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Isolation of Potato X-Virus from Collecting Plants

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Abstract. This article is about obtaining a purified preparation of potato virus X with the help of biological and physicochemical methods in the presence of D. tatula plant, in which the plant was infected with the virus by mechanical inoculation and the disease symptoms were observed in 18-20 days. TSK-65 was used to obtain a purified preparation of the virus. Viral fractions began to separate from 65-69 ml, and the highest concentration of virus corresponded to fractions of 80-85 ml.

Keywords: Potato X virus, D. tatula, D. stramonium, ammonium sulfate, centrifuge, phosphate buffer, systematic mosaic.

Introduction

To study the properties of viruses, it is appropriate to use their purified preparations. But their clean separation is one of the most difficult tasks [2,5,6]. The reason is that it is a multi-stage process, first biological treatment, then physical-chemical methods - centrifugation, treatment with chemicals. Methods for isolation of a pure preparation of viruses differ for different viruses [2,3,4,6]. Therefore, cleaning methods are used with some modifications.

Until now, purified preparations of KXV have been obtained by centrifugation at isoelectric point, using PEG and salts such as NaCl, ammonium sulfate, and high rotation speed [1,3,7]. Recently, the "soft" gel chromatography method has been used to isolate viruses [6,9]. Isolation of such viruses that harm the yield and quality of potatoes and obtaining a pure drug is important in developing measures to combat them.

For each studied phytopathogenic virus, it is necessary to select such indicator plants that the plant should produce specific symptoms characteristic of this virus [1,3,8].

KXV from plants belonging to the Solanaceae family, together with cultivated plants such as eggplant, tomato, pepper, black and red grapevine, D.stramonium, D.metel, Physalis floridana Rubd., Ph. angulota L., Nicandra physaloides L., Atropa beladona; Gompherena globosa, Amarantus refolexus L., A. hybrida, A. tricolor belonging to the Amarantaceae family; From the Compositae family, Chrisantemum morifolium Ram., Senecio sp. has been found to infect wild plants such as In some literature, there is information that the potato X-virus is also stored in the red sebar [6,7,9]. A virus of the same species causes different disease symptoms in different plants. Only KXV causes disease symptoms such as systemic mosaic in Datura

stramonium L., D. tatula L., green mosaic in D. metel L., and necrosis in Gomphrena globosa when mechanically inoculated [7].

Datura plants contain alkaloids of the tropane family: hyoscyamine, atropine, and scopolamine, which are 0.15% in the stem of the plant, 0.26% in the root, and 0.22% in the seed. Atropine and scopolamine alkaloids have been found to be high in the Datura tatula L. plant belonging to the Datura family[10,11,12]. When isolating viruses, it is necessary to take into account the properties of the virus as well as the substances contained in the plant. In Uzbekistan, KXV was isolated from D. stramonium, N. burley plants [6].

Based on the above information, we aimed to isolate KXV from Datura tatula L. plant.

Experience part. Based on these characteristics of viruses and the biology of the virus, biological purification is carried out, which allows to purify the viral sample from mixed infection. Taking this into account, biological purification of KXV was carried out according to the following scheme:

		D.stramonium,		*D. stramonium*
Sample =>	Homogeneous. => 0,02 M FB, pH 7-8	*D. tatula* => (sys. mosaic)	*G. globosa* => (necrosis)	*D. tatula* sys. mosaic

After the virus was purified three times according to the scheme above, it was studied using test-indicator plants. D.stramonium and D. tatula were used as virus collecting plants. They were infected by sprinkling corborundum on their leaves and applying infected aphids with clean, wet fingers [3]. KXV appeared on all leaves of the plant causing systemic mosaic symptoms in 18-20 days in these plants (picture). After sufficient accumulation of the virus in D. tatula plants, it was stored in polyethylene bags in a refrigerator (-4°C). These plants were later used to obtain a pure virus preparation

Figure 1. Potato leaves infected with KXV: healthy leaf on the left, infected leaf on the right

Figure 2. Disease symptoms of biologically purified KXV on leaves of Datura tatula plant

