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# Characterization of a new picorna-like virus, himetobi P virus, in planthoppers

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Picorna-like virus particles, 29 nm in diameter, were purified from apparently healthy *Laodelphax striatellus* Fallen. The virus particles had a buoyant density of 1·352 g/ml in CsCl and a sedimentation coefficient of 161 *s*. The virus capsid proteins consisted of three major polypeptides of $M_r$s 36500, 33000 and 28000, and three minor polypeptides. The virus contained a major ssRNA of $M_r$ $2{\cdot}8\times10^6$ and was also frequently associated with a minor dsRNA of $M_r$ $4\times10^6$. The 3′ end of the ssRNA had a poly(A) tract of about 60 adenine residues. The virus has been provisionally named himetobi P virus.

The small brown planthopper, *Laodelphax striatellus* (Fallen) is an important vector of virus diseases of graminaceous plants. It is the most important active vector of rice stripe tenuivirus (RSV) diseases in the rice-growing areas of Japan (Toriyama, 1983; Conti, 1985; Kisimoto & Yamada, 1986).

An isometric virus was detected while attempting to purify RSV from its planthopper vector, *L. striatellus*. The virus was detected, using an ELISA, in three planthopper species from Japan. High incidences of infection also occurred in some laboratory cultures of *L. striatellus*, *Sogatella furcifera* (Horv.) and *Nilaparvata lugens* (Stal.) (Toriyama *et al.*, 1991; Guy *et al.*, 1992). In the field, the virus was detected at a low incidence in these planthopper species. Here we report the characteristics of the virus from *L. striatellus*.

Small brown planthoppers were reared continuously on rice seedlings under laboratory conditions (courtesy of Dr T. Matsui of Agriculture Research Center, Tsukuba, Japan). Insects were harvested at the late adult growth stage and were stored frozen at −20 °C until needed. Virus purification followed, with slight modification, the procedure for RSV (Toriyama & Watanabe, 1989). One to 8 g of frozen planthoppers was macerated with 100 ml of extraction solution (100 mM-$Na_2HPO_4$, 10 mM-sodium diethyldithiocarbamate, ascorbic acid added to give pH 7·2) and clarified using Difron S-3 (40 ml, 28% final volume; Daikin). After a cycle of 4200 *g* and 123000 *g* centrifugation, the pellets were resuspended in 10 mM-potassium phosphate buffer pH 7·5. The preparation was centrifuged in 10 to 40% linear sucrose gradients. Contaminating host components were completely separated and removed by rate-zonal centrifugation in 1·4 M-$Cs_2SO_4$ for 16 h at 100000 *g* at 10 °C. The buoyant density of the virus particles was determined as described previously (Toriyama *et al.*, 1983). The virus preparations were centrifuged on CsCl gradients for 70 h at 90000 *g*. The sedimentation coefficient of the virus ($A_{260}$, 0·4 to 0·8) was measured with an Analytical Ultracentrifuge System (Absorption Scanner ABS 8, Hitachi). The $M_r$ values of viral capsid proteins were determined in 10% SDS–polyacrylamide gels (Laemmli, 1970) and 8 to 16% linear gradient SDS–polyacrylamide gels. Virus nucleic acid was characterized as described previously (Toriyama & Watanabe, 1989) and the 3′-terminal sequence of the viral RNA was determined by two-dimensional shift analysis and sequencing with an RNase sequencing kit (Pharmacia) (Wengler *et al.*, 1982; Takahashi *et al.*, 1990).

Double diffusion tests in 1% agar were done using the virus from *L. striatellus* and antisera to *Rhopalosiphum padi* virus (courtesy of Dr C. D'Arcy), sacbrood picornavirus (Dr D. Anderson), infectious flacherie virus (Dr M. Nagata) and flock house nodavirus (Dr P. Scotti). There were no serological reactions between these antisera and the virus from *L. striatellus*, but an antiserum produced against this virus had a reciprocal homologous titre of 4096.

