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## CULTIVATION OF POTATO LEAFROLL VIRUS (PLRV) IN MAMMALIAN CONTINUOUS CELL LINES

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**Aim.** To use the ability of potato leafroll virus (PLRV) to infect and multiply in mammalian continuous cell lines to purify PLRV isolates from the vegetative plant material, and to study the pathogenicity of those isolates for plants (after culturing in mammalian continuous cell line), to investigate morphological, physical-chemical, biological and antigen properties of PLRV isolates from mammalian cells and to study an alternative diagnostic method – the neutralization test in the mammalian continuous cell lines. **Methods.** The methods of cultivating animal viruses in the mammalian continuous cell line, microscopical biochemical, and serological methods, the method of artificial nutrition of aphids are detailed under Material and Methods. **Results.** It was demonstrated that successful cultivation of PLRV in mammalian continuous cell line allowed obtaining pure virus isolates from potato plants and aphids and preserving them for a long time (over a period of 7 years). The cultivation of PLRV in the mammalian continuous cell line did not impact its pathogenic properties and allowed transmitting the virus to plants. Continuous cell lines of pig embryonic kidney (PEKV), of kidney Syrian hamster (BHK-21), of testicles of piglets (PTP), of kidneys of the bull (MDBK), and of carcinoma rabbit kidney (RK-13) were found to be sensitive to PLRV, Continuous cell lines of human (HeLa, Hep-2 and of African green monkey kidney (Vero) were not infected by the virus. The infectious activity of PLRV in the sensitive continuous cell lines was 20–8.5 lg TCD<sub>50</sub>/ml depending on the cell line. The isolates of PLRV were resistant to lipid-dissolving solvents, multiplied in a pH range from 4.0 till 10.0 and were thermoresistant at 50 °C in the absence of bivalent ions of magnesium, TIP was in the range of 60–65 °C under our experimental conditions. The optimal temperature for the reproduction of PLRV in the cell culture was c. 24 °C. The use of neutralization test in the mammalian continuous cell line allowed isolation in pure culture and identification of PLRV reliably in a time span of c. 14 days. **Conclusions.** It was proven that PLRV can be cultivated in the mammalian continuous cell lines of PEKV, BHK-21, PTV, MDBK and RK-13. It was established that the cultivation of PLRV in these continuous cell lines did not impact its biological, pathogenic, antigenic and physical-chemical properties. The identification of pure cultures of PLRV obtained in mammalian cells can be reliably performed by the use of neutralization reaction.

**Keywords:** phytopathogenic viruse, mammalian cell culturing, neutralization test.

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### INTRODUCTION

Potato leafroll virus (PLRV), a representative of the *Potterovirus* genus, *Luteoviridae* family, is a single stranded RNA-virus, of which the sequence of nucleotides is completely determined [1]. The virions of PLRV are isometric, 23 to 25 nm in size and its pure RNA has ratio of  $A_{260}/A_{280}$  of 1.78. The virus remains

infectious when diluted up to  $10^{-4}$  in sap from infected plants, and in sap j after 5–10 days at 2 °C, the virus temperature inactivation point (TIP) in crude sap when heated for 10 minutes is 70 to 80 °C [2].

PLRV is an economically important phytopathogenic virus mainly infecting potato (*Solanum tuberosum*). Apart from potato, PLRV is able to infect about 40 plant species from different families: *Amaranthaceae*, *Cucurbitaceae*, *Chenopodiaceae*, *Cruciferae*, *Com-*

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*positae, Labiatae, Portulacaceae, Nolanaceae* and *Solanaceae* [3].

