MOLECULAR CLONING OF THE DOUBLE-STRANDED RNAS ASSOCIATED WITH STRAWBERRY MILD YELLOW-EDGE VIRUS

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Abstract

For many virus diseases of woody hosts it has not been possible to purify virus particles for the production of antisera or the extraction of genomic viral RNA for the production of cDNA probes. Sap of strawberry leaf tissues is very viscous and difficult to work with, and the successful transmission of strawberry mild yellow-edge virus (SMYEV) to *Rubus rosaefolius* has allowed us to purify microgram quantities of SMYEV dsRNA. The dsRNA was gel-purified and then denatured with methylmercuric hydroxide. First-strand cDNA synthesis was initiated with random primers and second-strand synthesis carried out by standard methods. The double-stranded DNA was size-fractionated through a CL-4B Sepharose column and larger cDNA recovered by ethanol precipitation. After C-tailing, the DNA was annealed into G-tailed pUC9 and transformed into *Escherichia coli*. About 800 transformants were obtained and 110 colonies selected on the basis of colony hybridization for plasmid isolation and characterization. The hybridization of ^{32}P-dATP oligo-labeled cDNA clones to Northern blots of SMYEV dsRNA from strawberry demonstrates that these cDNA clones are specific for SMYEV.

1. Introduction

Strawberry mild yellow-edge virus (SMYEV) is one of the most common viruses in cultivated strawberries (Converse et al., 1987). It is transmitted by *Chaetosiphon fragaefolii* in a persistent manner and is a tentative member of the luteovirus group (Matthews et al., 1982). The yellows complex, of which SMYEV is a component, is one of the most important diseases of strawberry in many parts of the world. It is difficult to assess the economic impact of SMYEV since most commercial cultivars of strawberry grown today are symptomless when inoculated with SMYEV alone. However, SMYEV seldom occurs alone, and in combination with other viruses, such as strawberry mottle, strawberry crinkle and strawberry veinbanding viruses, SMYEV can cause severe loss of plant vigor, yield and fruit quality.

Detection of SMYEV currently is done by graft analysis or aphid transmission tests, both of which are labor intensive, slow and relatively expensive methods of virus detection. Attempts to purify SMYEV from infected strawberry and produce antiserum for

serological detection yielded antisera that are not suitable for large-scale detection due to serious background problems (Martin and Converse, 1985). Recently, it has been possible to isolate dsRNA from SMYEV-infected strawberry (Spiegel, 1987), but the dsRNA extracted is contaminated with other nucleic acids and plant components. In addition to the lack of purity, the yield of dsRNA from strawberry is quite low.

In this paper we report the purification of dsRNA from *Rubus rosaefolius* which had been graft-inoculated with SMYEV. This dsRNA has been cloned successfully into pUC9. Selected clones are able to detect SMYEV dsRNA in Northern hybridizations.

2. Materials and methods

SMYEV isolate MY-18 (Martin and Converse, 1985) was graft-inoculated into *Rubus rosaefolius* Sm., which was then backgrafted to UC-4 to confirm SMYEV infection in the *Rubus*. Infected *Rubus* plants were maintained in an insect-free greenhouse and propagated by stem cuttings. DsRNA was extracted from virus-infected *Rubus* and strawberry leaves and from Coast Black oats infected with the PAV strain of barley yellow dwarf virus by the procedure described previously (Kurppa and Martin, 1986). For strawberry tissue, the CF-11 was washed twice with STE-EtOH before loading the columns. Nucleic acids were separated on 1% agarose slab gels and stained with ethidium bromide. Denaturing agarose gel electrophoresis using methylmercuric hydroxide was done as described by Maniatis et al. (1982). To achieve highly purified dsRNA template for cDNA synthesis, samples were separated on a low-melting-temperature agarose gel and the two top bands reextracted (Maniatis et al., 1982). Generation of cDNA clones using methylmercuric hydroxide-denatured dsRNA as template was basically according to Gubler and Hoffmann (1983) and will be described in detail elsewhere. Blotting and hybridization procedures were according to Maniatis et al. (1982) and Thomas (1980). Oligo-labeled DNA probes were prepared as described by Feinberg and Vogelstein (1983).

