

Viruses of *Spiroplasma citri* and Their Possible Effects on Pathogenicity

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Received January 4, 1983

Strains of *Spiroplasma citri* are persistently infected by viruses which have been separated into three groups on the basis of their morphology. The properties of each group are reviewed. Viruses normally only appear in spiroplasma cultures but recently all three types of particle have been identified in cells of a single strain of *S. citri* within an infected plant. Replication of a short-tailed polyhedral virus SP-V3 (*ai*) appears to be correlated with unusually mild symptom expression. Introduction of the virus with its host into plants already infected with a severe and potentially lethal strain of *S. citri* results in a marked suppression of symptoms and a reduction in the number of spiroplasmas.

One of the first features of *Spiroplasma citri* to be described [1] was its apparent infection by a long-tailed bacteriophage. Subsequently two other morphologically distinct types of virus have been shown to infect *S. citri* [2,3] (Table 1). Although virus infection of *S. citri* is common, until recently there was no evidence that any of these viruses were produced by spiroplasmas within infected plants and their significance in the pathogenicity of spiroplasmas was a matter of conjecture.

GROUP S1 SPIROPLASMA VIRUSES (SV1)

Members of this group are rod-shaped particles with a unique structure at one end by which the particles attach to host cells [4]. Multimers of the modal length are frequent. One isolate, KC3, which infects the honeybee spiroplasmas that are closely related to *S. citri*, has been partially characterized and shown to contain DNA [3]. A second isolate, SP-V3 (*aa*), from *S. citri* has been purified [5]. This virus contains DNA which is probably circular and single-stranded and has a genome size of about 4.5 Mdal [Dickinson MJ: personal communication].

Virus KC3 produces turbid plaques indicating that infected cells continue to grow but at a slower rate than uninfected cells. One-step growth curves confirm that infection is non-lytic and show that virus is released gradually over a period of hours without significant effect on host viability [3]. Virions probably assemble as they emerge through the cell membrane since no intracellular structures are apparent in infected cells. Electron microscopic observations and growth studies with *aa* indicate that continued virus production eventually causes degenerative changes in the host cell and loss of viability.

GROUP S2 SPIROPLASMA VIRUSES (SV2)

SV2 virions have only been observed in *S. citri* [1]; they have not been propagated. Virions resemble type B bacteriophage. The polyhedral head is hexagonal in outline

TABLE 1
Properties and Origins of *Spiroplasma citri* Viruses

Group	Morphology	Dimensions (nm)	Mode of Infection	Isolates*	References
SV1	Rods	230-280 × 10-15	Non-lytic	KC3 SP-V3(<i>aa</i>)	[3] [5]
SV2	Long-tailed polyhedra	48-51 × 52-58 tail 78-83 × 6-8			
SV3	Short-tailed polyhedra	37-44 × 35-37 tail 13-18 × 6-7	Lytic ^b	608 ASP-9(AV9/3) ASP-2(AV2/1) SP-V3(<i>ai</i>)	[2] [7] [7] [8,11]

* Designated according to strain of *S. citri* from which virus was isolated; laboratory designation in parentheses

^b See discussion of release of group 3 viruses.

and is attached to a long, non-contractile tail which terminates in a base plate structure. Evidence from electron microscopy suggests that the particles are released by lysis of the host cell [6].

GROUP S3 SPIROPLASMA VIRUSES (SV3)

SV3 viruses are widespread among *Spiroplasma*. Particles are polyhedral and hexagonal in outline with a short tail terminating in a base plate [6]. Two viruses of this group, 608 [2] and ASP-9 (AV9/3) [7] contain linear double-stranded DNA genomes of molecular weights between 13 Mdal and 14 Mdal but total DNA released from purified SP-V3 (*ai*) virions resolves into three bands after agarose gel electrophoresis. Evidence from DNA-DNA hybridization, restriction enzyme digests, and S_1 nuclease digestion supports the conclusion that this pattern represents a linear double-stranded molecule of molecular weight 10.3 Mdal which can circularize, due to cohesive ends, to form open circles as well as concatamers [8]. A similar pattern of DNA bands was obtained from another *S. citri* virus, AV2/1 [7].

Purified virions yield between five and seven major peptides when SDS denatured preparations are analyzed by polyacrylamide gel electrophoresis. Pattern differences suggest that there is some diversity among SV3 viruses.

Electron microscopy shows that infected cells contain large numbers of incomplete virus particles. Individual virions can be seen in the process of budding through the cell membrane, during which they acquire an envelope. This is rapidly lost and only naked virions are seen attached to host cells. This suggests that infection is non-lytic but one-step growth curves of *ai* show that 30 or more plaque-forming units (PFUs) are rapidly released about seven hours after infection. Premature lysis experiments show that infectious intracellular virus is present after five hours and this may be released by budding until the membrane becomes so weakened that cell lysis occurs and the remaining particles are released.