PLRV often occurs in potato plants along with other viruses, and to our knowledge there are no data in the scientific literature regarding the method of obtaining its isolates from plants with a mixed infection into pure culture. The above mentioned characteristics of PLRV complicate its detection and identification and hinders the development of fully specific diagnostic methods. The routine diagnosis of PLRV involves the application of immunological methods. These include the now outdated double immunodiffusion in gel, enzyme-linked immunosorbent assay (ELISA), the immunochromatographic assay detection of the virus (lateral flow immunochromatographic assay), Luminex × MAP® Technology – a novel method of analyzing different antigens, which combines immunological, fluorescent methods and laser technologies and allows the simultaneous detection of several viruses in plant material [4, 5]. Detecting virus by the method of reverse transcription polymerase chain reaction (RT-PCR) is ranked the first among the genetic molecular methods for the detection and identification of RNA viruses [6, 7].

Multiplication of PLRV is done using potato plants or indicator plants such as *Datura stramonium* L. and *Physalis angulata* L., onto which the virus is transmitted from a stock of known infected plants using grafting or aphids. After the multiplication period, which takes about 30 days, the leaves of infected plants are used to obtain PLRV preparations.

Cultivation and multiplication of PLRV can also be performed in protoplasts of tobacco or potato mesophyll. It envisages obtaining a culture of protoplasts, infecting it with the purified and concentrated preparation of PLRV using poly-L-ornithine and incubating protoplasts at permanent illumination and temperature [8].

In 2009 we detected a phenomenon of productive potato leafroll virus infection in mammalian continuous cell lines. The procedure to obtain an infected mammalian cell is, in short, as follows: To isolate PLRV vegetative parts of potato plants with the of PLRV (reference strain 879 from the Institute's phytopathogenic virus collection) are used, in which the presence of the virus was confirmed by electron microscopy (EM) and ELISA. Plants samples are clarified with chloroform and placed in culture vials with cultures cells of pigs embryonic kidney (PEKV). The inoculated culture vials with the continuous cell line are incubated in a thermostat at 37 °C for appearance of virus cytopathic

effect (CPE). Degenerative changes of PEKV showed appearance and gradual increase in number of single rounded cells with enhanced refractivity. The infected cells are moved away from glass. Areas without cells increase in size up to complete destruction of cell monolayer. On average the detection of CPE due to PLRV infection takes 5 to 15 days.

After adaptation of PLRV to the continuous cell line via four passages, the new system «virus-cell» is used for the accumulation of viral biomass. The EM of virus preparations after ultracentrifugation demonstrated aggregates of whole isometric particles and absence of empty capsids. After the manifestation of a CPE, the virus was identified using the reverse transcription polymerase chain reaction (RT-PCR) with the primer pair sense-5'-CgCgCTAACAgAgTTCAgCC and antisense-5'-gCAATgggggTCCAACAT, that should yield a 336 bp product, corresponding to the RNA of PLRV [6].

The aims of our present work were: 1) to isolate PLRV isolates, circulating in Ukraine's territory (Chernihiv's region); 2) to investigate physical-chemical, biological and antigen properties of PLRV isolates; 3) to study the pathogenicity of PLRV for plants, after it was multiplied and cultured in mammalian cells; 4) to investigate an alternative method of PLRV identification, namely the neutralization test in the continuous mammalian cell line.

## MATERIALS AND METHODS

Potato material (18 plants with symptoms of leafroll and three aphids samples (per sample 5–10 *Myzus persicae* aphids, directly collected from plants) selected in the fields of Chernihiv's region, and the reference strain of PLRV, kept in the virology laboratory of Institute in potato clone G 879, were used to conduct the virological research.

Plants and aphids samples were clarified with chloroform (1 : 4) and placed in culture vials with cultures cells of pigs embryonic kidney (PEKV). The multiplication of virus was done in these cell cultures which were grown in growth medium 199 («BioTestLab», Kyiv, Ukraine) at the culture vials. Prior to introducing the virus, the nutrient medium was drained, and the cell monolayer was rinsed twice with a 0.9 % NaCl solution and Hank's solution. An amount of 0.5 ml of the virus suspension was introduced per vial and placed in an incubator at 37 °C to contact for one hour. After the contact the cell monolayer was washed with Hank's solution, the solution was removed and the monolayer of

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cells further incubated at 37 °C up to a stage of 75 % CPE, subsequently the cultures were stored deep frozen at -18 °C in triplicate. Pure populations of PLRV were obtained via three times cloning of viruses by the method of limiting dilutions with subsequent three-times cloning by Melnick's method of plaque technique [9]. Virus accumulated in the previous dilution, was used for each subsequent passage.

