Pertanika 8(1), 73 – 77 (1985)

# Some properties of Potato Virus M (PVM) in Crude Sap and in Pure Preparations

ISMAIL AHMAD, R. STACE-SMITH* and N.S. WRIGHT*
*Department of Microbiology,*
*Faculty of Life Sciences,*
*Universiti Kebangsaan Malaysia,*
*Bangi, Selangor, Malaysia.*

**Key words: Potato Virus M; crude sap; pure preparations.**

## ABSTRAK

*Satu kajian telah dilakukan untuk menentukan beberapa ciri virus M (PVM) kentang dalam sap kasar dan dalam sediaan tulen. Dalam sap kasar virus tersebut telah ditunjukkan mempunyai nilai, titik pencairan terakhir sebanyak* $10^{-4}$, *pemandirian* in vitro *selama 3 hari, dan titik penyanaktifan terma setinggi 65 – 70°C. Zarah-zarah virus dalam sediaan tulen mendap sebagai satu puncak mempunyai pekali pemendapan bernilai 162 s. Ketumpatan apungan dalam sesium klorida adalah 1.304 g/cm*$^3$. *Elektroforesis dalam gel poliakrilamid menunjukkan virus tersebut mengandungi hanya satu subunit protein yang mempunyai berat molekul setinggi 39,300 dalton.*

## ABSTRACT

*A study was performed to determine some properties of the potato virus M (PVM) in crude sap and in pure preparations. In crude sap the virus was shown to have a dilution end point value of* $10^{-4}$, *longevity* in vitro *particles sedimented as one peak with a sedimentation coefficient value of 162 s. The buoyant density in cesium chloride was 1.304 gm/cm*$^3$. *Electrophoresis in polyacrylamide gels showed that the virus possessed only one protein subunit with a molecular weight of 39,300 daltons.*

## INTRODUCTION

A virus identified as potato virus M (PVM) was isolated in British Columbia, Canada, and studies on host range (Ahmad *et al.*, 1978) and purification (Ahmad, 1984) have been carried out. Some properties of the virus in crude sap and in pure preparations, albeit using different isolates, have been reported by several workers notably Bagnall *et al.* (1956), Hiruki (1973) and more recently Tavantzis (1982) and Ahmad (1984) (Table 1). However, the virus remains as one of the least characterised carlaviruses. Hence, this study aims to report some properties of the virus in crude sap and in pure preparations.

## MATERIALS AND METHODS

### *Viruses*

The PVM isolate used in this study was similarly maintained and purified as previously reported (Ahmad *et al.*, 1978; Ahmad, 1984). Purified $U_1$ strains of tobacco mosaic virus (TMV) for the centrifugation experiment was kindly provided by Dr. J.A. Dodds.*

*Canada Agriculture Research Station, Vancouver, Canada.
*(Former post-doctoral fellow, Canada Agriculture Research Station, Vancouver, Canada).

TABLE 1
Some properties of potato virus M (PVM) in crude sap and in pure preparation

| Properties | Reported values[1] |
|---|---|
| Particle length (nm) | 645 (g)[2], 650 (c), 651 (a), 660 (f) |
| Particle width (nm) | 13 (a, g) |
| Longevity *in vitro* (days) | 2 (b,c,d,e), 3 (j) |
| Thermal inactivation point (C) | 60 – 65 (h), 65 – 70 (b,c,e,j), 68 – 71 (i) |
| Dilution end point | $10^{-3}$ (e), $10^{-4}$ (d,h,j) |
| $A_{max}$ (nm) | 260 (a,g) |
| $A_{min}$ (nm) | 245 (a,g) |
| $A_{max}/A_{min}$ | 1.21 (a,g), 1.23 (a) |
| Sedimentation coefficient | 162 S (j)[3] |
| Buoyant density (g/cm$^3$) | 1.304 (j)[3] |
| Protein subunit molecular weight (daltons) | 39,300 (j)[3] |
| Estimated RNA content in virion (%) | 5 – 6 (g), 6 (j) |

[1]Selected references only.

[2]Sources of data: (a) Ahmad, 1984; (b) Bagnall *et al.*, 1956; (c) Bagnall and Larson, 1957; (d) Hiruki, 1973; (e) Hario *et al.*, 1969; (f) Rozendaal and van Slogteren, 1957; (g) Tavantzis, 1982; (h) Tu and Hiruki, 1970; (i) Wetter and Brandes, 1956; (j) present study.

[3]Not previously reported.

