A New Strain of Potato Carlavirus M

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ABSTRACT

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In 1994, potato samples for certification from Idaho seed fields reacted in enzyme-linked immunosorbent assay (ELISA) tests to a polyclonal potato carlavirus M (PVM) antiserum. Sample affinity to the antiserum was lower than control samples. Furthermore, ELISA-positive samples were obtained from both symptomatic as well as asymptomatic plants. A complementary DNA library was prepared using both reverse transcription-polymerase chain reaction and primers based on published PVM sequences, or oligo d(T) primed reverse transcribed sequences. The nucleotide sequence was determined for the 3'-terminus of the genome. Putative coat protein amino acid sequence was compared to published PVM and potato virus S coat protein sequences. While this new isolate is likely a strain of PVM, it is significantly different from known PVM coat protein sequences in the amino terminus region. These differences may explain the poor reactivity to other PVM antisera and suggest that it is a new strain of PVM, which we have designated PVM-ID.

Detection and elimination of viruses in potato seed stock is paramount to potato health and production worldwide. Consequently, seed certification programs are important in detecting, quantifying, and eliminating major potato viruses. When new virus isolates or strains occur, the spectrum of standard tests must be updated to provide the most efficacious detection possible.

Late in 1994, plants of potato breeding selection A82705-1R sent to New Brunswick, Canada, tested positive by enzyme-linked immunosorbent assay (ELISA) for potato carlavirus M (PVM). No symptoms of PVM infection were observed among previous lots grown in Idaho. However, during subsequent grow-out tests in California, two nuclear seed-lots produced from pre-nuclear stocks of this variety in southern Idaho contained plants with mosaic symptoms. PVM-infected plants were confirmed after testing with antisera for various mosaic-causing viruses.

These reports led us to investigate this isolate of PVM, characterize the virus, and produce a reliable antiserum to augment the potato seed certification tests in Idaho.

MATERIALS AND METHODS

Virus source, isolation and purification. Virus particles were initially extracted from infected potato leaf samples of A82705-1R. Because field samples contained mixed infection of potato virus S (PVS) and PVM, subsequent virus extractions were made from plants grown in tissue culture that tested free of PVS. Tissue culture samples were generated and provided by L. Ewing, Idaho Nuclear Seed Potato Program.

Plant tissue infected with PVM was titrated in three volumes of extraction buffer (100 mM Na-borate, pH 8.2, 100 mM EDTA, 2% [wt/vol] polyvinylpyrrolidone [PVP-40], 0.1% β -mercaptoethanol), expressed through cheesecloth, and centrifuged 10 min at $16,270 \times g$. The resulting supernatant was brought up to 200 mM with NaCl and 6% (wt/vol) polyethylene glycol (MW 8000) and stirred for 2 h at room temperature. The precipitate was collected by centrifugation for 20 min at $16,270 \times g$ at 4°C. The pellet was resuspended overnight in 20 ml of 10 mM Na-phosphate buffer, pH 7.5.

The resuspension was clarified by centrifugation for 10 min at $16,270 \times g$ at 4°C, then overlaid on a 20% sucrose cushion in 100 mM Na-phosphate buffer, pH 7.5. High speed centrifugation was performed for 1 h at $240,000 \times g$ at 4°C using a Type 70Ti rotor (Beckman Instruments, Inc., Palo Alto, CA). The resulting pellet was resuspended in 10 mM Na-phosphate buffer, clarified by low speed centrifugation, and overlaid on a 30% sucrose cushion. After the second high speed centrifur-

gation, the resuspended pellet was clarified by low speed centrifugation and subjected to cesium chloride density gradient centrifugation (density ≈ 1.28 g/ml). A single migrating band was collected after centrifugation for 8 h at $240,000 \times g$ at 20°C using a Sorvall TV-865 rotor (Du Pont Company, Wilmington, DE). The collected band was diluted in 10 mM Na-phosphate buffer, pH 7.5, pelleted by high speed centrifugation (1 h at $240,000 \times g$ at 4°C), and resuspended in 0.1 to 0.5 ml of 10 mM Na-phosphate buffer, pH 7.5, containing 0.04% sodium azide.

