ACTA BOT. CROAT. VOL. 39, 15—20, 1980.

UDC 58
CODEN: ABCRA2
YU ISSN 0365—0588

UDC 576.858:54.058 = 20

PURIFICATION OF BELLADONNA MOTTLE VIRUS BY AMMONIUM SULPHATE

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Received February 13, 1980

Introduction

Salt precipitation has often been used as a method for isolation of plant viruses since Stanley (1934) isolated tobacco mosaic virus by applying it. By means of that procedure isometric as well as anisometric viruses have been purified. Ammonium sulphate (a. s.) is often preferred to various inorganic salts which can be used for that purpose. The concentration of a. s. needed for precipitation is different for various viruses and includes the range from cca 1/3 to about 3/4 saturation of the solution (cf. Gibbs and Harrison 1976).

It seems that up till now 5 out of 16 tymoviruses (Koenig and Lesemann 1979) have been isolated by using salting out with a. s.. There is some difference in the salt concentration necessary for the precipitation of those viruses (cf. Discussion).

Attempts were made in the course of the present work to apply a. s. precipitation upon belladonna mottle virus (BdMV). A brief account on this subject was already given (Mamula 1977).

Material and Methods

The virus investigated was a Yugoslav isolate (BMV-M) of BdMV (Štefanac 1974) which was propagated in *Datura stramonium* L. plants as an appropriate source of the virus (cf. Moline 1973). In the isolation procedure systemically infected leaves of the plant were mainly used (Fig. 1a). Clarification of leaf sap, which usually had pH value of cca 5.3, was performed by emulsifying it with chloroform (10:4, v v), followed by low speed centrifugation.

Saturated a. s. solution was added successively to clarified plant sap under constant stirring. Precipitation at a given salt concentration was allowed to occur during the period of 60 min. Then the suspension was spun at a low centrifugal force and a further quantity of a. s. solution was added to the supernatant. Pellets were washed carefully once in distilled water and dissolved in it. When necessary, insoluble components and the remaining a. s. were removed from precipitate suspensions through low speed centrifugation and high speed centrifugation (average centrifugal force 90.000 g, 120 min), respectively. Low speed centrifugations were carried out at an average centrifugal force of 3.500g, 10 min. All the procedure except high speed centrifugation was performed at cca 22°C.

The amount of virus contained in precipitates was judged by means of infectivity test (*Nicotiana clevelandii* Gray; Fig. 1c) and by optical density of their suspensions at 260 nm wavelength. Electron microscope observation of virus particles was carried out in a Siemens Elmiskop I apparatus.

Results

To clarified leaf sap containing BMV-M isolate saturated a. s. solution was added to reach the ratio of 2:1 (v/v) and afterwards the ratios 1:1 and 1:2. This corresponded to 1/3, 1/2 and 2/3 saturation with a. s., i.e. to concentration of the salt 14.4%, 21.6% and 28.8%, respectively.

In comparison with other precipitates P3 precipitate obtained at 1/2 saturation (Table 1) was conspicuously the largest one and contained the comparatively smallest amount of insoluble substance. Indeed, fairly strong precipitation of BMV-M isolate took place already at a concentration somewhat lower than 1/2 saturation. Suspensions of the precipitates were brought to the same dilution and their equal aliquots inoculated onto the same number of *N. clevelandii* leaves. The results of two out several trials are presented in Table 1.

Fig. 1. *A* Systemic symptoms of isolate BMV—M on a leaf of *Datura stramonium*. *B* Necrotic local lesions on a *Nicotiana clevelandii* leaf after inoculation with a diluted suspension of purified BMV—M isolate. *C* Particles of BMV—M isolate in suspension of precipitate obtained from clarified sap of infected plants at 1/2 saturation with ammonium sulphate. Bar equals 75 nm.