*Physical Properties* in vitro

The dilution end point (DEP), longevity *in vitro* (LIV), and thermal inactivation point (TIP) were the properties of virus in crude sap being determined. Crude sap used for determing these properties was extracted from infected potato (*Solanum tuberosum* L. designated Banana selection) or tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) plants. Extraction was achieved by macerating leaves of either plant in a mortar and then filtering it through muslin cloth.

The determination of DEP was performed with crude sap which has been diluted in 0.005 M borate buffer, pH 7.8, to $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, or $10^{-6}$ of the original concentration. The determination of TIP was carried out as follows: Two milliliters of crude sap were placed in thin-walled serological test-tubes (75 × 10 mm) which were then incubated for 10 minutes in a water bath set at 30 C, 40 C, 50 C, 60 C, 65 C, 70 C, 75 C or 80 C. After 10 minutes the sap was immediately cooled by plunging the test-tubes into a beaker of ice for 30 seconds. In the determination of LIV, crude sap was incubated at room temperature for 1, 2, 3, 4, or 5 days.

The infectivity of the virus following each treatment was assayed onto the primary leaves of 10-12-day-old Red Kedney been plants (*Phaseolus vulgaris* L. cv. Red Kidney) (Hiruki, 1970). One half-leaf was inoculated with untreated freshly extracted sap while one-half leaf of the opposite primary leaf was inoculated with buffer only. The variously treated sap was then inoculated randomly onto the remaining half-leaves. The formation of local lesions was observed 6 – 12 days after inoculation.

*Properties in Purified Preparations*

Centrifugation experiments for the determination of sedimentation coefficient and buoyant density of the virus was carried out in a Spinco Mcdel E analytical ultracentrifuge. Purified virus at concentrations ranging from 0.25 to 0.8 mg per ml in 0.005 M borate buffer, pH 7.8, was centrifuged at 21,740 rpm and 20 C. The sedimentation coefficient was then estimated graphically (Markham, 1960) using pictures of Schlieren patterns taken at four-minute intervals.

The bouyant density was estimated by the equilibrium method in cesium chloride (Chervenka, 1973). Test samples containing 30 – 50 $\mu$g of PVM and 436.9 mg of cesium chloride per ml of 0.005 M borate buffer, pH 7.8, were centrifuged for 12 hours at 44,770 rpm and 25 C. The actual densities of the cesium chloride solutions were determined from their refractive indices which were read using a refractometer. In some rotor cells, 100 ug of tobacco mosaic virus (TMV) $U_1$ strain were added to PVM samples from comparison.

*Polyacrylamide Gel Electrophoresis*

Polyacrylamide gel electrophoresis for the determination of the subunit protein molecular weight was performed with gel concentrations of 4% – 9%. Virus particles were dissociated in 0.1 M phosphate buffer, pH 7.2, containing 4 M urea, 1% sodium dodecyl sulphase (SDS) and 1% mercaptoethanol (Dunker and Rueckert, 1969). Five proteins of known molecular weights were used as standards. Bovine serum albumin (62,000); ovalbumin (43,000); alcohol dehydrogenase (29,000); carbonic anhydrase (29,000); myoglobin (17,200). Electrophoresis was performed at a constant voltage of 50 V for 3 – 4 hours using 0.1 M phosphate-0.1% SDS solution at pH 7.2 as the running buffer (Weber and Osborne, 1969). The molecular weight of PVM protein subunit was estimated graphically from the relative mobilities of standard and test proteins (Shapiro *et al.*, 1969).

## RESULTS

*Physical Properties* in vitro

Local lesions were observed on Red Kidney bean half-leaves which had been inoculated with sap diluted up to $10^{-4}$. Thus, the DEP value of PVM in potato or tomato sap was $10^{-4}$. In the experiment to obtain the LIV value, virus in crude sap was infective after 1, 2, or 3 days of incubation at room temperature prior to inoculation onto Red Kidney bean half-leaves. Virus in sap incubated for 4 days perior to inoculation did not show any infectivity indicating that the LIV value was 3 days. In another experiment, virus in sap heated for 10 min at 65 C or lower remained infective but virus in sap heated at 70 C was not infective. Thus, TIP of PVM from tomato or potato sap was 65 – 70 C.

*Properties in Purified Preparations*

Studies with purified preparations showed that the virus sedimented as one peak during analytical ultracentrifugation. The individual values of sedimentation coefficient obtained from six determinations ranged from 159 S to 166 S with a mean value of 162 S. The Schlieren pattern obtained from equilibrium banding of virus in cesium chloride showed that PVM particles formed a band closer to the centre of the rotor cell than did TMV particles *(Fig. 1)*. The range of values obtained from four individual estimations was 1.301 – 1.309 g/cm$^3$ with a mean value of 1.304 g/cm$^3$. Tobacco mosaic virus which was run concurrently with PVM had a buoyant density of 1.324 g/cm$^3$.