RNA extraction. Genomic RNA was extracted from virions by combining 500 μ g/ml of purified virus with 5 μ l 20% sodium dodecyl sulfate (SDS) and 5 μ l of proteinase K (5 mg/ml) in a microcentrifuge tube and incubating the mixture at 37°C for 15 min. An additional 25 μ l of 20% SDS was added and the mixture heated to 65°C for 5 min. The mixture was extracted with an equal volume of Tris-EDTA (TE) 8 (10 mM Tris, 1 mM EDTA, pH 8.0)-saturated phenol at 65°C, vortexed briefly, and centrifuged in a benchtop centrifuge for 5 min at room temperature. This step was repeated three times. The upper phase was transferred to a clean tube and extracted five times with chloroform (24:1, chloroform:isoamyl alcohol), centrifuged as before, and the upper phase transferred to a clean tube. The genomic RNA was precipitated with 125 μ l of 7.5 M ammonium acetate, pH 5.2, and 750 μ l 100% ethanol, and incubated for 2 h at -80°C. The precipitated RNA was collected by centrifuging the solution for 30 min at $16,270 \times g$. The resulting pellet was washed once with 70% ethanol, centrifuged for 15 min at $16,270 \times g$, and resuspended in 50 μ l diethyl pyrocarbonate (DEPC)-treated water.

cDNA synthesis. Two methods were employed to generate cDNA for use in subsequent procedures. The first involved production of first-strand cDNA from intact virions as previously described (11), and the second from isolated RNA. When double-stranded cDNA was required, reactions from first-strand synthesis using Superscript II (GIBCO BRL, Gaithersburg, MD) were carried through to the second strand cDNA synthesis reactions, as described by the manufacturer's protocol. The resulting blunt-ended, double-stranded cDNA was used in subsequent ligation reactions.

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PVM-ID sequence has been deposited with GenBank, Accession # AF023877.

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RT-PCR. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using first-strand cDNA generated from genomic RNA or intact virions as described above. Generally, 4 µl of first-strand cDNA was combined on ice with 73.2 µl water, 10.0 µl 10× PCR buffer (GIBCO BRL), 2.5 µl 20 µM homologous-strand primer (5'-GGCGATGGGAGATTCAACGAAGAAAG-3'), 2.5 µl 20 µM complementary-strand primer (5'-GGA-GTAAAGCCACCTTGGTTACGTC-3'), 6.0 µl 25 mM MgCl₂, 0.8 µl 25 mM dNTP mix, and 5U *Taq* DNA polymerase (GIBCO BRL). Reactions were overlaid with Chill-out (MJ Research, Watertown, MA) and incubated for 5 min at 95°C, 5 min at 85°C, and 2 min at 60°C. Thirty-five cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C were performed, followed by 10 min at 72°C. Reactions were held at 9°C until assayed.

Cloning. The PCR-amplified fragments were electrophoresed on 1% agarose, excised, and purified using USB BioClean MP kit (USB, Cleveland, OH). Purified PCR fragments were cloned into pCRII (Invitrogen Corp., San Diego, CA) and transformed into INVαF¹-competent cells. Additionally, oligo d(T) primed cDNA was blunt-end cloned into the *EcoRV* site of pBSISK+, and DH10B-competent cells were transformed. Colonies positive for inserts were selected by blue/white screening on agar media supplemented with 100 µg/ml ampicillin, 40 µM isopropyl β-D-thiogalactopyranoside (IPTG), and 40 µM 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal; 9). Subclones were produced by restriction fragment digests and by exonuclease III digestion (9).

Nucleic acid sequencing and multiple sequence alignments. Sequencing reactions were performed using the Sequi-Therm Cycle Sequencing LongRead Kit (Epicentre Technologies Corporation, Madison, WI) and run on a LI-COR model 4000L Automated DNA Sequencer (LI-COR, Inc., Lincoln, NB). Sequence was collected and analyzed using BaseImager 2.20 software (LI-COR, Inc.). Fragment assembly, multiple sequence analysis, and antigenic site predictions were accomplished using individual programs from the Wisconsin GCG 8.0 software package (Genetics Computer Group, Madison, WI).

Predicted amino acid (a.a.) sequences used for comparison were taken from the literature and designated as follows: PVM-G (3), PVM-R (7), PVM-Z (12), PVS-An (5), and PVS-O (2). Amino acid sequence alignments were performed using Clustal V (EMBL Heidelberg, Germany) and displayed using SeqVu 1.0 (Garvan Institute, Sydney, Australia).

Antibody production. Polyclonal antibodies were produced using purified virions as antigen emulsified 1:1 with Titre-Max Adjuvant (Vaxcel, Inc., Norcross, GA), and injecting female New Zealand

white rabbits with 100 µl in two sites (one subcutaneous and one intramuscular). Serum was collected at one month post-injection, cross-absorbed with healthy potato extract, conjugated to alkaline phosphatase, and stored at 4°C with 0.04% sodium azide.

