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ВІКОВІ ОСОБЛИВОСТІ ТРАНСПОРТУ ВОДИ ТА ЕЛЕКТРОЛІТІВ ЧЕРЕЗ ЕПІТЕЛІЙ ТОВСТОЇ КИШКИ ЩУРІВ ТА ЇХ КОРЕКЦІЯ МУЛЬТИПРОБІОТИКОМ СИМБІТЕР АЦИДОФІЛЬНИЙ КОНЦЕНТРОВАНИЙ

Досліджено транспорт води і електролітів через епітелій товстої кишки у щурів різного віку. Встановлено, що у віці 21-го та 24-х місяців всмоктування води та іонів Na^+ і Cl^- значуще збільшується, що є однією з причин виникнення закрелів. Періодичне додавання до стандартного корму мультипробіотика "Симбітер® ацидофільний" концентрованою (0,14 мл/кг) запобігає віковим змінам у транспорті води і електролітів через епітелій товстої кишки.

Ключові слова: товстий кишечник, сумарний потік води та іонів Na^+ і Cl^- , мультипробіотик.

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ВОЗРАСТНЫЕ ИЗМЕНЕНИЯ ТРАНСПОРТА ВОДЫ И ЭЛЕКТРОЛИТОВ ЧЕРЕЗ ЭПИТЕЛИЙ ТОЛСТОГО КИШЕЧНИКА КРЫС И ИХ КОРЕКЦИЯ МУЛЬТИПРОБІОТИКОМ СИМБІТЕР АЦИДОФІЛЬНИЙ КОНЦЕНТРИРОВАННИЙ

Исследованы транспорт воды и электролитов через эпителий толстой кишки у крыс разного возраста. Установлено, что в возрасте 21-го и 24-х месяцев всасывания воды и ионов Na^+ и Cl^- значимо увеличивается, что является одной из причин возникновения заворотов у крыс пожилого и старческого возраста. Периодическое добавление к стандартному корму мультипробіотика "Симбітер® ацидофільний" концентрованою (0,14 мл/кг) предотвращает возрастные изменения в транспорте воды и электролитов через эпителий толстой кишки.

Ключевые слова: толстый кишечник, суммарный поток воды и ионов Na^+ и Cl^- , мультипробіотик.

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DETECTION OF SOME VIRUS PATHOGENS OF CACTUS IN UKRAINIAN BOTANICAL GARDENS

Cactus collections of some Ukrainian botanical gardens were analyzed for virus contamination. Different virus-like symptoms including mosaics, chlorosis and local necroses have been detected on cactus plants in these collections. Biological properties of isolated viruses were defined by the methods of bioassay, transmission electron microscopy and indirect ELISA.

Key words: ELISA, isolated viruses, cactus.

Introduction: The *Cactaceae* are mostly spiny succulents with photosynthetic stems comprising 200 genera and more than 2,000 species [1]. Cactuses have been the object of amateur and professional botanical collectors because of unusual structures and exceptionally colorful and beautiful blossoms. Huge variety of cactus species coupled with their distinctive ecological and biological characteristics cause a number of difficulties associated primarily with cultivation of these plants in the greenhouses. Due to the ordinary vegetative propagation and long-term cultivation of cactus plants in the same collections, these plants may serve as reservoirs of different viruses.

In accordance with literary data about 11 viruses are able to affect the members of *Cactaceae* family: *Cactus virus X* (CVX), *Schlumbergera virus X* (SVX), *Opuntia virus X* (OVX), *Zygocactus virus X* (ZVX), *Saguaro cactus virus* (SCV), *Sammons' Opuntia virus* (SOV), *Cactus virus 2* (CV2), *Cactus mild mottle virus* (CMMoV), *Rattail cactus necrosis-associated virus* (RCNAV), *Impatiens necrotic spot virus* (INSV) and *Tomato spot wilt virus* (TSWV) [3, 4, 5, 6, 7, 8].

That's why the aim of our work was to analyze collection of cactus in Ukrainian botanical gardens for virus contamination.

