition. World Patent 9816192. Publ. 23.04.1998.
- 29. Jing C., Bingbing Y. Sophorolipid fruit preservative and use thereof in fruit preservation. Chinese Patent CN 101886047. Publ. 17.11.2010.
- 30. Toral L., Rodríguez M., Béjar V., Sampedro I. Antifungal activity of lipopeptides from Bacillus XT1 CECT 8661 against Botrytis cinerea. Front. Microbiol. 2018, 9, 1315. https/doi.org/10.3389/fmicb.2018.01315.

ПІСЛЯВРОЖАЙНА ОБРОБКА ОВОЧІВ ЕКЗОМЕТАБОЛІТАМИ Nocardia vaccinii IMB B-7405, Acinetobacter calcoaceticus IMB B-7241 TA Rhodococcus erythropolis IMB AC-5017 ДЛЯ ПОДОВЖЕННЯ ТЕРМІНУ ЇХ ЗБЕРІГАННЯ

Т. П. Пирог, Б. С. Гейченко, А. О. Зварич

Національний університет харчових технологій, Київ, Україна

E-mail: tapirog@nuft.edu.ua

Мета роботи — дослідити можливість використання супернатантів *Nocardia vaccinii* IMB B-7405, *Acinetobacter calcoaceticus* IMB B-7241 та *Rhodococcus erythropolis* IMB Ac-5017 з різною концентрацією поверхнево-активних речовин (ПАР) для післяврожайної обробки овочів.

N. vaccinii IMB B-7405, A. calcoaceticus IMB B-7241 та R. erythropolis IMB Ac-5017 вирощували на відпрацьованій соняшниковій олії та етанолі. Для обробки овочів використовували супернатанти культуральної рідини з концентрацією ПАР 0,01–0,5 г/л. Концентрацію ПАР визначали ваговим методом після екстракції сумішшю Фолча. Загальну чисельність гетеротрофних бактерій і грибів на поверхні овочів визначали за методом Коха на м'ясо-пептонному агарі та сусло-агарі відповідно.

Показано, що обробка броколі, брюсельської капусти, солодкого перцю і томатів супернатантами N. vaccinii IMB B-7405, A. calcoaceticus IMB B-7241 та R. erythropolis IMB Ac-5017 супроводжувалася зниженням чисельності бактерій і грибів на їхній поверхні у 6–17 і 8–50 разів відповідно порівняно з кількістю на поверхні митих водою овочів. Встановлено можливість двократного використання одного й того самого супернатанту для миття різних партій овочів. Необроблені та миті водою овочі швидше піддавалися гниттю порівняно з обробленими ПАР-вмісними супернатантами.

Екзометаболіти N. vaccinii IMB B-7405, R. erythropolis IMB Ac-5017 і A. calcoaceticus IMB B-7241, використовувані для обробки овочів з метою подовження терміну їх зберігання, порівняно з відомими мікробними поверхнево-активними речовинами мають такі переваги: виявляють високу антимікробну активність за нижчих у кілька разів концентрацій ПАР і у вигляді супернатанту, що дає змогу виключити з технологічного процесу високовартісну стадію виділення та очищення цільового продукту. Крім того, ПАР-вмісні супернатанти характеризуються високою ефективністю у разі їх повторного використання.

Ключові слова: зберігання овочів, мікробне псування, поверхнево-активні речовини.

ПОСЛЕУБОРОЧНАЯ ОБРАБОТКА ОВОЩЕЙ ЭКЗОМЕТАБОЛИТАМИ Nocardia vaccinii ИМВ В-7405, Acinetobacter calcoaceticus ИМВ В-7241 И Rhodococcus erythropolis ИМВ АС-5017 ДЛЯ ПРОДЛЕНИЯ СРОКА ИХ ХРАНЕНИЯ

Т. П. Пирог, Б. С. Гейченко, А. О. Зварыч

Национальный университет пищевых технологий, Киев,Украина

E-mail: tapirog@nuft.edu.ua

Цель работы — исследовать возможность использования супернатантов Nocardia vaccinii ИМВ В-7405, Acinetobacter calcoaceticus ИМВ В-7241 и Rhodococcus erythropolis ИМВ Ас-5017 с различной концентрацией поверхностно-активных веществ (ПАВ) для послеуборочной обработки овощей.

N. vaccinii ИМВ В-7405, A. calcoaceticus ИМВ В-7241 и R. erythropolis ИМВ Ас-5017 выращивали на отработанном подсолнечном масле и этаноле. Для обработки овощей использовали супернатанты культуральной жидкости с концентрацией ПАВ 0,01–0,5 г/л. Концентрацию ПАВ определяли весовым методом после экстракции смесью Фолча. Общую численность гетеротрофных бактерий и грибов на поверхности овощей определяли по методу Коха на мясо-пептонном агаре и сусло-агаре соответственно.

Показано, что обработка брокколи, брюссельской капусты, сладкого перца и томатов супернатантами N. vaccinii ИМВ В-7405. A. calcoaceticus ИМВ B-7241 и R. erythropolis ИМВ Ас-5017 сопровождалась снижением численности бактерий и грибов на их поверхности в 6-17 и 8-50 раз соответственно по сравнению с количеством на поверхности мытых водой овощей. Установлена возможность двукратного использования одного и того же супернатанта для обработки различных партий овощей. Необработанные и мытые водой овощи быстрее подвергались гниению по сравнению с обработанными ПАВ-содержащими супернатантами. Экзометаболиты N. vaccinii ИМВ B-7405, R. erythropolis ИМВ Ac-5017 и A. calcoaceticus ИМВ B-7241, используемые для обработки овощей с целью продления срока их хранения, по сравнению с известными микробными поверхностно-активными веществами имеют следующие преимущества: проявляют высокую антимикробную активность в более низких концентрациях и в виде супернатанта, что позволяет исключить из технологического процесса дорогостоящую стадию выделения и очистки целевого продукта. Кроме того, ПАВ-содержащие супернатанты характеризуются высокой эффективностью при их повторном использовании.

Ключевые слова: хранение овощей, микробная порча, поверхностно-активные вещества.