

Cell Biology

Membrane Phospholipid Ratio and Cell Stability

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ABSTRACT. The results of quantitative description of *II⁵⁽¹⁾* y *Nocardioopsis dassonvillei* cells phospholipids exposed to different doses of UV irradiation are presented. Spectral methods are applied to measure the amount of total, amino and choline comprising phospholipids. Additionally, stability of *II⁵⁽¹⁾* y *Nocardioopsis dassonvillei* cells irradiated with the same doses was examined. Different pattern of quantitative changes of amino and choline comprising phospholipids was detected. Functional relationship between phospholipid composition and cell stability is discussed. © 2009 Bull. Georg. Natl. Acad. Sci.

Key words: phospholipids, UV irradiation, microorganisms.

UV radiation because its absorption by important biomolecules causes deleterious effect to biological systems. Several studies conducted under laboratory and/or natural conditions have revealed the harmful effect of UV radiation on growth, survival, nutrient uptake and various metabolic processes of different microorganisms [1]. These effects are in part due to the direct effect on cell membranes, enzymes as well as indirect effect through the formation of reactive oxygen species [2]. Among several targets of UV damage that have been investigated, cell membranes are recognized as the most predominant action sites. Consequently, considerable changes of membrane permeability leading to the disturbance of metabolic and functional activity take place. In view of our previous publications it is evident that UV radiation leads to a considerable change of functional characteristics determining the destructive activity of *II⁵⁽¹⁾* y *Nocardioopsis dassonvillei* [3]. The nature of the revealed changes and complete correlative correspondence prompt us to ascertain the possible interdependence among membranes' structural condition and *II⁵⁽¹⁾* y *Nocardioopsis dassonvillei* cells stability—one of the determinants of microbial destructive activity. For that reason we have investigated the phospholipid com-

position and viability of *II⁵⁽¹⁾* y *Nocardioopsis dassonvillei* cells exposed to different doses of UV irradiation. Increasing doses of UV irradiation was employed to study the possible role of radiation on the amount of total, amino/choline comprising phospholipids and stability of *II⁵⁽¹⁾* y *Nocardioopsis dassonvillei* cells as well.

Materials and methods. Experiments were performed on the well established biodestructor *II⁵⁽¹⁾* y *Nocardioopsis dassonvillei* cells. *II⁵⁽¹⁾* y *Nocardioopsis dassonvillei* cells culture was inoculated (1% [vol/vol]) into 100 mL of mineral liquid medium containing KNO₃-1g; K₂HPO₄-0,5g; MgSO₄-0,5g; NaCl-0,5g; FeSO₄-trace amount; CaCO₃-1g; glucose-20g; agar-agar-20 g; water-1l, incubated for 9 days at 28 °C. *II⁵⁽¹⁾* y *Nocardioopsis dassonvillei* cells cultures were irradiated by rising doses (200j/m²; 400j/m²; 600j/m²; 800j/m²; 1000j/m²), settling for 30 min. The radiation source was a BUV-ZOP lamp. Lipids extracted from cells homogenate were dissolved in CCl₄, final concentration 100g/ml. Quantity of phospholipids was determined using spectrophotometric methods elaborated by the staff of a former Faculty of Biology (see acting patents) [4-6]. Total, amino and choline comprising phospholipids were assessed spec-

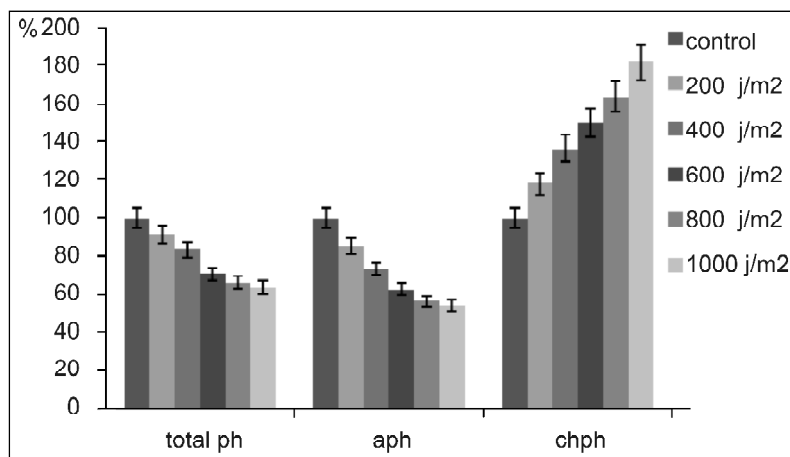


Fig. 1. Quantitative changes of phospholipids (total ph-total phospholipids, Aph-amino comprising phospholipids and Chph-choline comprising phospholipids) in 11⁵⁽¹⁾ y *Nocardioopsis dassonvillei* cells subjected to different doses of UV irradiation. The values are the means of triplicate ± standard deviations.

trophotometrically in overall of lipid fraction. The amount of total, amino and choline comprising phospholipids was determined according to phosphatidylethanolamine and phosphatidylcholine calibration plot.