3. Results

DsRNA extractions from SMYEV-infected *R. rosaefolius* showed four detectable bands (1, 2, 3 and 4) in agarose gel electrophoresis with sizes of 6.2, 4.3, 1.9 and 0.8 kbp in size, respectively (figure 1). When compared with SMYEV dsRNAs that were extracted from virus-infected strawberries, the same electrophoretic pattern was observed but dsRNAs extracted from *R. rosaefolius* were less contaminated with plant components. DsRNA yield was about 300 ng per 20 g of leaf tissue.

Molecular cloning of dsRNAs-1 and -2 resulted in a library of about 800 clones, of which 110 were analysed by rapid plasmid isolation and restriction enzyme digestion. Insert sizes of

these clones ranged between 400 and 3,000 bps. The usefulness of the generated clones for virus detection was demonstrated in Northern hybridizations to dsRNAs with clones p309 and p463. While clone p309 hybridized to RNAs-1 and -2, a different reaction was observed with clone p463 hybridizing to RNAs-1 and -3 (figure 2).

4. Discussion

DsRNA extraction from SMYEV-infected strawberries was first reported by Spiegel (1987). However, graft inoculation of SMYEV into *R. rosaefolius* allowed us to extract dsRNA less contaminated with plant components. Using this dsRNA, we were able to generate cDNA clones which, as was demonstrated by Northern hybridizations, have the ability to provide a rapid and sensitive in vitro test. The development of dot hybridization assays like those which have been applied to detect tobacco streak virus from total strawberry RNA (Stenger et al., 1987), strawberry vein banding virus from total strawberry DNA (Stenger et al., 1988) and arabis mosaic virus from strawberry sap (Jelkmann et al., 1988) are objectives of future studies.

Identical dsRNA profiles from SMYEV-infected strawberry and SMYEV-infected *R. rosaefolius* along with back grafting of the SMYEV-infected *R. rosaefolius* to SMYEV indicators confirm that the dsRNA extracted was that of SMYEV. The different reactions of clones p309 and p463 in Northern hybridizations indicate that these two clones cover different regions of the SMYEV genome.

References

Converse, R.H., Martin, R.R., and Spiegel, S., 1987. Strawberry mild yellow-edge. pp. 25-29 in Converse, R.H. (ed.). Virus Diseases of Small Fruits. U.S. Dept. of Agric., Agriculture Handbook no. 631. Washington D.C. 277 pp.

Feinberg, A.P., and Vogelstein, B., 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6-13.

Gubler, U., and Hoffmann, B.J., 1983. A simple and efficient method for generating cDNA libraries. Gene 25:263-269.

Jelkmann, W., Maiss, E., Breyel, E., and Casper, R., 1988. Preparation of cDNA clones and analysis of arabis mosaic virus (AMV). Ann. Appl. Biol. (in press).

Kurppa, A., and Martin, R.R., 1986. Use of double-stranded RNA for detection and identification of virus diseases of Rubus species. Acta Hortic. 186:51-62.

Maniatis, T., Fritsch, E.F., and Sambrook, J., 1982. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, New York. 545 pp.

Martin, R.R., and Converse, R.H., 1985. Purification, properties and serology of strawberry mild yellow-edge virus. Phytopathol. Z. 114:21-30.

Matthews, R.E.F., 1982. Classification and nomenclature of viruses. Intervirology 17: 23-179.
Spiegel, S., 1987. Double-stranded RNA in strawberry plants infected with strawberry mild yellow-edge virus. Phytopathology 77:1492-1494.
Stenger, D.C., Mullin, R.H., and Morris, T.J., 1987. Characterization and detection of strawberry necrotic shock isolate of tobacco streak virus. Phytopathology 77:1330-1337.
Stenger, D.C., Mullin, R.H., and Morris, T.J., 1988. Isolation, molecular cloning, and detection of strawberry vein banding virus DNA. Phytopathology 78:154-159.
Thomas, P.S., 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA 77: 5201-5205.