Viral preparations formed a sharp band on $Cs_2SO_4$ gradients which contained homogeneous isometric particles approximately 29 nm (28 to 30 nm) in diameter (Fig. 1). The virus yield was 20 to 50 μg/g of planthoppers. The buoyant density of the virus particles

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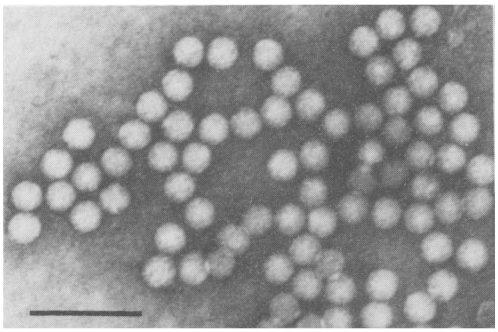

Fig. 1. Virus particles purified from *L. striatellus* and stained with 2% phosphotungstate. Bar marker represents 100 nm.

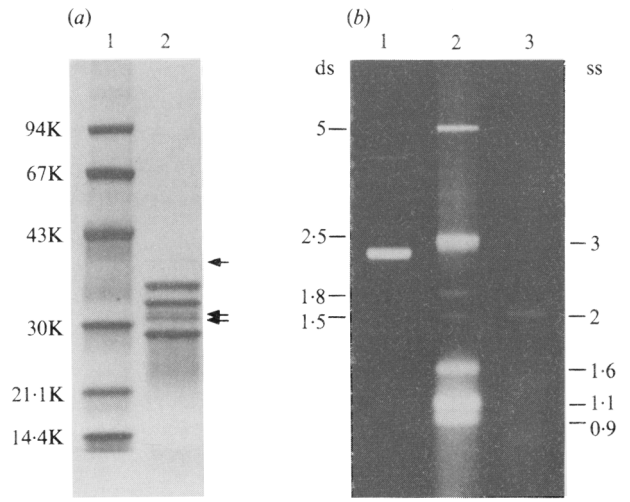


Fig. 2. (*a*) Analysis of viral proteins of *L. striatellus*, subjected to electrophoresis through an 8 to 16% linear SDS–polyacrylamide gel (Funakoshi Ltd) and stained with Coomassie blue. Lane 1, marker proteins (Pharmacia), from upper band: phosphorylase b (94K), albumin (67K), ovalbumin (43K), carbonic anhydrase (30K), trypsin inhibitor (21·1K) and lactalbumin (14·4K). Lane 2, capsid proteins of the virus. Arrows show the positions of the minor proteins. (*b*). Nucleic acid extracted from the virus, subjected to electrophoresis through a 1% agarose gel and stained with ethidium bromide. Lane 1 (from top), minor dsRNA and major ssRNA. Lanes 2 and 3, ss- and dsRNAs for $M_r$ markers. Position and $M_r$ ($\times 10^{-6}$) of RNA markers are shown on the right (ssRNAs) and left (dsRNAs); these were ssRNAs of RSV (3, 1·6, 1·1 and 0·9), tobacco mosaic virus (OM strain) (2) and dsRNAs of RSV (5, 2·5, 1·8 and 1·5) (Toriyama & Watanabe, 1989).

was 1·352 ± 2 g/ml and the sedimentation coefficient was estimated to be 161 *s* (160 to 162 *s*). As shown in Fig. 2(*a*), the three major capsid proteins, $M_r$ 36500, $M_r$ 33000 and $M_r$ 28000, had similar staining density ratios. Another three minor components with $M_r$s of 41500, 31500 and 31000 were always associated with the viral preparations. In some insect picornaviruses, a minor protein component of $M_r$ 36500, which is assumed to correspond to VP0 of mammalian picornaviruses, is present (Moore