Sl. 1. *A Datura stramonium*, list sistemično inficiran izolatom BMV—M. *B* Nekrotične lokalne lezije na listu vrste *Nicotiana clevelandii* inokuliranom razrijeđenom suspenzijom purificiranog izolata BMV—M. *C* Virusne čestice izolata BMV—M u suspenziji precipitata koji je nastao u klarificiranom soku inficirane biljke pri 1/2 zasićenosti amonijevim sulfatom. Linija u C označava 75 nm.

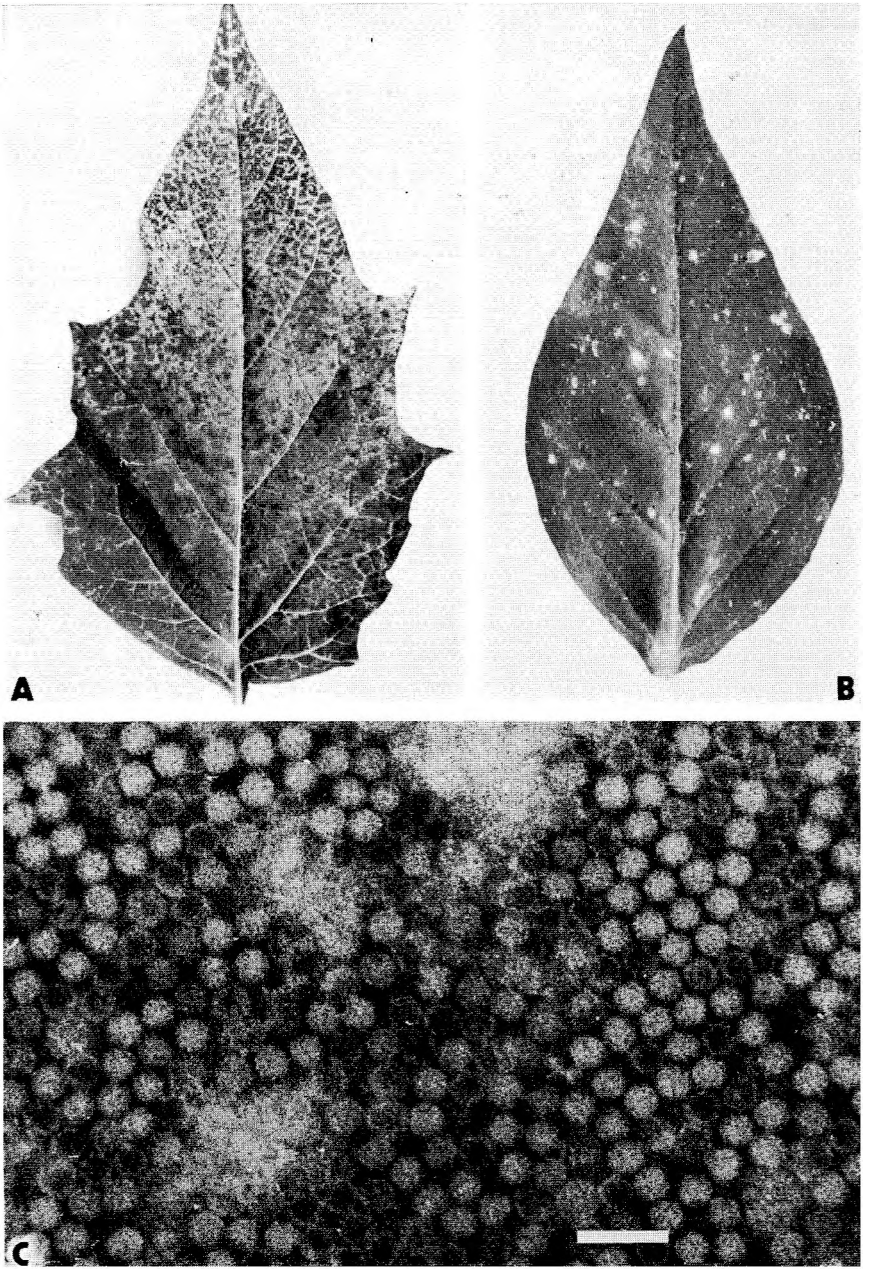

Fig. 1. — Sl. 1.