Plaques produced by 608 on indicator-lawns of *S. citri* are clear [2] but *ai* gives rise to a variety of different plaques, ranging from clear to turbid depending on the indicator strain and the lawn density. The most common plaque type, formed on lawns of *S. citri* strains SP-A or ASP-1, has a small clear center surrounded by a wide zone of increased turbidity which reflects the growth of virus-resistant cells (Fig. 1). Extended incubation can result in the formation of concentric rings of in-

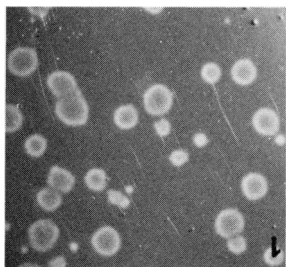


FIG. 1. Plaques formed by SP-V3 (*ai*) on a lawn of *S. citri* SP-A.

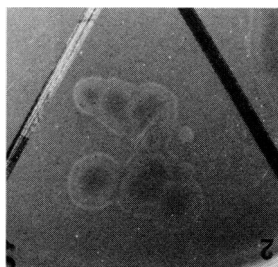


FIG. 2. Concentric ring plaques formed by SP-V3 (*ai*) on a lawn of *S. citri* SP-A.

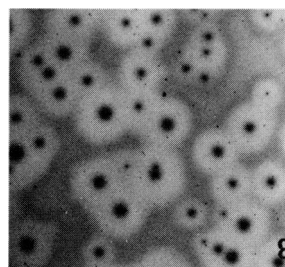


FIG. 3. Colony-centered plaques formed by *S. citri* SP-A resistant to SP-V3 (*ai*) overlaid with SP-A.

creased turbidity (Fig. 2). Electron microscopy has confirmed that very few cells in the turbid areas are infected with virus. Such plaques are similar to those produced by temperate bacteriophages [9]. Infection of the bacteria at low multiplicities more often results in a lytic response, thus accounting for the clear center to the plaque, while at high multiplicities of infection, lysogeny is the more frequent outcome. So, as the concentration of virus in the plaque increases, cells will become lysogenized and consequently resistant to further infection. A few of these cells release low numbers of virus particles which cause a second zone of lysis.

Infection of SP-A or ASP-1 cultures with *ai* is characterized by cellular deformation and a rapid decline in host viability. However, if incubation is continued the titer of viable spiroplasmas begins to rise, presumably reflecting the growth of virus-resistant cells. If SP-A cells are spread on agar layers containing *ai*, colonies of resistant spiroplasmas arise at a frequency of about 1×10^{-5} . After repeated subculture in *ai*-specific antiserum these clones are still resistant to superinfection and liberate virus as demonstrated by their capacity to give rise to colony-centered plaques when overlaid with SP-A host cells (Fig. 3). This preliminary evidence together with the plaque morphology and the ability of the genome to circularize suggests that this virus might be a temperate phage, albeit with a low efficiency of lysogenization of *S. citri*.

OCCURRENCE

Cole et al. [4] examined 23 different isolates of *S. citri* and identified virus particles in 16 of them. Some isolates contained all three types of virus. Stephens [7] found that of supernatants from 22 different strains of *S. citri*, 11 formed plaques on a combination of different lawns, but no attempt to characterize the infectious agents was made. Several of the virus-producing strains of *S. citri* were in their second or third subculture after isolation from diseased plants or insects; therefore it is surprising that, until very recently, there was only one report of spiroplasma viruses occurring in an *S. citri*-infected host [10]. In that instance, particles resembling an SV2 virus were observed in association with spiroplasmas within the salivary glands of leafhoppers rendered infective by microinjection with cultured *S. citri* (SP-A). Even then there was no evidence that plants subsequently infected by such insects contained virus.

EVIDENCE THAT VIRUS INFECTION CAN AFFECT PATHOGENICITY

Alivizatos et al. [11] have reported the observation of an SV3-type virus, SP-V3 (*ai*), apparently multiplying in spiroplasma cells within a periwinkle (*Catharanthus*

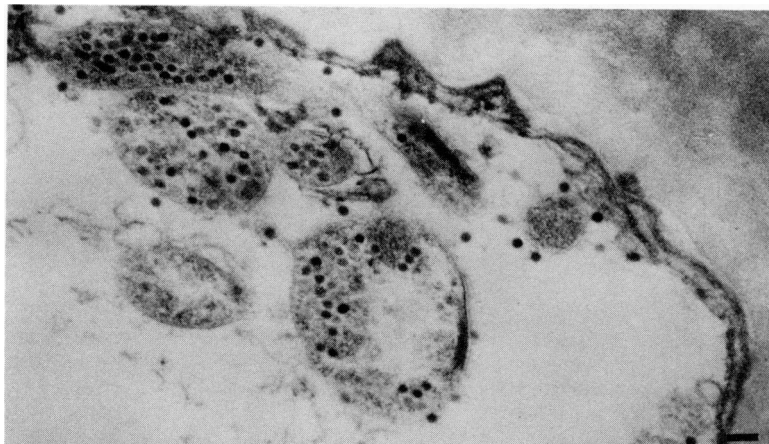


FIG. 4. Section of *C. roseus* infected with *S. citri* SP-V3 showing the presence of SV3-type virus. Bar represents 100 nm. Courtesy of A.S. Alivizatos.

roseus) infected with *S. citri* (SP-V3) (Fig. 4). All stages of virus replication were observed, including the release of enveloped virions, and sap extracts from the plant contained up to 1×10^6 PFUs per gram of tissue. Normally symptoms of *S. citri* (SP-A) in periwinkle at 30°C are severe and invariably lethal in eight to ten weeks, but symptoms produced by SP-V3 were very mild even at 30°C. This plant, and four others infected by grafting with SP-V3 tissue, developed only mild symptoms and have continued to survive and flower.