The isolation, concentration and purification of PLRV from the infected mammalian cell lines was performed as follows: Two parts of virus suspension were added to one part of chloroform with subsequent hand shaken homogenization for 30 min. Then the mixture was kept for 12–18 h at 4 °C, and subsequently centrifuged for 20 min at 1500 g. To the supernatant ammonium sulfate was added until saturation of 50 % and the suspension kept at 4 °C for 1 h. The precipitate was removed by centrifugation at 5–10 °C for 20 min at 1500 g, resuspended in 0.9 % NaCl, and dialyzed against the same solution for 16–18 h. Control of purity of virus preparations was done spectrophotometrically using a spectrophotometer (SF-46, Leningrad, USSR) by measuring the ratio of  $A_{260}/A_{280}$ , and by electron-microscopy using a transmission electron microscope (UEMB-100V, Sumy, USSR).

For the hyperimmunization of rabbits purified and concentrated virus-containing suspensions of PLRV were used. To the prepared antigen, used for subcutaneous administration, adjuvant Montanide ISA 25 (SEPPIC, France) was added according to the manufacturer's instructions. The immunization of rabbits was done according to the scheme, developed by us. Immunization of rabbits is carried out through five-time administration of concentrated virus antigen in turns subcutaneously with adjuvant Montanide ISA 25 in an amount of 1 mg of protein/2 ml intracutaneously without adjuvant along spinal column to 8–10 points in amount of 1 mg of protein/1 ml with an interval between introductions of 7, 3, 4, 3 days respectively.

We did not determine genetic markers of PLRV when cultured in mammalian cells, but we analysed resistance of virus isolates to lipid-dissolving solvents [11], the sensitivity to certain media at different pH values, thermo-resistance [12] and thermal stability in order to properly identify the isolates as PLRV.

The stability of isolates of PLRV at various pH values of the solution (0,1 M Na<sub>2</sub>CO<sub>3</sub>, 0.1 M Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> and their combinations) was studied in a BHK-21 cell line. For this purpose, the virus isolates LT, LB, LS at a dose of 1 lgTCID<sub>50</sub> were kept in the solution with a pH value

of 2.0, 3.0, 4.0, 7.2, 10.0 and 11.0 respectively at room temperature for 10 minutes. After that, in all samples, the pH was adjusted to 7.2. The sensitivity of the virus to the acid and alkaline pH values was determined by the difference between the titre of the virus isolates as compared to that of the PLRV control strain (pH 7.2). The experiment was performed in three replications.

Thermo-resistance was studied via determining the infectious activity of the three PLRV isolates in cell culture after heating them at 50 °C for 1 h in the presence of 1 M of a MgCl<sub>2</sub> solution and without it. The experiment was performed in three replications.

The impact of temperature on the functioning of the PLRV-animal cell system was studied in the BHK-21 cell line, infected with isolate LT at a dose of 1 lgTCID<sub>50</sub>. The incubation was conducted at 2, 10, 24 and 37 °C. The degeneration changes in the monolayer, the time of their occurrence and infectious activity of the virus were noted. The experiment was performed in three replications.

