A NEW BENY-LIKE SUGAR BEET VIRUS EMERGING IN THE UNITED STATES

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ABSTRACT

A virus with rigid rod-shaped particles was isolated in addition to *Beet necrotic yellow vein virus* (BNYVV) from rhizomania infested fields in California. The infected sugar beet leaves showed oak-leaf pattern symptoms different from rhizomania. For purposes of discussion this unnamed virus will be tentatively called Beet oat-leaf virus (BOLV). BOLV is serologically distinct from BNYVV, *Beet soil-borne mosaic virus* (BSBMV), and *Beet soil-borne virus* (BSBV). The host range of BOLV is similar to BNYVV and BSBMV mostly infecting Chenopodiaceous plants. BOLV produces chlorotic local lesions with a necrotic ring after mechanical inoculated to *Chenopodium quinoa*. Particles were 20 nm wide and ranged from 80 to 640 nm long with three modal lengths: 180-200 nm, 260-280 nm, and 300-320 nm. *Polymyxa betae* transmission of BOLV was demonstrated through a bioassay by using BOLV-infected cystosori and sugar beet as bait. BOLV has been purified from *Spinacia oleracea*. The molecular mass of the capsid protein was estimated to be 46.0 kDa. A polyclonal antibody from rabbits has been produced and can be used in ELISA, western blot, and immunogold labeling tests. BOLV appears to be wide spread in U.S. It has been found also in Colorado, Michigan, Minnesota, Nebraska, and Wyoming. BOLV was found in sugar beet alone or co-infected with BNYVV and/or BSBMV. The economic significance of BOLV and its interaction with other benyviruses are not known.

INTRODUCTION

During the survey for rhizomania disease, an unnamed virus showed oak-leaf pattern symptoms on sugar beet (Fig. 1) was found in California. A virus with rod-shaped particles was isolated in addition to *Beet necrotic yellow vein virus* (BNYVV), the causal agent of rhizomania. This unnamed virus of sugar beet is tentatively called Beet oak leaf virus (BOLV). BOLV and BNYVV serologically are distinct. Taproots of beets infected with BOLV often appear healthy, unlike those of beets infected with BNYVV. The objectives of this study are to determine some of the physical, biological, and serological characteristics of BOLV.

MATERIALS AND METHODS

Symptomatic field sugar beet leaves were ground in 0.1 M phosphate buffer, pH 7.0, and mechanically inoculated to *Chenopodium quinoa* Willd. Each single

local lesion was subinoculated to *C. quinoa*. The local lesions were freeze dried for virus source. In host range tests, the selected host plant species were mechanically inoculated as above.

BOLV was purified from *Spinacia oleracea*. Infected spinach plants were homogenized with two volumes of 0.1 M phosphate buffer and clarified with 1/2 volume of carbon tetrachloride. Virions were precipitated with 6% polyethylene glycol (mol. wt 6,000) and 0.2 M sodium chloride. The virions were further purified and concentrated by two cycles of differential centrifugation, followed by centrifugation through a 10-35 % sucrose density gradient. Purified virus particles were analyzed by SDS-PAGE for its coat protein.

Antiserum to the purified virions was prepared in New Zealand white rabbits. Freund's complete adjuvant and 500 µg of virus were used for the first injection and incomplete adjuvant with 250 µg of virus was used in four subsequent injections. The double antibody sandwich (DAS)-ELISA, Western blot procedure, and immunoelectron microscopy technique were conducted essentially as described in the literatures (Clark and Adams, 1977, Towbin, et al, and Lin, 1984).

BOLV infested soil or BOLV infected *Polymyxa betae* cystosori in sugar beet roots were air-dried for 3 weeks to provide inocula for transmission tests. The air-dried roots were ground to a fine powder and mixed with pasteurized potting soil. Sugar beet seeds were added to the pots and covered with pasteurized sand. The pots were kept in insect-proof greenhouse and temperature controlled at about 80 F for 40 to 50 days. Plants were then harvested, tested for BOLV using DAS-ELISA and microscopic examination for *P. betae*.

RESULTS

In host range tests, 15 species of 5 families were mechanically inoculated. *C. amaranticolor*, *C. murale*, and *C. quinoa* showed local lesions and *Beta macrocarpa*, *B. vulgaris*, *Spinacia oleracea*, *Nicotiana benthamiana* and *Tetragonia expansa* produced systemic infection.

In both soil testing and *P. betae* transmission tests sugar beet roots were positive for BOLV in ELISA tests and *P. betae* was found in the infected roots under light microscope. BOLV was recovered by mechanical inoculation to *C. quinoa* plants.