Materials and methods: The material for investigation was collected in different Ukrainian botanical gardens: Donetsk botanical garden of the National Academy of Sci-

ences of Ukraine, Botanical garden of Ivan Franko National university of Lviv, Botanical garden of Odessa I.I. Mechnikov National university, Karazin' Botanic Garden of Kharkiv National University, Nikitsky Botanical Garden – National Scientific Centre. For detection and identification of viruses 67 samples of different cactus cultivars were selected. For the biological characteristics of the pathogen we used the method of bioassay. Infectivity of plant sap was confirmed proved using indicators plants such as *Chenopodium murale*, *Celosia cristata*, *Datura stramonium*, *Gomphrena globosa*, *Chenopodium murale*, *Nicotiana tabacum*, *Nicotiana rustica*. These plants are typical test-plants for majority cactus viruses.

Virus identification was carried out using indirect ELISA [2]. Samples were tested by ELISA with serums to *Tobacco mosaic virus* (TMV) (antiserum obtained at the virology department, the sensitivity and specificity confirmed experimentally), *Potato virus S* (PVS), *Potato virus M* (PVM), *Potato virus X* (PVX) (Institute of Agricultural Microbiology, Ukrainian Academy of Agrarian Sciences, Chernigiv), *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) (Loewe, Germany).

The morphology of virions was studied in leaf dip preparations negatively stained with 2% uranyl acetate.

electron microscopy was carried out using a JEOL-1400 electron microscope at the magnification 30 000 [7].

Results and discussion: Cactus plants demonstrated different symptoms of virus infection. On plants *Mammillaria zeilmanniana*, *Opuntia* sp. (from collection of the Donetsk botanical garden of the National Academy of Sciences of Ukraine), *Ferocactus echidne*, *Gymnocalycium* sp., *Opuntia* sp. (from collection of Botanical garden of Ivan Franko National university of Lviv), *Mammillaria nivosa*, *Opuntia* sp. (from collection of Botanical garden of Odessa I.I. Mechnikov National university), *Mammillaria microhelia*, *Ferocactus* sp., *Thelocactus chrenbergii*, *Trichocereus*

bridgesii, *Mammillaria magnimamma*, *Opuntia* sp., *Cereus* sp., *Astrophytum capricorne*, *Cereus* sp., *Astrophytum myriostigma* (from greenhouse collection of Nikitsky Botanical Garden) and *Ritterocereus pruinosus* (from the collection of Karazin' Botanic Garden of Kharkiv National University) we observed mosaic symptoms. On plants *Ausrocylindropuntia tunicata*, *Monvillea* sp., *Sulcorebutia* sp., *Bolivicereus samupatanus*, *Opuntia* sp., *Astrophytum myriostigma* v. *nudum* from greenhouse collection of Nikitsky Botanical Garden – National Scientific Centre the symptoms of necrosis had been detected (Fig.1. A., B.).



Fig.1. Symptoms of cactus plants:

- A. Mosaic on stem of *Opuntia* sp. from the collection of Botanical garden of Odessa I.I. Mechnikov National University
 B. Chlorosis and necrotic spots on stem of *Opuntia microdasys* from the collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv

To investigate the biological characteristics of viruses and their diversity samples of cactus with visual symptoms of disease were selected. All samples were tested for indicator plants typical for cactus virus (*Celosia argentea*, *Celosia cristata*, *Gomphrena globosa*, *Chenopodium amaranticolor*, *Chenopodium murale*, *Nicotiana tabacum*, *Nicotiana rustica*). Local necrosis on the leaves of plants *Chenopodium murale* and *Gomphrena globosa* are typical evidence of a cactus X virus [3, 5, 6]. In *Nicotiana tabacum* and *Nicotiana rustica* the symptoms observed included small localized chlorotic lesions and in some plants systemic damage.