The antigenic affinity between virus isolates was established in the cross reaction of virus neutralization using a stable dose of the virus (100 TCID<sub>50</sub>) and antiserum to PLRV isolates (20 neutralizing doses) and 10-times dilutions of the virus with a stable dose of antiserum (20 neutralizing doses). Normal rabbit serum in 1 : 5 dilution and blood serum, obtained from the culture of BHK-21 cells, were used for the control. The antigenic affinity was calculated by the formula [9]:

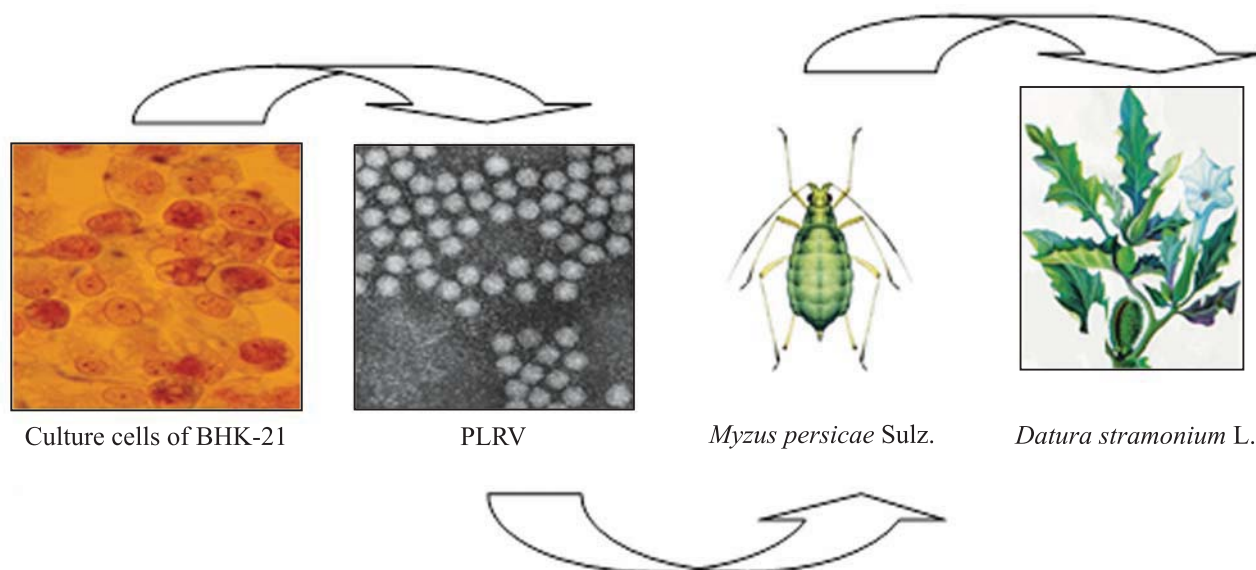
$$A = 100\sqrt{r_1 \times r_2}$$

$r_1$  = heterologous titer/homologous titer, for strain 1;

$r_2$  = heterologous titer/homologous titer, for strain 2.

To study biological properties of PLRV the sensitivity of different cultures of mammalian cells to PLRV, we conducted the studies of virus replication in continuous cell lines (from the Institute's continuous cell line collection) such as: a continuous cell line of pigs embryonic kidney (PEKV), of kidney Syrian hamster (BHK-21), of testicles of piglets (PTP), of kidneys of the bull (MDBC), of rabbit kidney carcinoma (RK-13), of human (HeLa, Hep-2) and of African green monkey kidney (Vero). Reference strain 879 of PLRV was adapted to the mammalian continuous cell lines with 4 passages.

To study the pathogenicity of PLRV for plants, after it was multiplied and cultured in mammalian cells, the method of Rochow [13] was used to transmit the virus to plants using aphids (*Myzus persicae* Sulz.), which were fed through an artificial membrane (Fig. 1).



**Fig. 1.** The scheme of transmitting PLRV by aphids from the infected continuous mammalian cell line to plants-indicators

We have made a ‘aphid nursery’ in order to obtain “sterile” (not infected) clones of aphids. After consecutively obtaining four generations of *M. persicae* on *Brassica pekinensis* (Lour.) Rupr. plants (under greenhouse conditions, 18–25 °C, natural light), the insects are deemed free from PLRV. After five weeks of replication, homogeneous colonies of *M. persicae* were obtained. “Sterile” aphids (10–20 insects from the replication nursery) were fed for 24 h via a “sandwich” of membranes of *Parafilm M* (52 × 52 mesh), fixed at the end of a small glass tube with the size of 15 mm × 5 cm. To that end 0.1 µl of the PLRV preparation solution (obtained in the BHK-21 cell line and cleared with chloroform, concentrated by centrifugation via a 20 % sucrose cushion at 37,000 g) was introduced between the membranes. Negative control was a similar combination with 5 % saccharose solution only. For the experiment we used 5 passage reference strain 879 in the BHK-21 cell line.