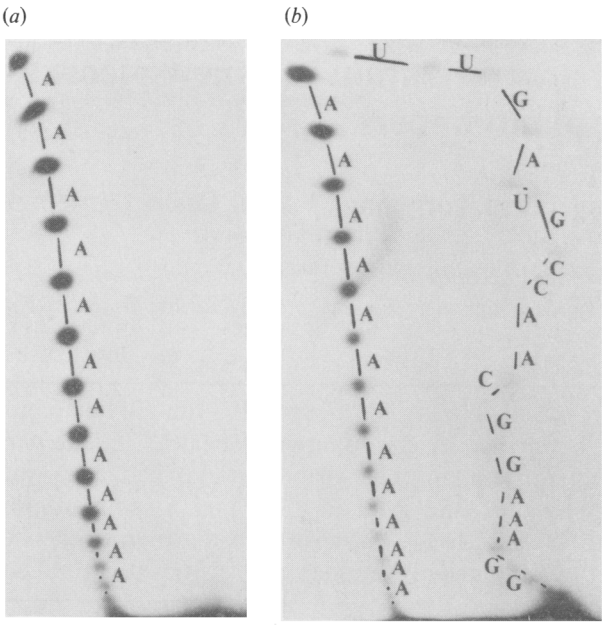


Fig. 3. Autoradiograms of wandering spots of (*a*) ss- and (*b*) dsRNAs of the virus from *L. striatellus*. The 3′ end of the RNA was labelled with 5′ [$^{32}$P]pCp using T4 RNA ligase and subjected to electrophoresis through a 1% agarose gel. Each band of ss- and dsRNA was cut out, RNA was electrophoretically eluted and this was followed by two-dimensional shift analysis (Takahashi *et al.*, 1990). The line of wandering spots on the right side of (*b*) was more visible after longer exposure.

*et al.*, 1985). However, the origin and function of the other two minor components are unclear.

Electrophoresis of nucleic acid extracts in 1% agarose gels revealed two components, although the amount of the slower migrating component varied in different viral preparations (Fig. 2*b*). DNase treatment (DNase 1, RNase-free; Boehringer Mannheim) did not digest either component, whereas λ phage DNA (*Hin*dIII digest) was completely digested under these experimental conditions. The faster migrating major component was digested by RNase A under high and low salt (0·3 M- and 0·03 M-NaCl) conditions whereas the slower migrating minor component was resistant to RNase A in 0·3 M-NaCl. This indicated that the major component was ssRNA ($M_r$ 2·8 × 10$^6$) and that the minor component was dsRNA ($M_r$ 4 × 10$^6$). Most of the 3′ $^{32}$P-labelled viral RNA was bound to an oligo(dT)–cellulose column (Type 7, Pharmacia) and eluted with the appropriate elution buffer, showing that the RNA contained a poly(A) tract (data not shown). This was confirmed by the two-dimensional shift analysis. The terminal nucleotide of ssRNA was adenine (A) and the wandering spots in Fig. 3(*a*) resolved an ssRNA 3′-terminal tract of 14 A residues. A tract of approximately 60 A residues was confirmed by RNA sequencing

(RNase kit, Pharmacia), suggesting a poly(A) tract of variable length. The 3′ end of the dsRNA was also labelled and a similar poly(A) tract was determined (Fig. 3*b*). An additional line of poorly labelled wandering spots was observed in the dsRNA after long exposure (Fig. 3*b*, right side). A similar wandering spot pattern has been reported in the replicative form of Sindbis virus RNA with a poly(A) tract (Wengler *et al.*, 1982). The terminal nucleotides of the dsRNA were A (major component) and uridine (U) (minor component). The terminal nucleotide U may correspond to the poorly labelled 3′ end sequence which is complementary to the 5′ end of the RNA molecule with the 3′ end poly(A) tract of dsRNA (Fig. 3*b*).

As the dsRNA is found in highly purified viral preparations, it seems unlikely to be a replicative form RNA. It is similar to the dsRNA that was found in RSV ss- and dsRNAs (Toriyama & Watanabe, 1989).

The characteristics of the viral capsid proteins and the ssRNA, and physical properties of the viral particles suggest that the virus of small brown planthoppers may belong to the Picornaviridae (Francki *et al.*, 1991; Moore *et al.*, 1985, 1987; Williamson *et al.*, 1988). No serological relationships were found between the picorna-like virus of *L. striatellus*, the aphid virus of *R. padi*, infectious flacherie virus or sacbrood virus. Cricket paralysis and *Drosophila* C picornaviruses are serologically related to a number of other insect picornaviruses. Unfortunately antisera to these viruses were unavailable. The capsid proteins of *Drosophila* C virus and cricket paralysis virus (Victoria isolate) include a small protein with an $M_r$ of approximately 8000 (Jousset *et al.*, 1977; Scotti *et al.*, 1981), which was not observed in the picorna-like virus of planthoppers; however the presence of a minor dsRNA component seems to be a characteristic of this virus. We named the new picorna-like virus of planthoppers himetobi P virus after the Japanese name for the small brown planthopper.

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