Table 1. Infectivity of precipitates containing BMV-M isolate obtained at different $(NH_4)_2SO_4$ concentrations.

Precipitate	$(NH_4)_2SO_4$ saturation	Lesion number (Infectivity index)°	
		Trial I	Trial II
P2	1/3	310 (7.7)	49 (3)
P3	1/2	1021 (25.5)	241 (15.3)
P4	2/3	82 (2)	16 (1)
S	above 2/3	40 (1)	—

° Dilution of suspensions was 10^{-3} in trial I and $2 \cdot 10^{-4}$ in trial II with respect to plant sap; S = supernatant which remained after precipitate P4 was pelleted; — = not tested.

Evidence quoted in Table 1 shows that the main infectivity was contained in precipitate P3 obtained in 1/2 saturated solution, i.e. at 21.6% salt concentration. That infectivity was about 3—5 times and 13—15 times higher than in precipitates P2 and P4, respectively. However, measurements of optical density revealed larger differences in the virus content between the precipitates. Following this, precipitate P3 (A260 = 1.50) was found to contain cca 13 times as much virus as precipitate P2 (A260 = 0.115), while virus content in P4 and S (cf. Table 1) was comparatively negligible. The UV light absorption curve of P3 suspension was typical of a nucleoprotein. The purity of the preparation (P3) was of moderate grade (A260/A280 = cca 1.50; cf. Discussion).

The suspension of P3 precipitate was examined in electron microcope, which revealed numerous apparently unaffected virus particles. The ratio of nucleoprotein to protein particles in the preparation was approximately 2.5:1 (Fig. 1c).

The efficiency of a. s. precipitation for isolating BMV-M was compared with a standard purification method for BdMV (Paul et al. 1968) which includes chloroform/n-butanol clarification and isolation of the virus by high speed centrifugation. Two equal aliquots of the same leaf sap containing BMV-M were processed by either procedure. The precipitate P3 was compared with the preparation obtained in the other procedure. It was found that A260 of P3 was usually cca 0.85 of A260 of the other preparation. Consequently, the a. s. procedure yielded about 15% less virus than the method after Paul et al.

Discussion

According to literature data turnip yellow mosaic (Markham and Smith 1949), wild cucumber mosaic (Symons et al 1963) and plantago mottle (Granett 1973) viruses were precipitated at an a. s. concentration which was equal to 1/3 saturation. On the other hand cacao yellow mosaic, CYMV, (Brunt et al. 1965) and Andean potato latent, APLV, (Gibbs et al. 1966) viruses required a somewhat higher concentration corresponding to 1/2 saturation. The present paper brings evidence that the main amount of BdMV in solution is precipitated

from it near 1/2 saturation with a. s. Therefore, with respect to that property BdMV is more similar to CYMV and APLV than to the other three above mentioned tymoviruses. Concerning that property it can be noted that the relationship among the six tymoviruses is not congruent with the serological relationship among them (cf. Koenig 1976). It may be useful here to mention that salt concentration necessary for the initial precipitation of a substance (virus) can vary somewhat, as this depends on the concentration of the substance precipitated (cf. J. Barry and E. Barry 1971).

TYMV yields constantly large isotropic octahedra crystals when precipitated with a. s. (Markham and Smith 1949; cf. Mamula 1968). Contrary to this, adequate but comparatively very small crystals were observed with the isolate BMV-M in the suspension of precipitate P3 (cf. Table 1.), however not regularly. Small isotropic octahedra crystals were found in CYMV (Brunt et al. 1965) too, the precipitations having been done from suspension of partially purified preparations.

Regarding some discrepances between the data on virus concentration obtained in the course of the present work by infectivity test and by UV light absorption measurement, it seems that higher dilutions of precipitate suspensions should have been used in infectivity test. Besides, a more suitable local lesion host for the isolate BMV-M than *N. clevelandii* (possibly *N. glutinosa)* should be used.