After artificial nutrition, aphids were transmitted in batches of 10 insects on five indicator plants of *Datura stramonium* and five indicator plants of *Physalis angulata* (under greenhouse conditions, 18–25 °C, ...% relative humidity (RH) and natural light). One day later the aphids were killed with insecticide.

To study an alternative method of PLRV identification, namely the neutralization test, two samples of potato leaves (on 1 sheet with 10 plants) of variety *Suvenir Chernihivsky* with mild mosaic symptoms and variety *Tyras* without any disease symptoms. Samples were selected in the hydroponic greenhouse of Scientific Production Association “Chernihivelitkartoplia”.

A pure culture of PLRV reference strain obtained via the PEKV cell line, was used to obtain the reference antiserum.

The preparations, obtained from both samples of potato leaves, were used to infect the PEKV cell line and incubated in the thermostat at 37 °C till the manifestation of the features of cytopathic effect of the virus, which appeared on the third day.

The samples were typed in the serological neutralization test in the PEKV cell line. Prior to that, the titer of the obtained reference antiserum was determined to be 1:256 in the neutralization test.

To identify the isolated viruses in the neutralization test, 0.5 ml, containing 100 TCID<sub>50</sub> of virus antigen in 0.1 ml, was mixed with 0.5 ml of reference serum to PLRV, containing 20 neutralizing doses per milliliter. After incubating the mixture at 37 °C for 60–90 min, 0.2 ml was introduced into a test tube to which 0.8 ml of the supporting medium was added before. Control and experiment test tubes were further incubated at 37 °C and the results registered on the 4<sup>th</sup> and 7<sup>th</sup> day.

## RESULTS AND DISCUSSION

Three isolates (LB, LS and LT) of PLRV were obtained from 18 symptomatic potato and 3 aphid samples after being cultured in the PEKV cell line (Table 1).

PLRV isolates were extracted in 3, 4 and 5 passages, their infection titers were 6.5–8.5 lg TCID<sub>50</sub>/ml and typed with rabbit serum to the reference strain 879 in the PEKV cell line, where the neutralization test estab-

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lished their homology to the reference strain 879 of PLRV

All three isolates of PLRV, when multiplied in the cell culture under agar cover, formed small plaques of 1 mm in diameter on days 3–4.

The LB, LS, LT isolates of PLRV were kept at  $-18^{\circ}\text{C}$  in a domestic freezer and did not lose their infectivity over a period of 7 years and maintained in a pure culture by passing them in PEKV and BHK-21 cell cultures.

Virus isolates, extracted from plant samples and aphids, were found to be resistant to lipid-dissolving solvents (ether, chloroform) which demonstrated the absence of a lipid-containing envelope in them.

As seen from the results, presented in Table 2, at different pH values the infectious titer almost did not change in the range from 4 to 10.0, decreased by 4 lg TCD<sub>50</sub>/ml at pH 3.0, and at pH 2.0 there was complete inactivation.

The study of thermal stability of extracted PLRV isolates established that the thermal TIP in the culture of PEKV after heating for 10 min was in the range from  $60^{\circ}\text{C}$  to  $65^{\circ}\text{C}$ . It may be that the difference in TIP values was conditioned by different chemical composition of the media, where the viruses were placed while heated, although we did not test this supposition.

While studying the thermal resistance, the infectivity of the three PLRV isolates, heated without 1 M of the solution of MgCl<sub>2</sub>, did not change compared to the unheated control, and in the presence of 1 M of MgCl<sub>2</sub>, it decreased by 2–3.5 lg TCID<sub>50</sub>/ml, which demonstrated the absence of stabilization of virions with bivalent cations of magnesium (Table 3).