According to ELISA datas antigens of CVX were detected in plants material of *Mammillaria zeilmanniana*, *Opuntia* sp., *Ferocactus echidne*, *Gymnocalycium* sp., *Opuntia* sp., *Mammillaria nivosa*, *Opuntia* sp., *Consolea rubescens*, *Caralluma* sp., *Echinocereus* sp., *Opuntia brasiliensis*, *Opuntia microdasys* v. *rufida*, *Opuntia* sp. and *Pereskia aculeata* v. *godseffiana*, *Monvillea* sp., *Sulcorebutia* sp., *Bolivicereus samupatanus*, *Opuntia* sp., *Ferocactus* sp. *Chamaecereus silvestrii* f. *cristata*, *Echinopsis* sp. f. *cristata*, *Echinocereus pectinatus* f. *cristata*, and *Mammillaria elongata* f. *cristata*, *Ritterocereus pruinosus*, *Opuntia* sp., *Cereus* sp., *Astrophytum capricorne*, *Astrophytum myriostigma*, *Astrophytum myriostigma* v. *nudum*. According to ELISA datas antigens of CV2 were detected in plants *Mammillaria zeilmanniana*, *Opuntia* sp., *Ferocactus echidne*, *Gymnocalycium* sp., *Opuntia* sp., *Mammillaria nivosa*, *Opuntia* sp., *Consolea rubescens*, *Caralluma* sp., *Echinocereus* sp., *Opuntia brasiliensis*, *Opuntia microdasys* v. *rufida*, *Opuntia* sp. and *Pereskia aculeata* v. *godseffiana* and *Monvillea* sp., *Sulcorebutia* sp., *Bolivicereus samupatanus*, *Opuntia* sp., *Ferocactus* sp.

To study the morphology of the pathogen we carried out transmission electron microscopy. In the plant sap of *Mammillaria zeilmanniana*, *Opuntia* sp. (collection of the

Donetsk botanical garden of the National Academy of Sciences of Ukraine), *Ferocactus echidne*, *Gymnocalycium* sp., *Opuntia* sp. (collection of Botanical garden of Ivan Franko National university of Lviv), *Mammillaria nivosa*, *Opuntia* sp. (collection of Botanical garden of Odessa I.I. Mechnikov National university), *Consolea rubescens*, *Caralluma* sp., *Echinocereus* sp., *Opuntia brasiliensis*, *Opuntia microdasys* v. *rufida*, *Opuntia* sp. and *Pereskia aculeata* v. *godseffiana* (collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv) and *Monvillea* sp., *Sulcorebutia* sp., *Bolivicereus samupatanus*, *Opuntia* sp., *Ferocactus* sp. (collection of Nikitsky Botanical Garden – National Scientific Centre) we registered filamentous virions with size 580 × 13 nm which is typical *Cactus virus* X. In the plant sap of *Opuntia* sp. (collection of Botanical garden of Odessa I.I. Mechnikov National university), *Opuntia* sp. (collection of Botanical garden of Ivan Franko National university of Lviv), *Gymnocalycium mihanovichii* v. *friedrichii*, *Mammillaria magnimamma*, *Mammillaria microhelia*, *Opuntia* sp. and fasciated plants of *Chamaecereus silvestrii* f. *cristata*, *Echinopsis* sp. f. *cristata*, *Echinocereus pectinatus* f. *cristata*, and *Mammillaria elongata* f. *cristata* (collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv), *Ritterocereus pruinosus* from the collection of Karazin' Botanic Garden of Kharkiv National University, *Opuntia* sp., *Cereus* sp., *Astrophytum capricorne*, *Astrophytum myriostigma*, *Astrophytum myriostigma* v. *nudum* (collection of Nikitsky Botanical Garden – National Scientific Centre) we registered filamentous virions with 650 × 12 nm (Fig.2), which is typical for *Cactus virus* 2. It was also found rod-shaped particle with size of 317 × 18 nm, which is typical for tobamovirus in fasciated plants of *Echinopsis* sp. f. *cristata* and *Mammillaria elongata* f. *cristata*.