As a method for purification of BdMV a. s. precipitation has no special advantages over some other methods (cf. Results). However, it can be useful, because of its simplicity, especially for large scale work. Consequently two precipitations are to be done, i. e. at 1/3 (removing of some plant proteins) and at 1/2 saturation with a. s. Each may last 20—30 min as no considerable increase in the amount of precipitate occurs after a prolonged precipitation. If a purer virus preparation is needed, one or several reprecipitations at 1/2 saturation can be made.

Summary

The isolation of belladonna mottle virus (BdMV) by means of salting out with ammonium sulphate (a. s.) is reported. An isolate (BMV-M) of the virus from Yugoslavia was propagated in *Datura stramonium* and investigated. By adding saturated a. s. solution to clarified sap of infected leaves we tried to precipitate the virus and also plant proteins present in the sap. A part of proteins was precipitated from the suspension when it was 1/3 saturated with the salt. The main quantity of the virus was precipitated at salt concentration which corresponded roughly to 1/2 saturation. This was proved by measuring optical density (light wavelength 260 nm) of precipitate suspensions obtained at different a. s. concentrations and by infectivity test. With respect to the property of the precipitation BdMV shows some similarity with two other tymoviruses, i. e. with cacao yellow mosaic and Andean potato latent viruses which are precipitated from suspension at 1/2 saturation with a. s., too.

Following the treatment with a. s. infectivity as well as integrity of virus particles were preserved greatly, which was confirmed by electron microscope observation, in addition to the assaying methods quoted. Yield and purity of virus preparate obtained were of a satisfactory degree, altogether suggesting that the procedure, because of its simplicity, can be useful in the work with this virus.

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Acknowledgement. The author is thankful to Dr. N. Ljubešić, Ruđer Bošković Institute, Zagreb, for making electron micrograph, and also to Miss Lidija Zidar, for her helpful assistance in the work.

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SAŽETAK

PURIFIKACIJA VIRUSA IŠARANOSTI VELEBILJA S POMOĆU AMONIJEVA SULFATA

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U nazočnosti amonijeva sulfata (a. s.) članovi skupine timovirusa precipitiraju iz suspenzije kad stupanj zasićenosti dosegne 1/3, odnosno 1/2 u odnosu na koncentraciju zasićene otopine te soli. Istraživanja koja se iznose u ovom radu imala su za cilj da pokažu pri kojoj koncentraciji a. s. precipitira virus išaranosti velebilja (VIV), koji također pripada spomenutoj virusnoj skupini. Pokusi su bili vršeni izolatom BMV-M iz Jugoslavije koji je bio umnožen u vrsti *Datura stramonium*. Iscijeđeni sok inficiranih listova bio je djelomično pročišćen kloroformom i niskookretajnim centrifugiranjem nakon čega mu je dodavana zasićena vodena otopina a. s. Precipitati nastali pri različitim stupnjevima (1/3, 1/2 i 2/3) zasićenosti bili su u obliku suspenzija u dest. vodi ispitani u smislu optičke gustoće (UV područje) i infektivnosti.

Ustanovljeno je da se najveći dio virusa istaložio iz suspenzije kad je stupanj zasićenja a. s. dosegao približno 1/2, pa u tom pogledu VIV pokazuje veću sličnost s druga dva timovirusa, tj. s virusom žutog mozaika kakaovca i latentnim virusom andskog krumpira. Proteini i druge komponente u biljnom soku, koje su precipitirale pri istom tom kao i pri nižem (1/3) stupnju zasićenosti, uklonjene su iz virusnog preparata kao netopivi talozi. Opisanim načinom može se iz suspenzije BMV-M izolata izolirati njegov veći dio, pri čemu razmjerno čist preparat sadržava neoštećene virusne čestice. Stoga, te zbog jednostavnosti postupka, opisani način izolacije izolata BMV-M prihvatljiv je kao purifikacijski postupak za VIV ili barem za neke njegove izolate.

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