The results of the determination of temperature impact are presented in Table 4.

There was a noted considerable slowing down of PLRV replication at temperatures below  $24^{\circ}\text{C}$ . For in-

stance, after 7 days of incubation at 2 and  $10^{\circ}\text{C}$ , there were no degenerative changes observed in the monolayer, and the infectious titer of PLRV decreased to 2 lg TCID<sub>50</sub>/ml. At  $24^{\circ}\text{C}$  on the 3<sup>rd</sup> and 4<sup>th</sup> day after the inoculation, single round cells were observed. The destruction of 75 % of the monolayer was observed after 7 days. On the 3<sup>rd</sup> and 4<sup>th</sup> day, the infectious activity was 4.5–5.5 lg TCID<sub>50</sub>/ml respectively, on the 7<sup>th</sup> day it was 6.5 lg TCID<sub>50</sub>/ml. At the optimal temperature of incubation, *i.e.*  $37^{\circ}\text{C}$ , the cytopathic action of the virus developed already after 24 h, and the infectious titer of the virus was 7.5 lg TCID<sub>50</sub>/ml.

The antigenic affinity of the three PRLV isolates from three different sources, namely aphids, leaves and tubers of potato, was 100 % *i.e.* they were serologically identical.

PLRV had a cytopathic effect on the PTP cell line 12–24 h after inoculation, for the PEKV and the BHK-21 cell lines it was 24–48 h after inoculation. The cytopathic effect was visible as symptoms of degeneration in the cell culture in the form of single rounded cells with increased refractivity, in increasing numbers. The affected cells came loose from glass, and clear empty spots appeared in the monolayer, increasing in size up to complete and visible destruction of cell groups.

**Table 1.** The three isolates of PLRV extracted from plant samples and aphids used in our study

Source of extracting the isolate	Code of the isolate	Passage of extracting	Infection titer (lg TCID <sub>50</sub> /ml)
Potato tubers, Skarb variety	LB	4	$6.5 \pm 0.12$
Potato leaves, Suvenir Chernihivsky variety	LS	3	$8.5 \pm 0.18$
Aphids from potato leaves, Tyras variety	LT	5	$7.0 \pm 0.10$

**Table 2.** The impact of pH value of the solution on the infectivity of three PLRV isolates

PLRV isolates	Virus titer (lg TCD <sub>50</sub> /ml) by variants					
	pH 7.2	pH 2.0	pH 3.0	pH 4.0	pH 10.0	pH 11.0
LT	$8.5 \pm 0.14$	0	$4.0 \pm 0.12$	$8.0 \pm 0.12$	$8.0 \pm 0.12$	$6.0 \pm 0.14$
LB	$8.5 \pm 0.10$	0	$4.5 \pm 0.14$	$8.5 \pm 0.18$	$8.0 \pm 0.12$	$5.5 \pm 0.10$
LS	$8.0 \pm 0.12$	0	$4.0 \pm 0.10$	$8.0 \pm 0.14$	$7.0 \pm 0.24$	$6.0 \pm 0.18$

After 48–96 h, destructive changes were also observed in the cultures of the RK-13 and MDBK cell lines, here the cells also formed symplasts.

No degenerate changes were detected in the cultures of HeLa, Hep-2 and Vero cells after consecutive passaging, PLRV did not multiply in these cell lines.

The infectious activity of PLRV in the PTP cell line was 7.5–8.5 lg TCID<sub>50</sub>/ml, in the BHK-21 cell line – 6.0–8.5 lg TCID<sub>50</sub>/ml, in the PEKV cell line – 7.5–8.5 lg TCID<sub>50</sub>/ml.

Twenty-four days after the 10 *M. persicae* aphids per PLRV isolate had been feeding for 24 h and were killed by insecticides there was a noted manifestation of interveinal chlorotic zones on old and young leaves in *P. angulata* plants and a delay in growth of *D. stramonium* plants, which indicated successful transmission of PLRV with the preservation of virus pathogenicity after long-term cultivation in mammalian cell culture.