The next step in virus purification is a physico-chemical method, first the viral sample is homogenized using phosphate buffer pH 7.4, 0.02M. Viral sample from the solid components of the cell at 5000 m/min. It was separated by centrifugation for 20 min. After removing the supernatant, chloroform (1/8) was added to remove the pigments, shaken vigorously for 20 minutes and centrifuged at 5500 rpm for 30 minutes. In this case, the sample formed 3 layers, and the viral layer was carefully collected in a separate container. To precipitate the virus, a 1:0.5 ml volume of a saturated solution of ammonium sulfate (40%) was added to the viral sample and stored overnight in a refrigerator (+4°C). The viral pellet was separated by centrifugation at 5800 rpm/30 min and dissolved in 0.02 M phosphate buffer, pH 7.4. To get rid of salts, it was dialyzed 3-4 times using distilled water 100 times the volume of the sample. Excess ions in the dialysis product were removed by centrifugation at 3000 rpm/5 minutes. The supernatant was used for separation in the next step, i.e. gel chromatography. Fractions separated by TSK-65 gel were measured in a spectrophotometer at a wavelength of 260/280 nm. The result of SF was graphically represented (Figure 3).

Due to the low amount of purified virus preparation and high impurities in the partially purified virus sample, the viral sample was re-concentrated. In this case, the precipitation of the virus was carried out in the same way as above: that is, it was precipitated using a saturated solution of (NH4)2SO4 for 2 hours, a partially purified preparation of the virus was obtained by centrifugation, dialysis and centrifugation again. This sample differed from the previous drug in that it was clear and had few impurities. The sample was passed through TSK-65. The separated fractions were measured at a light wavelength of 260/280 nm. Result excl. graphically represented in the program (Fig. 4).

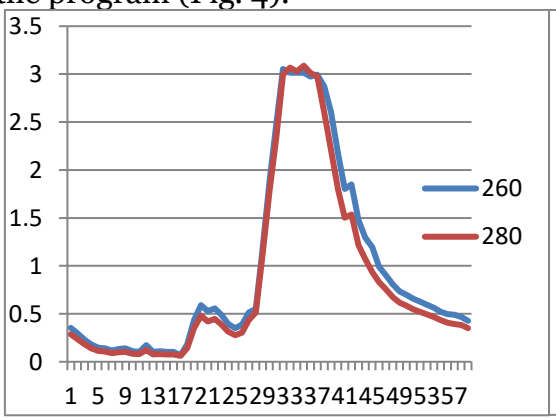

Figure 3. Graphic representation of the purity of the initial fractions of KXV separated by TSK-65 (Experiment 1).

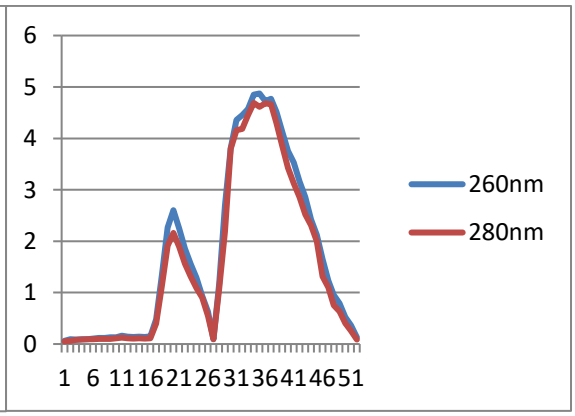

Figure 4. Graphic representation of the results of further fractionation of KXV by TSK-65 (Experiment 2)

After the viral sample was completely passed through the gel, the viral fractions were collected and again concentrated using a saturated solution of ammonium sulfate and centrifuged (12,000 rpm/30 minutes). The precipitate was dissolved in buffer and the supernatant was dialyzed several times to remove excess salts. Deionization of dialysis was carried out by centrifugation at 3000 rpm/5 minutes. The supernatant was removed, the pellet was again dissolved in a small amount of buffer, centrifuged again, and the supernatant was added to the previous viral sample. This was

considered to be a pure preparation of the virus and was withdrawn to study the properties of the virus.

Analysis of results and conclusion part. Symptoms were observed in 18-20 days when potato X virus was mechanically infected with D. tatula. Disease symptoms on the leaves persisted even at temperatures above 30°C. During the transfer of the viral sample from the gel, the cellular components of the plant did not interfere with the process of separation of viral fractions. It follows that it is possible to use TSK-65 gel for pure isolation of potato X virus from this plant and gel chromatography method. This information does not represent a complete result. Searching for the type of plant used for virus isolation and the way to get rid of the metabolites found in it without reducing the amount of virus requires further research.

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