The amount of viable cells was calculated by the number of colonies grown on solid medium. All the experiments were repeated at least twice and the samples were assayed in triplicate. The data points represent the mean

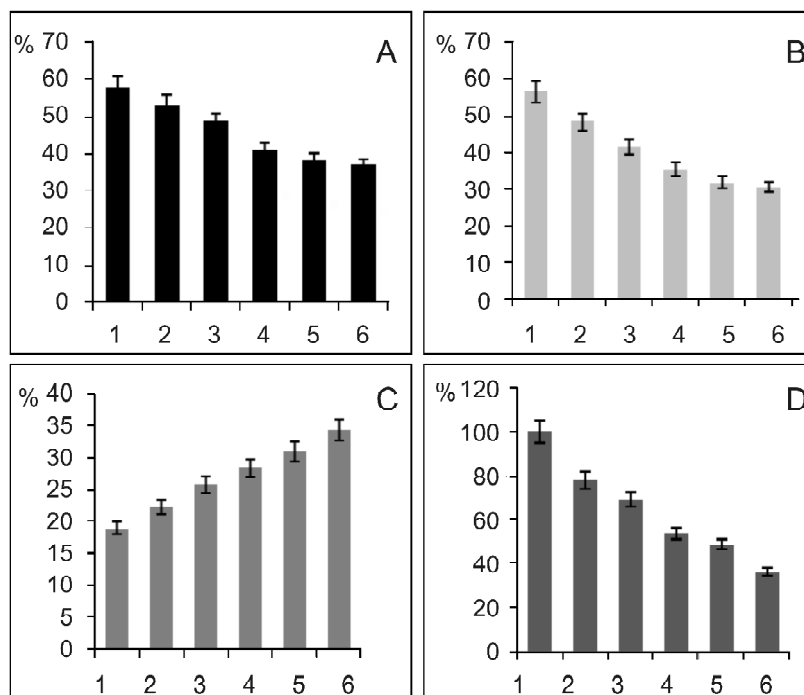


Fig. 2. Quantitative change of total, amino/choline comprising phospholipids and cell viability in 11⁵⁽¹⁾ y *Nocardioopsis dassonvillei* cells subjected to different doses of UV irradiation. The values are the means of triplicate ± standard deviations. Keys: 1- control; 2-200j/m²; 3-400j/m²; 4-600j/m²; 5-800j/m²; 6-1000j/m².

- A- Quantitative change of total phospholipid in 11⁵⁽¹⁾ y *Nocardioopsis dassonvillei* cells subjected to different doses of UV irradiation. Values are given in % against overall lipid fraction. Quantitative determination of total phospholipids was carried out using complex solution containing ammonium molybdate, mercury, ethanol, HCL and H₂SO₄. Quantity of total phospholipids was assessed by absorbency at 670 nm.
- B - Quantitative change of amino comprising phospholipid in 11⁵⁽¹⁾ y *Nocardioopsis dassonvillei* cells subjected to different doses of UV irradiation. Values are given in % against total phospholipids. Quantitative determination of amino comprising phospholipids was carried out spectrometrically at 403 nm using ninhydrin and 1M CaCl₂.
- C- Quantitative change of cholin ecomprising phospholipid in 11⁵⁽¹⁾ y *Nocardioopsis dassonvillei* cells subjected to different doses of UV irradiation. Values are given in % against total phospholipids. Quantitative determination of cholin ecomprising phospholipids was carried out spectrometrically at 500nm using Dragendorph reagent.
- D-Viability of 11⁵⁽¹⁾ y *Nocardioopsis dassonvillei* cells subjected to different doses of UV irradiation. The quantity of viable cells was calculated according to the number of colonies grown on solid medium.

values within ± 4 to 5% of the individual values. The data were treated by one-way ANOVA analysis.

Results and Discussion. Obtained experimental results are summarized in Figs. 1 and 2 (A;B;C;D). Data given on figures correspond to the alteration of percentage composition of $11^{5(1)}$ y *Nocardioopsis dassonvillei* cells phospholipids treated with UV irradiation. Fig.1 demonstrates quantitative changes of total, amino and choline comprising phospholipids compared to control samples. Amount of tested phospholipids in control samples is considered as 100%. Fig. 2 shows changes of phospholipid content: total phospholipids against mg/ml of overall lipid fraction; amino and choline comprising phospholipids against total phospholipid amount.

Illustrated data clearly demonstrate that treatment of $11^{5(1)}$ y *Nocardioopsis dassonvillei* cells with different doses of irradiation reveal dose dependant variations of total, amino and choline comprising phospholipid amount. In particular, the quantity of choline comprising phospholipids is increased, while the quantity of amino comprising as well as total phospholipids decreases related to elevated doses of irradiation. Referring to our experimental data viable cells amount under the same experimental conditions show dose-

dependent character i.e. appliance of rising doses of irradiation decreases viable cell quantity. Lethal dose LD_{50} at $800 J/m^2$ was established as well. According to our experimental data, recognized quantitative changes of phospholipids and viable cells reveal complete correlative interdependence. Specifically, complete negative correlation in the case of choline comprising phospholipids was established - ($r = - 0.99 \pm 0.09$). At the same time complete positive correlation was observed between viable cell amount changes and amino comprising phospholipid content ($r = 0.99 \pm 0.009$). It must be admitted that during the appliance of LD_{50} composition of amino/choline comprising phospholipids in overall fraction of phospholipids is equalized. Concurrently, no correlation was established between quantitative changes of total phospholipids/cells stability, testifying to the absence of functional relationship between cell viability and membrane phospholipid amount. Thus, we suppose that membrane's total phospholipid content should not predetermine cell stability against a variety of injuring factors. We assume that from this point of view the ratio of amino and choline comprising phospholipids in total fraction of phospholipids seems to be more favorable.

უჯრედული ბიოლოგია

მემბრანის ფოსფოლიპიდების თანაფარდობა და უჯრედების სტაბილურობა

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შესწავლილია ულტრაიისფერი გამოსხივების გავლენა $11^{5(1)}$ y *Nocardioopsis dassonvillei* უჯრედების ფოსფოლიპიდურ შედგენილობასა და უჯრედების სტაბილურობაზე. გამოვლენილია ამინო/ქოლინშემცველი ფოსფოლიპიდების თანაფარდობის როლი უჯრედების სტაბილურობაში.

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Received April, 2009