We also studied the possibility of using the mammalian cell culture for PLRV diagnostics in plant samples.

The neutralization of viruses with the reference serum of rabbit blood demonstrated PLRV infection in

potato leaves of varieties Suvenir chernihivsky and Tyras. The virological analysis with the mammalian cell culture takes c. 10 days. Thus, it was established that the application of neutralization test – a method, previously not applicable for identification of phyto-viruses – in the continuous mammalian cell culture allows isolation and identification of PLRV in potato plants reliably.

## CONCLUSIONS

It was demonstrated that the cultivation of PLRV in some mammalian continuous cell lines allowed isolation of pure virus isolates from potato plants and aphids and preserving them for a long time (up to 7 years).

The investigated three isolates of PLRV were resistant to lipid-dissolving solvents, were multiplying in media with pH values from 4.0 till 10.0 and were thermo-resistant at 50 °C in the absence of bivalent ions of magnesium; TIP was in the range of 60–65 °C under our experimental conditions. The optimal temperature for the replication of PLRV was c.24 °C.

It was established that continuous cell line lines of PEKV, BHK-21, PTP, MDBK and RK-13 were sensitive to PLRV. The human cell lines HeLa and Hep-2, and a primate cell line (Vero) were not infected by the virus. The infectivity of PLRV in the sensitive cell cultures was 2.0–8.5 lg TCID<sub>50</sub>/ml depending on the cell culture.

The cultivation of PLRV in continuous mammalian cell lines did not impact its pathogenic and other physico-chemical properties and allowed transmitting the virus to plants.

The application of neutralization test can be reliably used to identify pure isolates of PLRV obtained from mammalian continuous cell lines.

**Table 3.** Thermal resistance of three PLRV isolates in the cell culture of PEKV

PLRV isolates	Virus titer (lg TCD <sub>50</sub> /ml)		
	Control (without heating)	Heating at 50 °C, 1 h	Heating at 50 °C, 1 h, 1 M, solution of MgCl <sub>2</sub>
LT	8.5 ± 0.12	8.5 ±	5.0 ±
LB	8.0 ± 0.12	8.0 ±	5.0 ±
LS	6.5 ± 0.14	6.5 ±	4.0 ±

**Table 4.** The impact of temperature on PLRV multiplication in the cell culture of BHK-21

Incubation temperature, °C	Degenerate changes in the monolayer of the cell culture	Period of observations, days	Infectious titer of the virus (lg TCD <sub>50</sub> /ml)
2	not observed	7	2.0 ± 0.12
10	not observed	7	2.0 ± 0.12
24	single round cells	3	4.5 ± 0.12
24	single round cells	4	5.5 ± 0.12
24	destruction of 75 % monolayer	7	6.5 ± 0.12
37	destruction of 75 % monolayer	1	7.5 ± 0.12

