Viruses of Cucumber Isolated from Some Regions of Ukraine

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Abstract

Properties of viruses isolated from cucumbers in greenhouses of Ukraine are characterized in this article by electrophoresis, ELISA, electron microscopy, and immunoblotting.

Keywords: isolate; serological relationships; *Tobacco mosaic virus*; *Cucumber green mottle mosaic virus*

INTRODUCTION

Despite the wide spectra of viruses infecting representatives of *Cucurbitaceae* family, *Cucumber green mottle mosaic virus* (CGMMV) remains one of the most dangerous viral agents causing significant losses in cucumber greenhouse yield. CGMMV is an RNA tobamovirus related to TMV – the most studied tobamovirus – in morphology, biochemical and biophysical properties (Virus Taxonomy 2000). Regardless of general similarity in spatial organization, homology in primary structure between TMV and CGMMV makes up only 36%. Numerous CGMMV strains isolated in Europe and Asia are divided in four groups according to Okada's classification: common cucumber virus 3, cucumber virus 4, watermelon CGMMV-W, cucumber CGMMV-C (ODINTSOVA *et al*. 2000). The point of this work was to study properties of two virus isolates extracted from cucumbers of different greenhouses of Ukraine.

MATERIALS AND METHODS

Plant samples were collected by visual screening of greenhouses on presence of symptoms of viral etiology. Extraction and purification of viruses from *Cucumis sativus* was conducted following the standard technique. Virus concentration was determined spectrophotometrically. Electron microscopy was carried out on JEM-100B. 5% uranil acetate was used as a contraster. Rabbits were immunized with purified virus preparate in order to obtain specific polyclonal antiserum. Immunization was conducted in weekly three steps. Reimmunization of animals has been done in 30 days after the last immunization. Indirect ELISA was carried out on Labsystem polystirole plates. We used "Jackson Laboratories Inc" USA antirabbit antibodies conjugated with alkaline phosphatase as second antibodies. Measurements were done on "Dynatech" reader at the wavelength of 405 nm. In order to determine the molecular weight of virus protein, polyacrylamide gel electrophoresis (PAGE) by Laemmli was used with 14% gel (LAEMMLI 1970). We used protein markers "Pharmacia", Sweden. Gel was coloured with Coomassie R-250 solution ("Serva", Germany). Specificity of obtained polyclonal antiserum was proved by Southern immunoelectroblotting with nitrocellulose membrane "Schleicher & Schuel".

RESULTS AND DISCUSSION

Viruses were isolated from *Cucumis sativus* cv. Marinda plants (Mykolajiv region) – *isolate 1*, and *Cucumis sativus* cv. Bianka plants (Dniepropetrovsk region) – isolate *2* of greenhouses of Ukraine that are known for growing of cucumbers. There was similar manifestation of systemic infection in both greenhouses – leaves' mosaic, suppression of plant growth, flowers drop, fruits' deformation. (Figure 1). $E_{260/280}$ of both purified virus samples was 1.36. Electron microscopy of sap of infected plants and of purified concentrated virus preparations demonstrated presence of rod-shaped flexible structures 280–300 × 15 nm in size (Figure 2). Electrophoretic studies revealed one

Figure 1. Symptoms of cucumbers' virus infection caused by isolate 2

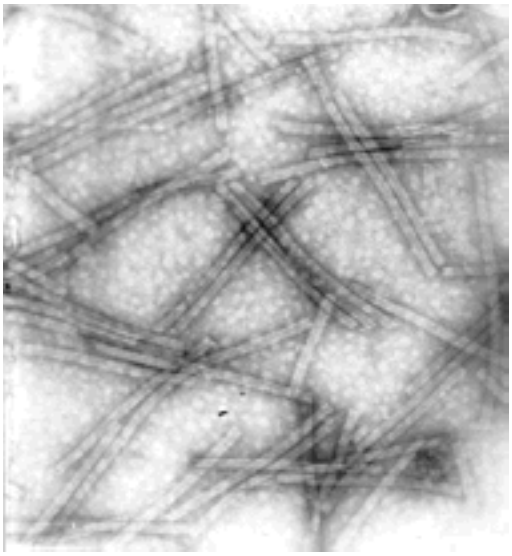
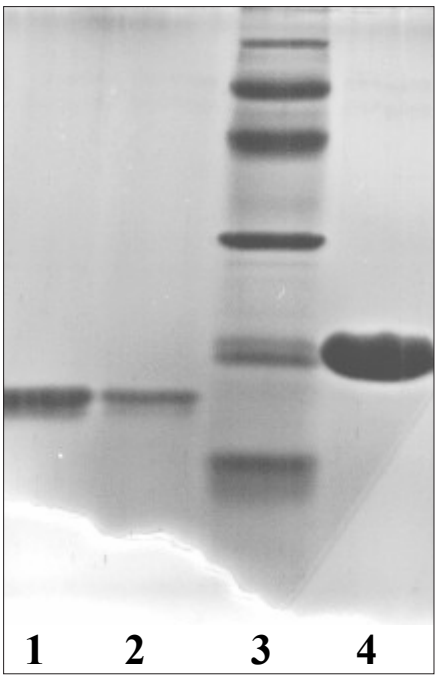