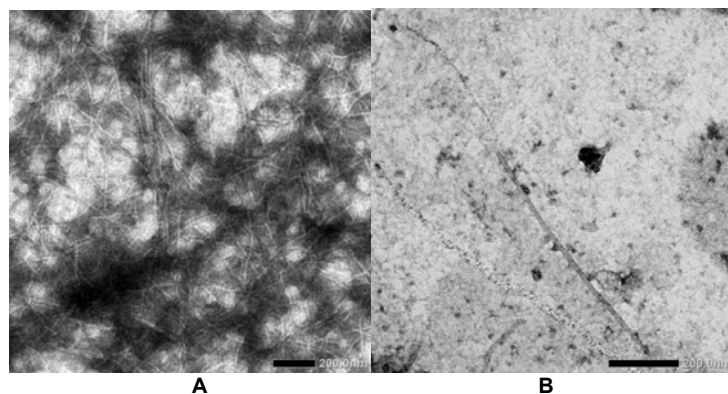


Fig.2. Electron micrograph of filamentous virus-like particles from plant material from:
 A- *Mammillaria elongata* f. *cristata*
 B- *Opuntia brasiliensis*

Comparing the results of bioassay, EM and ELISA tests CV2 had been detected in such plants as: *Mammillaria zeilmanniana*, *Opuntia* sp. (collection of the Donetsk botanical garden of the National Academy of Sciences of Ukraine), *Ferocactus echidne*, *Gymnocalycium* sp., *Opuntia* sp. (collection of Botanical garden of Ivan Franko National university of Lviv), *Mammillaria nivos*a, *Opuntia* sp. (collection of Botanical garden of Odessa I.I. Mechnikov National university), *Consolea rubescens*, *Caralluma* sp., *Echinocereus* sp., *Opuntia brasiliensis*, *Opuntia microdasys* v. *rufida*, *Opuntia* sp. and *Pereskia aculeata* v. *godsefiana* (collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv) and *Monvillea* sp., *Sulcorebutia* sp., *Bolivicereus samupatanus*, *Opuntia* sp., *Ferocactus* sp. (collection of Nikitsky Botanical Garden – National Scientific Centre).

CVX has been identified in plants of *Opuntia* sp. from collection of Botanical garden of Odessa I.I. Mechnikov National university, *Opuntia* sp. from collection of Botanical garden of Ivan Franko National university of Lviv, *Gymnocalycium mihanovichii* v. *friedrichii*, *Mammillaria magnimamma*, *Mammillaria microhelia*, *Opuntia* sp. and fasciated plants of *Chamaecereus silvestrii* f. *cristata*, *Echinopsis* sp. f. *cristata*, *Echinocereus pectinatus* f. *cristata*, and *Mammillaria elongata* f. *cristata* from the collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv, in plants of *Ritterocereus pruinosus* from the collection of Karazin' Botanic Garden of Kharkiv National University, *Opuntia* sp., *Cereus* sp., *Astrophytum capricorne*, *Astrophytum myriostigma*, *Astrophytum myriostigma* v. *nudum* from the collection of Nikitsky Botanical Garden – National Scientific Centre. Attracts attention the fact that CVX was in the all botanical gardens, but the species composition of affected plants was different. It should be noted that in all the studied collections plants of the genus *Opuntia* were the most infected

Moreover, virus related to tobamoviruses has been detected in plants of *Echinopsis* sp. f. *cristata* and *Mammillaria elongata* f. *cristata* from the collection of Fomin Botanical Garden of Taras Shevchenko National university of Kyiv. Definitive identification of this virus requires additional

research because there are three different tobamoviruses which are able to infect the cactus plants.