**Культивування вірусу скручування листя картоплі в культурах клітин ссавців**

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**Мета.** Використати явище продуктивної інфекції вірусу скручування листя картоплі в культурах клітин ссавців для виділення ізолятів ВСЛК з рослинного матеріалу, вивчення патогенності ВСЛК для рослин при культивуванні в культурі клітин ссавців, дослідження морфологічних, фізико-хімічних, біологічних й антигенних властивостей ізолятів ВСЛК та випробування альтернативного методу діагностики – реакції нейтралізації в культурі клітин ссавців. **Методи.** Використано загальноприйняті методи культивування вірусів тварин в культурі клітин ссавців, біохімічні методи, серологічні методи, метод штучного живлення попельці. **Результати.** Показано, що культивування ВСЛК в культурі клітин ссавців робить можливим виділення чистих ізолятів вірусу з рослин картоплі та попельці та їх підтримання тривалий час. Культивування ВСЛК в культурі клітин ссавців не впливає на його патогенні властивості та дозволяє передавати вірус на рослини. Перещеплювані лінії культур клітин нирки ембріона свині (СНЕВ), нирки сірійського хом'яка (ВНК-21), тестикул поросят (ПТП), нирки бика (МДБК) та карциноми нирки кроля (РК-13) виявилися чутливими до ВСЛК. Інфекційна активність ВСЛК в чутливих культурах клітин становила 2,0–8,5 Іг ТЦД<sub>50</sub>/см<sup>3</sup> в залежності від культури клітин. Ізоляти ВСЛК стійкі до ліпідорозчинників, до середовищ із значеннями рН від 4,0 до 10,0 та терморезистентні при 50 °С за відсутності двовалентних іонів магнію, ТТІ знаходиться в межах 60–65 °С. Оптимальною температурою для репродукції ВСЛК в культурі клітин є +24 °С та вище. Використання реакції нейтралізації в культурі клітин ссавців дозволяє швидко та надійно діагностувати ВСЛК у рослинах картоплі. **Висновки.** Доведено, що ВСЛК можна культивувати в культурах клітин ссавців СНЕВ, ВНК-21, ПТП, МДБК, РК-13. Встановлено, що культивування ВСЛК у цих культурах клітин не впливає на його біологічну активність, патогенні, антигенні, та фізико-хімічні властивості. За результатами досліджень рекомендовано використання реакції нейтралізації для ідентифікації ВСЛК.

**Ключові слова:** фітопатогенний вірус, культивування клітин ссавців, реакція нейтралізації.

**Культивирование вируса скручивания листьев картофеля в культурах клеток млекопитающих**

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**Цель.** Использовать явление продуктивной инфекции вируса скручивания листьев картофеля в культурах клеток млекопитающих для выделения изолятов ВСЛК из растительного материала, изучение патогенности ВСЛК для растений при культивировании в культуре клеток млекопитающих, исследования морфологических, физико-химических, биологических и антигенных свойств изолятов ВСЛК и использование альтернативного метода диагностики – реакции нейтрализации в культуре клеток млекопитающих. **Методы.** Используются общепринятые методы культивирования вирусов животных в культуре клеток млекопитающих, биохимические методы, серологические методы, метод искусственного питания тлей. **Результаты.** Показано, что культивирование ВСЛК в культуре клеток млекопитающих делает возможным выделение чистых изолятов вируса из растений картофеля и тлей, их поддержание длительное время. Культивирование ВСЛК в культуре клеток млекопитающих не влияет на его патогенные свойства и позволяет передавать вирус на растения. Перевиваемые линии культур клеток почки эмбриона свиньи (СПЭВ), почки сирийского хомяка (ВНК-21), тестикул поросят (ПТП), почки быка (МДБК) и карциномы почки кролика (РК-13) оказались чувствительными к ВСЛК. Инфекционная активность ВСЛК в чувствительных культурах клеток составляла 2,0–8,5 Іг ТЦД<sub>50</sub>/см<sup>3</sup> в зависимости от культуры клеток. Изоляты ВСЛК устойчивы к липидоразтворителям, к среде со значениями рН от 4,0 до 10,0 и терморезистентны при 50 °С в отсутствие двухвалентных ионов магния, ТТИ находится в пределах 60–65 °С. Оптимальной температурой для репродукции ВСЛК в культуре клеток является 24 °С и выше. Использование реакции нейтрализации в культуре клеток млекопитающих позволяет быстро и надежно диагностировать ВСЛК в растениях картофеля. **Выводы.** Доведено, что ВСЛК можно культивировать в культурах клеток млекопитающих СПЭВ, ВНК-21, ПТП, МДБК, РК-13. Установлено, что культивирование ВСЛК в этих культурах клеток не влияет на его биологическую активность, патогенные, антигенные и физико-химические свойства. По результатам исследований реко-

мендовано использование реакции нейтрализации для идентификации ВСЛК.

**Ключевые слова:** фитопатогенный вирус, культивирование клеток млекопитающих, реакция нейтрализации.

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