Figure 2. Electron microscopy of isolate 1 (× 80 000)

structural component with molecular weight of about 17 kD for both isolates. Indirect ELISA with specific antiserum to CGMMV (specific antiserum to CGMMV and corresponding positive control were kindly provided by Prof. Thomas Kuhne, Aschersleben, Germany) showed positive results. Basing on obtained results these viruses were classified as cucumber isolates of CGMMV – CGMMV-C. Then further researches on establishment of relationships among these isolates and typical tobamovirus – TMV – were conducted. Results of comparative electrophoresis showed that isolated viruses had less molecular weight of the protein than in case of TMV, molecular weight of the coat protein for which was around 20 kD (Figure 3). Specific antiserums were obtained to the isolates with titer 1:120 000 for following experiments. Henceforth, indirect ELISA was conducted with antiserums to the isolates, specific antiserum to CGMMV and antiserum to TMV (antiserum to TMV was given from Virology Department, Taras Shevchenko Kiev National University). ELISA results demonstrated that obtained polyclonal antiserum to the isolates reacted



1 – isolate 1*;* 2 – isolate 2*;* 3 – markers (phosphorylase B – 94 kDa; bovine serum albumin – 67 kDa; ovalbumin – 43 kDa; carbonic anhydrase – 30 kDa; soybean trypsis inhibitor – 20 kDa; alpha-lactalbumin – 14.4 kDa); 4 – TMV

Figure 3. Electrophoretic separation of coat proteins of the isolates (1, 2) and TMV (4)

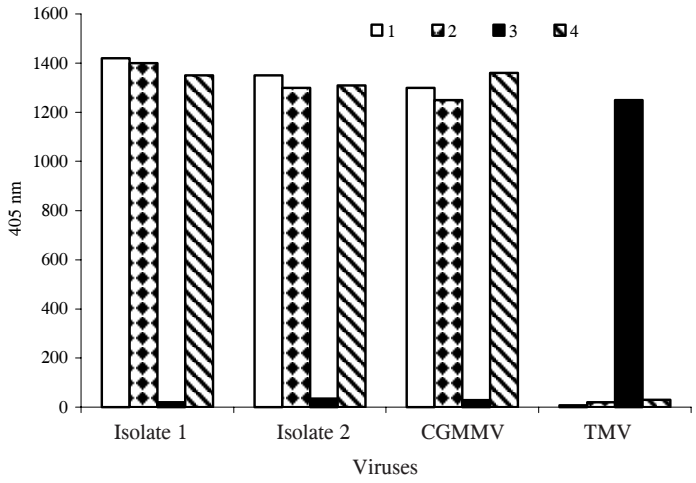


Figure 4. ELISA results. 1 – specific antiserum to isolate 1; 2 – specific antiserum to isolate 2; 3 – specific antiserum to TMV; 4 – specific antiserum to CGMMV

with positive control, specific antiserum to CGMMV reacted with both isolates when antiserum to TMV was specific only to TMV (Figure 4). These data was confirmed by results of immunoelectroblotting. These data was confirmed by results of immunoelectroblotting. These results demonstrate that isolated viruses belong to cucumber isolates of *Cucumber green mottle mosaic virus* (CGMMV-C). It has been revealed that contrary to the rigid structure of TMV, studied viruses are flexible structures according to the data of electron microscopy. Also, differences in molecular weights of coat proteins of these viruses and TMV are shown. Following the results of ELISA and immunoelectroblotting, these viruses are not related serologically to TMV.

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