*Polyacrylamide Gel Electrophoresis*

Dissociated virus moved through the polyacrylamide gel as one band. This indicated that PVM contained only one protein subunit. The molecular weight of the protein subunit differed slightly when different gel concentrations were used. The values estimated were 38,900, 39,100, 39,00, 39,600, 39,700 and 39,800 daltons for 4, 5, 6, 7, 8 and 9% polyacrylamide gels, respectively. The mean value for PVM protein subunit modelucar weight was 39,300 daltons *(Fig. 2)*.

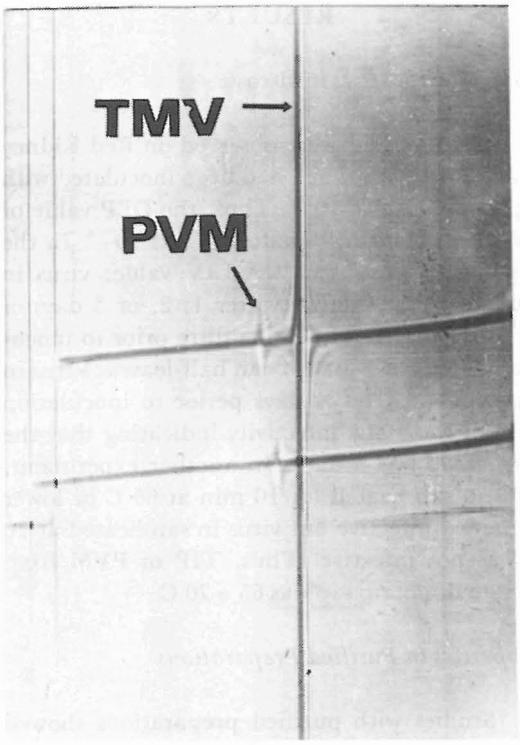


*Fig. 1. Schlieren pattern of PVM and TMV bands following isopycnic centrifugation in cesium chloride.*

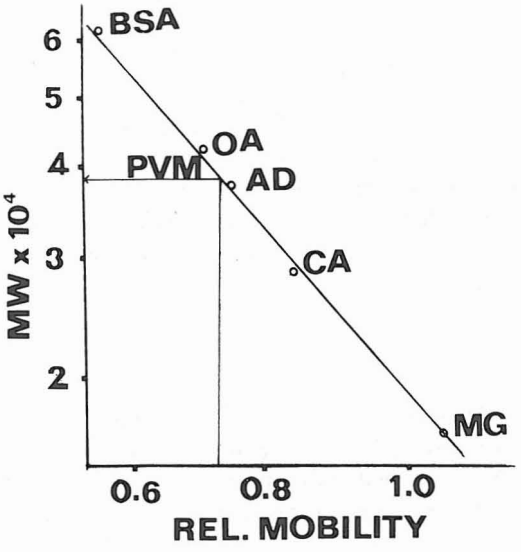


*Fig. 2. Electrophoresis of PVM protein subunits through 7% polyacrylamide gel using bovine serum albumin (BSA), ovalbumin (OA), alcohol dehydrogenase (AD), carbonic anlydrase (CA) and myoglobin (MG) as protein standards.*

## DISCUSSION

The present study showed that the physical properties *in vitro* of PVM, such a DEP value of $10^{-4}$ and a TIP value of 65 – 70 C were similar to some previously reported values (Table 1). However, LIV value of 3 days found in this study is slightly higher than the value of 2 days reported by Bagnal *et al.* (1956) and Hiruki (1973). This difference is probably not important since slight variations in physical properties *in vitro* of plant viruses is a common phenomenon especially when experiments were performed using different isolates (Francki, 1980).

This is the first report on sedimentation properties of PVM. The values of 162 s and 1.304 g/cm$^3$ for the sedimentation coefficient and the buoyant density, respectively, reported in this paper were typical properties of viruses of the carlavirus group (Matthews, 1981). These values indicated that PVM has an RNA content of about 6% (Gibbs and Harrison, 1976; Sehgal *et al.*, 1976). The closeness of buoyant density estimation was confirmed by the value of 1.324 g/cm$^3$ obtained for the bouyant densities of TMV $U_1$ strain are 1.325 g/cm$^3$ (Siegal and Hudson, 1959) and 1.324 g/cm$^3$ (Sehgal *et al.*, 1970).