The authors obtained further evidence that mild symptoms were a consequence of virus infection from a series of dual-infection experiments, the results of which are summarized in Table 2. Ten plants were grafted with the lethal strain SP-A and the infection was allowed to establish for 15 days, by which time the first symptoms had become apparent; five were then grafted with tissue carrying SP-V3. All control plants which did not receive the second graft developed severe symptoms and died within 12 weeks. No virus was isolated from them. All dually infected plants developed only mild symptoms and yielded high concentrations of SV3-type virus. They contained far fewer spiroplasmas than plants infected with SP-A alone, but primary isolates derived from these surviving organisms were resistant to infection with *ai* and may therefore have been lysogens.

The authors emphasized that these results did not exclude the possibility that symptom reduction in dually infected plants was a consequence of competition between the two strains of *S. citri* and was unrelated to virus infection. However, it is difficult to conceive of a situation in which a small inoculum of SP-V3 could com-

TABLE 2
Concentrations of Virus and Spiroplasmas in Plants Infected with a Normal Strain of *S. citri* (SP-A) and a Virus-Producing Strain (SP-V3)^a

Plants Grafted With	Concentration of Virus (PFU/g Tissue)	Concentration of Spiroplasmas (CFU/g Tissue)
Healthy	0	0
SP-A	0	5×10^6
SP-A + SP-V3	1×10^8	2×10^3

^aFrom [11]

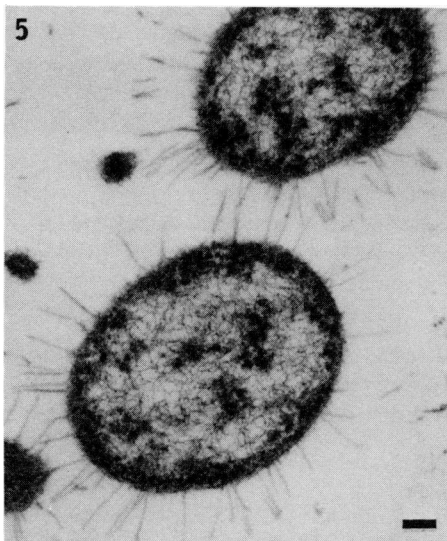


FIG. 5. As Fig. 4, but showing the presence of SV1-type virus. Bar represents 100 nm. Courtesy of P.G. Markham and K.A. Plaskitt.

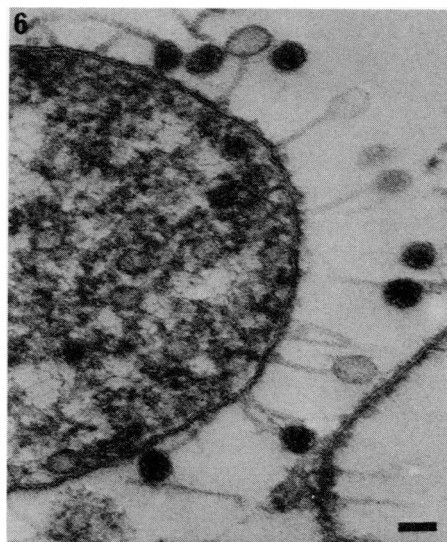


FIG. 6. As Fig. 4, but showing the presence of SV2-type virus. Bar represents 50 nm. Courtesy of P.G. Markham and K.A. Plaskitt.

pete with a well-established infection unless the greater part of the SP-A population was rapidly killed as a consequence of virus infection.

Plants infected with *S. citri* SP-V3 continued to survive for more than two years without significant change in symptom severity, but the occurrence of spiroplasma cells apparently supporting replication of SV3-type virus as demonstrated by electron microscopy declined considerably [12]. In view of the preliminary evidence that *ai* can lysogenize its host, the interesting possibility arises that lysogenization could result in a phenotypic conversion affecting phytopathogenicity of the host spiroplasma.

A second unusual feature of these plants was the subsequent appearance of spiroplasma cells apparently supporting replication of an SV1-type virus, SP-V3 (*aa*) (Fig. 5), and in a single instance also an SV2-like virus (Fig. 6) [5,13]. In view of the non-lytic nature of SV1 infections, it is possible that this virus could establish a persistent infection of SP-V3 which might slow the pathogen's growth and result in amelioration of symptoms. However, there was no evidence of SV1-type virus production during the experiments reported by Alivizatos et al. [11]. A further possibility is that replication of the SV1-type virus is correlated with persistent infection by *ai*.

Although these observations suggest that the situation may be more complex than was at first envisaged, they do not invalidate the basic premise that virus infections are an important factor in the symptomatology of *S. citri* infections.

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