SYSTEMIC VIRAL MATERIAL IN THE CELLS OF THE FRESHWATER RED ALGA SIRODOTIA TENUISSIMA (HOLDEN) SKUJA

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SUMMARY
A cytoplasmic inclusion consisting of polygonal viral particles surrounding a spherical viroplasm is described in the cells of *Sirodotia tenuissima* but not in other species of *Sirodotia* and *Batrachospernum* investigated. Some polygonal viral particles are also found associated with nucleolar material in the nucleus of *S. tenuissima*. The viral material is present in the apical cell and appears to be distributed to the daughter cells during division of the apical cell.

INTRODUCTION
While various types of viral particles have been described in the blue-green algae (Safferman & Morris, 1963; Ueda, 1965; Smith, Brown, Goldstein & Walne, 1966; Safferman, Morris, Sherman & Haselkorn, 1969; Jensen & Bowen, 1970) there have been no reports of any such viral particles in eucaryotic algal cells. The following investigation describes an association of viral particles with one species of the freshwater red alga *Sirodotia*.

MATERIALS AND METHODS
The following algal isolates were obtained from the Culture Collection of Algae at Indiana University (Starr, 1966); *Sirodotia suecica* Kylin (LB 1497), *S. suecica* Kylin (LB 1498), *S. tenuissima* (Holden) Skuja (LB 1499), *S. sp.* (LB 1500) and *Batrachospermum moniliforme* Kylin (LB 1493). All of the algae were maintained in Bold's 3N Bristols (Starr, 1966) in either a 12 h light-12 h dark cycle at 15 °C or a 16 h light-8 h dark cycle at 20 °C. The illumination was provided by two 20-W daylight fluorescent bulbs at an intensity of 300-500 ft. cand. (1029-1715lx).

The organisms were fixed in either (1) a solution of 3 % glutaraldehyde in 0.025 M phosphate buffer pH 7.2 at room temperature followed by a wash in 0.1 M phosphate buffer pH 7.2 and post-osmication in 1 % OsO4 in 0.1 M phosphate buffer for 1 h at room temperature; or (2) in 1 % OsO4 in 0.1 M phosphate buffer for 1 h at room temperature. After fixation the algae were washed twice in distilled water and dehydrated in a standard ethanol series to 100 % ethanol. The material was then placed in three 1-h changes of 100 % ethanol and a 1-h and overnight change of Spurr's (1969) 'soft' resin plus accelerator. The specimens were embedded in the last change of resin at 60 °C. The trimmed blocks were sectioned with glass knives on a Reichert Om U2 ultramicrotome. After staining with 2 % uranyl acetate and Reynold's (1963) lead citrate the material was observed in a Zeiss EM 9A electron microscope.

The cells were fragmented by blending them in a Virtis homogenizer at full speed for 1 min

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followed by sonication in a Branson Sonicator at 5 A for three 1-min intervals. The vessels containing the samples were packed in ice in both fragmentation procedures. The fragmented cells were then dried on 200-mesh Formvar-covered grids, stained with 2% uranyl acetate and observed in the electron microscope.

RESULTS AND DISCUSSION

The cells of one of the organisms investigated, *Sirodotia tenuissima*, were found to have an unusual inclusion in their cytoplasm. This inclusion consisted of a central electron-dense body 1.0-1.5 μm in diameter (Figs. 2-6) which had an electron-transparent area near the centre (Fig. 3). This body resembles the viroplasm or viral organizing areas found in maize rough dwarf virus (Gerola & Bassi, 1966) and in wound tumour virus (Shikata & Maramorosch, 1967). Surrounding the viroplasm are many 50-60 nm polygonal bodies that are found in crystalline arrays (Figs. 1-4, 7) which resemble virus particles found in many higher plant cells (Esau, 1968). In the mature cells the viroplasm with its associated viral particles is in close proximity to the nucleus (Figs. 1-4) which in turn has a few virus particles around the periphery of the nucleolus (Figs. 1, 2, 5). The viral material has the same appearance in the cells of the filamentous *Chantransia* phase (Fig. 4) and in the lateral (Figs. 1-3) and axis cells (Fig. 5) of the thallus stage.

*Sirodotia* is an organism which grows by means of an apical cell and the viral material in the cells of the organism is apparently derived from the viroplasm of the apical cell. The apical cells contain an inclusion of viroplasm about the same size as that in the mature cells but which has only a few viral particles around its periphery (Figs. 5, 6). The apical cell also has polygonal viral particles associated with the nucleolus. The cells that have been recently cut off from the apical cell typically have a nucleus on one side of the cell and the mass of viroplasm on the other (Fig. 5). As the cells mature more polygonal virus particles are found surrounding the viroplasm (Figs. 1-4).

The other species of *Sirodotia* investigated as well as a member of the other genus of the Batrachospermaceae, *Batrachospermum moniliforme*, did not contain any of the viral material. In order to see if the viral material in *Sirodotia tenuissima* was acquired while I had it in culture in my laboratory a second sample was obtained from the culture collection and the alga was fixed upon receipt of the material. This sample also contained the viral material. The viral-containing *S. tenuissima* was cultured in flasks that contained the uninfected species of *Sirodotia* and *Batrachospermum* to see whether the viral material could infect them. After a month of such culture no evidence of any transmittance of the viral material could be detected at the ultrastructural level.

After disruption of the cells by blending and sonication the viral particles still maintained their basic polygonal shape (Fig. 7, inset).
REFERENCES


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The material in all the micrographs was fixed in the glutaraldehyde-osmium mixture with the exception of Fig. 2 which was fixed only in the osmium mixture. The line at the bottom right of each micrograph represents 1 μm.

Fig. 1. A section of a lateral cell of *Sirodotia tenuissima*. Viral particles (v) can be seen adjacent to the nucleus. A single viral particle (arrow) is near the nucleolus. × 16000.

Fig. 2. A micrograph of a lateral cell of *S. tenuissima* showing viroplasm (vp) surrounded by viral particles (v). Viral particles (arrows) can also be seen in the nucleus. × 15000.
Viral material in Sirodotia
Fig. 3. Lateral cells of *S. tenuissima*. An electron-transparent vacuole can be seen in the viroplasm (vp). \( \times 14,000 \).

Fig. 4. A cell of the filamentous *Chantransia* stage of *S. tenuissima*. Viroplasm (vp) and viral particles (v) can be seen in the cytoplasm. \( \times 14,500 \).

Fig. 5. Cells that have been recently cut off from the apical cell. Viroplasm (vp) and viral particles can be seen in the cytoplasm and viral particles (arrows) can be seen in the nucleolar material. \( \times 12,000 \).
Viral material in Sirodotia
Fig. 6. Micrograph of the apical cell (left) and the sub-apical cells of the thallus stage of *S. tenuissima*. Viroplasm (*vp*) and a few viral particles can be seen in the apical cells as well as in one of the sub-apical cells. $\times 12,500$.

Fig. 7. Section of the viral particles of *S. tenuissima*. Inset at the upper left shows viral particles isolated from the cells and stained with uranyl acetate. $\times 130,000$. 
Viral material in Sirodotia