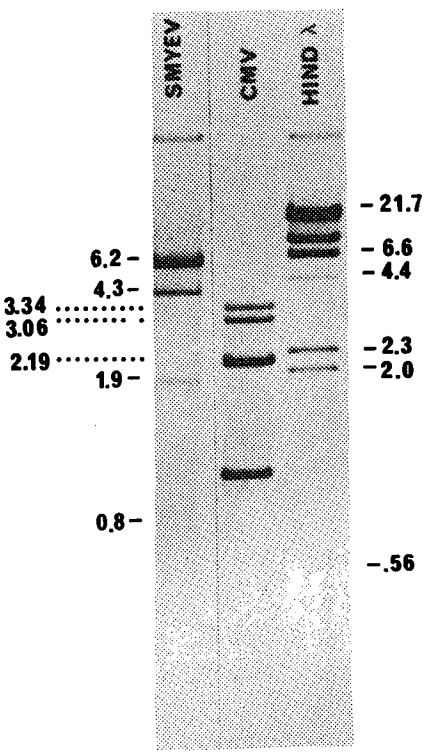

Figure 1 - Size determination of dsRNAs associated with strawberry mild yellow-edge virus (SMYEV)-infected *Rubus rosaefolius*. (Left): SMYEV dsRNA from *Rubus* (sizes in kilobase pairs given beside dashes at left); (center) cucumber mosaic virus (CMV) dsRNAs used as molecular weight markers (sizes in kilobase pairs given beside dotted lines on left); (right) lambda bacteriophage DNA cut with Hind III (sizes in kilobase pairs shown on right).

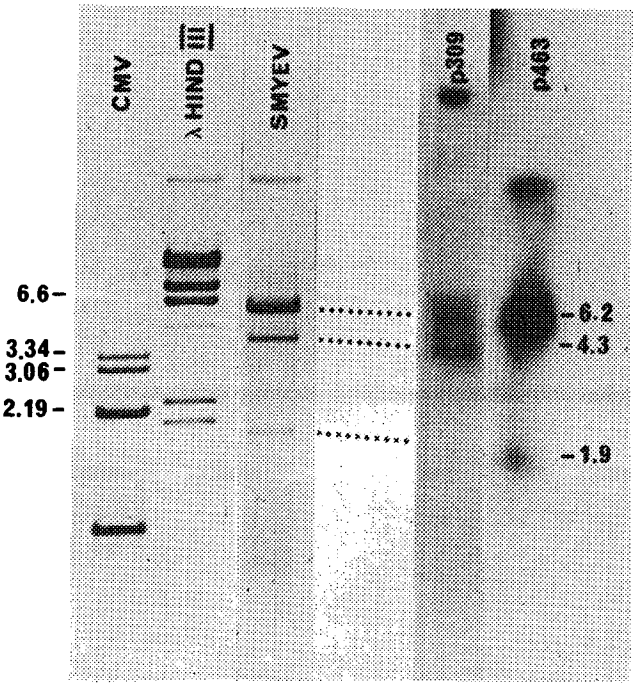

Figure 2 - DsRNA of strawberry mild-yellow edge virus (SMYEV) from *Rubus* and Northern blots of SMYEV dsRNA probed with cDNA clones. (Left to right): Cucumber mosaic virus (CMV) dsRNAs as markers (sizes in kilobase pairs shown at left); lambda bacteriophage DNA cut with Hind III (size in kilobase pairs shown at left for the third band); dsRNA from SMYEV-infected *R. rosaefolius*; Northern blots of SMYEV probed with SMYEV cDNA clones p309 and p463 respectively (values on the right are the sizes in kilobase pairs of the three upper bands of SMYEV).