Although the molecular weight of the PVM protein subunit appeared to increase with higher gel concentrations, the differences were not significant ($P > 0.05$). The average value of 39,000 daltons was higher when compared with those of other carlaviruses for which a range from 31,000 – 36,700 daltons has been recorded (Wetter and Milne, 1981). In our tests, the PVM protein subunit consistently moved slower than alcohol dehydrogenase which has a molecular weight of 37,000 daltons *(Fig. 3)*. This confirms the conclusion that the molecular weight of PVM protein subunits of other carlaviruses.

### REFERENCES

AHMAD, I.B. (1984): Purification of potato virus M (PVM). *Sains Malaysia* (in press).

AHMAD, I.B., WRIGHT, N.S. and STACE-SMITH, R. (1978): Potato virus M (PVM): Suscepti-

bility and host range studies. *Proc. Pl. Port. Conf.* Kuala Lumpur, 1978. pp. 93 – 100.

BAGNALL, R.H. and LARSON, R.H. (1957): Potato virus M (Abstr.) *Phytopath.* **47:** 3.

BAGNALL, R.H., LARSON, R.H. and WALKER, J.C. (1956): Potato virus M, S, and X in relation to interveinal mosaic of the Irish Cobbler variety. *Res. Bull. Agric. Exp. Stain. Univ. Wish.* No. 198.

CHERVENKA, C.H. (1973): *A Manual of Methods for the Analytical Ultracentrifuge.* Backman Instrument, Palo Alto, California. USA.

DUNKER, A.K. and RUECKERT, R. (1969): Observations on molecular weight determination on polyacrylamide gel. *J. Biol. Chem.* **244:** 5074 – 5080.

FRANCKI, R.I.B. (1980): Limited value of the thermal inactivation point, longevity *in vitro* and dilution end point as criteria for the characterization, identification and classification of plant viruses. *Intervirology.* **13:** 91 – 98.

GIBBS, A. and HARRISON, B. (1976): *Plant Virology: The Principles.* Edward Arnold LTD, London. p. 121.

HIRUKI, C. (1970): Red Kidney bean, a successful bioassay host for quantitative qualitative work with potato virus M. *Phytopath.* **60:** 739 – 740.

HIRUKI, C. (1980): Detection of potato virus M in tubers, sprouts, and leaves of potato by French bean test. *Potato Res.* **16:** 202 – 212.

HORIO, Y., YANO, I. and ESUMI, K. (1969): Potato virus M found in Japan. *Ann. Phyopath. Soc. Japan.* **35:** 47 – 54.

MARKHAM, R. (1960): A graphical method for rapid determination of sedimentation coefficient. *Biochem. J.* **77:** 516 – 515.

MATTHEWS, R.E.F. (1982): Classification and nomenclature of viruses. *Intervirology.* **15:** 1 – 199.

ROZENDAAL, R and VAN SLOGTEREN, D.H.M. (1957): A potato virus identified with potato virus M and its relationship with potato virus S. *Pro. 3rd. Conf. Potato Virus Diseases.* Lisse-Wageningen, pp. 20 – 37.

SEHGAL, O.P., JONG-HO, J., BHALL, R.B., SOONG, M.M. and KRAUSE, F.F. (1970): Corellation between bouyant density and ribonucleic acid content in viruses. *Phytopathology.* **60:** 1778 – 1783.

SHAPIRO, A.L., VINULA, E. and MAIZEL, J.V. (1967): Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. *Biochem. Biophs. Res. Comm.* **28:** 815 – 820.

SIEGEL, A. and HUDSON, W. (1959): Equilibrium centrifugation of two strains of tobacco mosaic virus in density gradient. *Biochim. Biophs. Acta.* **34:** 3514 – 3515.

TAVANTZIS, S.M. (1982): Improved purification of two potato carlaviruses. *Phytopath.* **73:** 190 – 194.

TU, J.C. and HIRUKI, C. (1970): Ultrastructure of potato infected with potato virus M. *Virology.* **42:** 238 – 242.

WEBER, K. and OSBORNE, M. (1969): The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **224:** 4406 – 4412.

WETTER, C. and BRANDES, J. (1956): Untersuchungen uber das Kartoffel-S-Virus. *Phytopath. Z.* **26:** 81 – 92.

WETTER, C. and MILNE, R.G. (1981): Carlaviruses. Pages 696 – 730 in: *Handbook of Plant Virus Infections and Comparative Diagnosis.* E. Kurstak, ed. Elsevier/North-Holland Biomedical Press, Amsterdam.

*(Received 27 September, 1984)*