Conclusions: Summarizing the obtained results it is possible to assert that collections of Donetsk botanical garden of the National Academy of Sciences of Ukraine, Botanical garden of Ivan Franko National university of Lviv, Botanical garden of Odessa I.I. Mechnikov National university, Karazin' Botanic Garden of Kharkiv National University, Nikitsky Botanical Garden – National Scientific Centre were contaminated by *Cactus virus X* and *Cactus virus 2*. It should be noted that before being placed in a greenhouse cactuses had not been tested for presence of viral pathogens. Besides the pests (nematodes) and mites had been registered in some plants with the viral symptoms. Although ability of vector transmission not proven for majority of cactus viruses, it is possible that the presence of pests and non-viral disease causing deterioration of the physiological state of the plants. In addition, cactuses could support reproduction of viruses other types of plants and, thus, be the reservoirs of plant virus infections. A timely detection and continuous monitoring of cactus viruses is a main part of the system of plant virus protection.

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ДЕТЕКЦІЯ ДЕЯКИХ ВІРУСНИХ ПАТОГЕНІВ КАКТУСІВ В БОТАНІЧНИХ САДАХ УКРАЇНИ

Колекції кактусових провідних українських ботанічних садів були проаналізовані на наявність вірусної інфекції. На кактусах були виявлені різні вірусоподібні симптоми, включаючи мозаїки, хлорози та локальні некрози. Біологічні властивості детектованих вірусів визначали методами біологічного тестування, електронної мікроскопії та непрямого імуноферментного аналізу.

Ключові слова: імуноферментний аналіз, віруси рослин, кактуси.

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ДЕТЕКЦИЯ НЕКОТОРЫХ ВИРУСНЫХ ПАТОГЕНОВ КАКТУСОВ В БОТАНИЧЕСКИХ САДАХ УКРАИНЫ

Коллекции кактусовых ведущих украинских ботанических садов были проанализированы на наличие вирусной инфекции. На кактусах были выявлены различные вирусоподобные симптомы, включая мозаики, хлороз и локальные некрозы. Биологические свойства детектированных вирусов определяли методами биологического тестирования, электронной микроскопии и косвенного иммуноферментного анализа.

Ключевые слова: иммуноферментный анализ, вирусы растений, кактусы.

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ETHANOL EFFECT ON BIOPRODUCTIVITY, PHOTOSYNTHESIS AND RESPIRATION OF MICROALGA *CHLAMYDOMONAS REINHARDTII*

Some organic compounds may significantly stimulate the growth of unicellular green alga *Chlamydomonas reinhardtii*. Among them the most effective growth enhancers are acetate and monohydric alcohol methanol. The aim of the present work was studying the effect on the productivity of *C. reinhardtii* another alcohol – ethanol, which transforms into acetic acid in the process of intracellular oxidation. The results showed that in the presence of ethanol respiration was stimulated, photosynthesis inhibited and the growth of the culture stopped. We concluded that the cause of growth inhibition of *C. reinhardtii* was pH decline of the cultural medium due to oxidation of ethanol to acetic acid.

Keywords: *Chlamydomonas reinhardtii*, photosynthesis, respiration, ethanol, mixotrophy.

Introduction. The green alga *Chlamydomonas reinhardtii* has importance as model for many biotechnological processes and algal biofuels [17]. Availability of a sequenced genome [10], a proteomic database [9], and metabolomics protocols [8] benefits the use of *Chlamydomonas* to establish many fundamental aspects of metabolic control in photoautotrophic organisms [8]. It can grow either photosynthetically in the light with atmospheric CO₂ as the sole carbon source, or under heterotrophic conditions in the dark using various exogenic carbon sources added to the growth medium or else mixotrophic conditions (light and carbon sources). Under all conditions, *C. reinhardtii* remains green and retains a normally developed chloroplast, which can thus metabolize a variety of carbon sources as located in the chloroplast (starch) or assimilated through the cytosol of the cultural medium [12].

Significant stimulation of microalgae growth by exogenic methanol at mixotrophic cultivation was shown for unicellular green algae *Chlorella minutissima*, *Scenedesmus obliquus* [16], *Botryococcus braunii* [12] as well as *C. reinhardtii* [2]. Another alcohol – 2-carbon ethanol, following methanol in the homologous series of monohydric alcohols, is able to enhance the growth of microalga *Euglena gracilis*, being one of the most efficient carbon sources for this microalga [19]. Acetate, the product of ethanol oxidation, strongly stimulates *C. reinhardtii* growth [6]. *C. reinhardtii* is capable of heterotrophic and mixotrophic growth utilizing acetate as a source of carbon and energy.

