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BRIEF COMMUNICATION

Effect of the storage conditions on the activity of the potato virus A in ELISA

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Abstract

We followed the effect of storage conditions on the serological activity of the potato virus A (PVA) in the ELISA test. Storage of the purified PVA in 0.05 M borate buffer, pH 8.3 at -70 °C represents the optimum conditions. The activity decreased 40% in comparison to the original activity after four months. Lyophilization of infected leaves or the leaf homogenate in the extraction buffer were found to be the best methods for the storage of the PVA in natural conditions. After 12 months, the activity of PVA decreased to 80 % and 50 % of the original activity in the case of lyophilized leaves and leaf homogenates, respectively.

For checking the health of the plants, enzymoimmunological tests are usually used which enquire a positive control for their evaluation. The aim of our work was to find optimum conditions for the storage of the potato virus A in plant tissues and in the purified form.

The host plants *Nicotiana tabacum* L. cv. Samsun were inoculated mechanically with the potato virus A (isolate of PVA-LI was obtained from Dr. Dědič (Potato Research Institute, Havlíčkův Brod). The symptomatic leaves from the infected plants were grounded in a mortar with the extraction solution (0.02 M phosphate buffer, pH 7.4 containing 0.8% NaCl, 0.05 % Tween 20, 2% polyvinylpyrrolidone, 0.2% bovine serum albumin (BSA) and 0.05% NaN_3) at the ratios of 1:5 and 1:20. A negative control was prepared by the same method from healthy leaves. 1 ml portions of prepared antigen were stored at +4 °C, -20 °C, -70 °C and in a lyophilized form. Serological activity was tested by the double antibody sandwich ELISA according to Clark and Adams (1977).

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We used rabbit IgG antibodies isolated from the antiserum by precipitation with caprylic acid (Steinbuch and Audran 1969) and IgG antibodies conjugated to alkaline phosphatase (Avrameas 1969) for the test.

The values given in the tables are means of three replicate experiments measured in duplicate.

We followed the serological activity of the potato virus A in the sap stored at +4 °C in the course of weeks. In long-term experiments (lyophilized samples or samples stored at -20 °C and -70 °C) we tested the virus activity after 1, 4, 9 and 12 months. Simultaneously, a further month after unfreezing or dilution of the appropriate sample stored at +4 °C the virus activity was tested.

Table 1. Effect of the storage conditions on the serologic activity of PVA.

Time of antigen activity [month]	Lyophilized homogenate dilution 1:5	dilution 1:20	Leaves lyophilized	-20 °C
Original activity	100	100	100	100
4	59	45	100	50
9	55	42	100	30
12	50	37	78	0

Direct lyophilization of the infected leaves was the best method for long-term storage of PVA (Table 1). The average decrease of the serological activity of PVA after 12 months was only about 20 %. In samples stored in the form of a lyophilized homogenate in the extraction buffer, the activity dropped in comparison to that measured after the lyophilization by 50 to 60 %, depending on the dilution.

In leaves stored at -20 °C, the virus lost 70 % of its activity already after 9 months, after 12 months it was serologically quite inactive. Table 2 illustrates the loss of the serological activity of PVA in the sap during the short-term storage at +4 °C. Samples were prepared in the extraction buffer. On the other hand, the activity of PVA in samples lyophilized from the extraction buffer and then diluted dropped to these levels already after three weeks and after four weeks it was impossible to prove the presence of PVA in the samples by ELISA (Table 3).

Table 2. Effect of dilution of homogenate in infected leaves stored at +4 °C on the serological activity of PVA. The numbers in the table show % of activity, 100 % = absorbance at 405 nm > 2.5.

Time of homogenate storage [week]	Dilution of homogenate 1:5	1:20
Original activity	100	100
1	100	44
3	61	42
4	22	18

Table 3. Effect of time and dilution of lyophilized homogenate on the activity of PVA. The numbers in the table show % of activity, 100 % = absorbance at 405 nm > 2.5.

Time of homogenate storage [week]	Dilution of lyophilized homogenate 1:5	1:20
Original activity	100	100
1	54	34
3	34	24

The activity of the purified PVA was preserved best in the 0.05 M borate buffer, pH 8.3 frozen at -20 °C or -70 °C (Table 4). In average, the activity of preparations stored under these conditions decreased after 4 weeks only by 8 %, in comparison to the starting values of the isolated PVA. Different temperatures (-20 °C and -70 °C) influenced the activity only in the case of long-term storage. The activity of the sample stored at -20 °C was zero after 30 days, whereas samples stored at -70 °C retained from 50 to 60 % of their original activity.

Table 4. Effect of storage conditions on the activity of purified PVA in 0.05 M phosphate buffer, pH 8.3 (P) and 0.05 M borate buffer, pH 8.3 (B). *A* - absorbance at 405 nm in ELISA immediately (A^1), 30 d (A^{30}) and 120 d (A^{120}) after purification or lyophilization, respectively.

Buffer	Purified virus					Lyophilized		
		+4 °C	-20 °C	-20 °C	-70 °C			
	A^1	A^{30}		A^{120}		A^1	A^{30}	A^{120}
P	1.15	0	0.50	0	0.40	0.80	0.20	0
B	1.30	0	1.16	0	0.70	0.90	0.40	0

Lyophilization of the purified PVA preparations in both the used buffers resulted in a 30 % decrease of the activity, independently of the addition of stabilizing protein agents (ovalbumin, BSA). The lyophilized purified preparation became serologically inactive after four months.

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