Purified virions were rigid rod-shaped particles with a central canal (Fig. 2). More than 350 virus particles were measured in the leaf dip preparations (Liu, et al, 2000). The virus particles were about 20 nm wide and of three predominant lengths, 180-200 nm, 260-280 nm, and 300-320 nm (Fig.3). The virus particles were capsided by single protein subunits of 46.0 kDa (Fig. 4). The antisera to BOLV produced from purified virions were specific to BOLV in DAS-ELISA and western blot analyses. BOLV-infected plants were successfully identified by immunogold labeling in leaf dips (Fig. 5).

Fig. 1. Beta vulgaris infected with Beet oak-leaf virus showing oak-Leaf virus showing oak-leaf Pattern symptoms.

Fig. 2. Purified Beet oak-leaf virus particles are straight, rod-shaped with a central canal. The bar represents 100 nm.

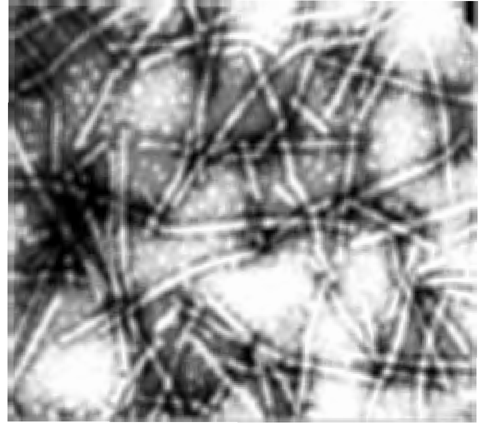


Fig. 3. Particle Length Distributions of Beet Oak-Leaf Virus in Leaf Dip Preparations

Frequency

Particle length (nm)

Fig. 4. Sodium dodecyl sulfate-polyacrylamide (12% acrylamide) slab gel showing virion capsid protein. Lane 1, molecular weight standards in order of decreasing mass: phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, B-lactoglo-bulin, and lysozyme. Lane 2, Beet oak-leaf virus.

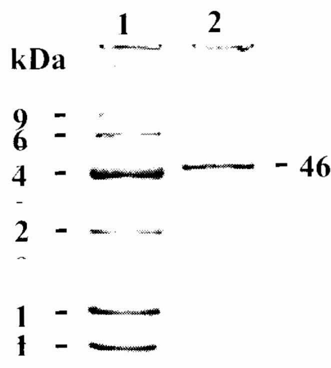
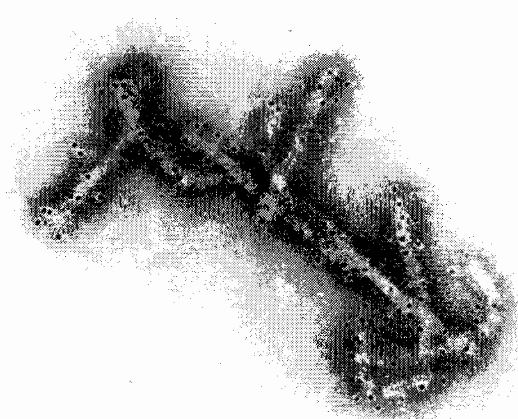


Fig. 5. Immunoelectron microscopy. BOLV-anti-BOLV followed by gold-IgG complexes, showing a direct association of virus particles and labelled gold-IgG complexes.



CONCLUSIONS

BOLV was isolated from sugar beet leaves with oak-leaf pattern symptoms from a rhizomania field in California. Like BNYVV, it causes local lesions on *C. quinoa*, but those of BOLV always had a necrotic ring surrounding the chlorotic local lesions. In the later stages, all lesions became large irregular shaped necrotic lesions. Systemic infection of *C. quinoa* were not observed. BOLV was difficult to pruify, probably because it is unstable in vitro, tends to aggregate during purification, and/or occurs within plants in low concentration; nevertheless, an antiserum was obtained with partially purified virus preparations. BOLV antiserum was specific and can be used in ELISA tests, Western blots, and immunoelectron microscopy. BOLV coat protein molecular

weight was estimated at 46.0 kDa. The reported molecular weight of BNYVV coat protein is 22 kDa and BSBMV is 24 kDa (Wisler, G. et al, 1994). BOLV was distinct from beet infecting benyviruses serologically. It was also distinct from *Beet virus Q* biologically (Koenig, R. et al, 1998), e.g. symptom expression on *C. quinoa* and systemic infection on *N. benthamiana*.

BOLV seems to be a multiparticulate virus, made up of 3 particles. The molecular weight of BOLV RNAs has not yet been determined. Whether BOLV belongs to benyvirus or other fungal-transmitted rod-shaped viruses will require additional study.

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