In microanaerobiosis, which is naturally formed in habitat of microalgae when the respiration rate exceeds the rate of photosynthesis, the cells of *C. reinhardtii* excrete ethanol, formate and acetate [7]. Acetate can be metabolized to triose by an ATP-dependent entry into the glyoxylate or Krebs cycle to produce reducing equivalents, which can be used to reduce the plastoquinone pool [11]. It is incorporated into acetyl coenzyme A (acetyl-CoA) following two possible pathways: a direct conversion with acetyl-CoA synthetase or a two-step reaction involving acetate kinase and phosphate acetyltransferase. Acetyl-CoA enters into the glyoxylate cycle, where it is converted to succinate. Succinate is further utilized in the Krebs cycle. The carbon of ethanol, like in methanol, is oxidised to CO₂ at the final stage and may supplied as a substrate for photosynthesis.

From other hand, it was established that the addition of 0.3% v/v ethanol in the culture medium of *Dunaliella viridis*

accompanied by cessation of culture growth and increased intracellular concentrations of DNA, RNA and total protein [1]. Ethanol increases ploidy of the cells and inhibits their metabolism. Microalgae pass from dormancy to intense growth after removal of ethanol from the cultural medium. It was found toxic effects of ethanol on the growth of *Chlorella vulgaris* and *Selenastrum capricornutum*, ethanol inhibited the growth of these algae at a concentration of 0.05% [4].

The ability of exogenic ethanol to regulate productivity of *C. reinhardtii* under aerobic conditions in the light and in the dark has not been investigated. The aim of our study was to determine the effect of exogenic ethanol on productivity of batch culture of *C. reinhardtii* and its effect on photosynthesis and respiration.

Materials and methods. Unicellular green alga *Chlamydomonas reinhardtii* was obtained from the microalgae collection of Kholodny Botany Institute of NAS of Ukraine (IBASU-B – 163). Batch autotrophic cultures were grown on liquid Kessler's medium [3] in 0.5 l flasks with magnetic stirrer agitation at room temperature. 24 h white fluorescent light with 100 μmol photons·m⁻²·s⁻¹ on the surface of flasks was used. The ethanol effects were studied at the stage of exponential growth phase of batch culture. The packed cell volume (PCV) was determined as a measure for the biomass accumulation. The PCV, the volume of the cell pellet in μl, was measured by the centrifugation of a defined volume of the cell suspensions at 1400×g for 5 min in haematocrite tubes [13]. The chlorophylls (Chl) were determined spectrophotometrically in ethanolic extracts by the method of Wintermans and De-Mots [18]. The concentration of chlorophylls was calculated using the formulas: Chl a = 13,70(A₆₆₅-A₇₅₀)-5,76(A₆₄₉-A₇₅₀); Chl b = 25,80(A₆₄₉-A₇₅₀)-7,60(A₆₆₅-A₇₅₀); Chl a + b = 6,10(A₆₆₅-A₇₅₀)+20,04(A₆₄₉-A₇₅₀).

Intensity of visible photosynthesis (A) and dark respiration (R) was determined in the gas phase above the suspension of algae by IRGA method with QUBIT Systems S151 Carbon Dioxide Analyzer (Canada). Gas flow rate was 0.4 l/min and the concentration of carbon dioxide – 700-800 μM. Gas exchange measurements were carried out in a thermostated glass cell filled by 2 ml of concentrated suspension of microalgae (30-40 mg/l of chlorophyll). The rate of carbon dioxide uptake was determined under illumination with light intensity of 350 mol photons·m⁻²·s⁻¹. Dark respiration measured with a low content CO₂ in the gas space above the suspension of microalgae after turn-