SDS-PAGE electrophoresis and Western blotting. Samples were prepared by grinding leaf disks (~1-cm diameter) 1:35 (wt/vol) in disassociation buffer (2.5% SDS, 2.5% β-mercaptoethanol, 20% sucrose), boiling for 10 min, and centrifuging in a benchtop microcentrifuge for 4 min. Total plant protein samples were separated on duplicate 12.5% SDS-polyacrylamide gel electrophoresis (PAGE) gels and elec-

trophoresed at 6 W constant power for 4 h in a Hoefer Model SE400 Vertical Slab Gel Unit (Hoefer Scientific, San Francisco, CA). One gel was used for staining with Coomassie brilliant blue R-250, and one gel soaked in transfer buffer (25 mM Tris, 250 mM glycine, 0.05% SDS) for 10 min and electroblotted to nitrocellulose for 1 h at 5 watts, using a semi-dry electroblotter (ISS, Natick, MA). Blots were incubated with phosphate buffered saline (PBS) containing 5% Carnation non-fat milk for 1 h at room temperature. Primary polyclonal antibody was added at 1:2000 and incubated overnight at room temperature on an orbital shaker. Nitrocellulose blots were

Table 1. Indirect enzyme-linked immunosorbent assay (ELISA) data from potato leaf samples for the Idaho strain of potato carlavirus M (PVM-ID)^a

Sample	Number	Antiserum ^b			
		#1	#2	#3	#4
Collection PVM ^c	n = 3	0.65 ± 0.07	> 2.00	1.76 ± 0.28	0.06 ± 0.01
A82705-1R (virus free)	n = 6	0.03 ± 0.01	0.03 ± 0.02	0.01 ± 0.00	0.04 ± 0.03
A82705-1R (PVS) ^d	n = 6	0.07 ± 0.02	0.26 ± 0.03	0.04 ± 0.02	0.01 ± 0.00
A82705-1R (PVM-ID)	n = 6	0.68 ± 0.18	0.41 ± 0.16	0.03 ± 0.02	0.51 ± 0.03
A82705-1R (PVM-ID + PVS)	n = 6	0.72 ± 0.20	0.51 ± 0.16	0.05 ± 0.02	0.42 ± 0.01

^a ELISA data for antiserum #4 were collected in a separate experiment but are included in the table for comparison with earlier ELISA results. ELISA readings in all experiments were at 405 nm using alkaline phosphatase. Numbers to right of each value are standard deviations.

^b Antiserum #1, Bioreba Corp. (lot #110221) South Bend, IN; antiserum #2, Idaho Crop Improvement Association (Thomas), Idaho Falls, ID; antiserum #3, Agdia Corp. (lot #0188) Elkhart, IN; and antiserum #4, University of Idaho, PSES Dept., Moscow, ID.

^c Supplied by R. Goth, USDA-ARS, Beltsville, MD; originally collected by E. S. Shultz.

^d PVS = potato virus S.

Fig. 1. Replicate Western blots of total protein samples extracted from plant tissue. (A) Blot probed with potato carlavirus M (PVM) antiserum (Idaho Crop Improvement Association [ICIA]); (B) blot probed with PVM-ID antiserum, and (C) blot probed with potato virus S (PVS) antiserum (ICIA). Arrow indicates 41.8-kDa prestained marker. Lanes in all three gels are (1) kaleidoscope markers (BioRad, Richmond, CA), (2) PVS-infected tobacco, (3) non-infected potato, and (4) PVM-ID infected potato.

washed twice in PBS and once in Tris-buffered saline (TBS) for 10 min at room temperature. Blots were then incubated for 5 h in TBS (containing 5% Carnation non-fat milk) and goat anti-rabbit alkaline phosphatase (1:2000) or goat anti-sheep alkaline phosphatase (1:2000) for PVM and PVS, respectively. The blot was washed twice with TBS and once with AP buffer (100 mM NaCl, 50 mM MgCl₂, 100 mM Tris, pH 9.5) for 10 min at room temperature. Color development was accomplished using Western Blue stabilized substrate (Promega, Corp., Madison, WI).

RESULTS

Virus extractions from field samples contained both PVM and PVS. After passage in tissue culture, using techniques to produce potato tissue free of PVS, plants were obtained with only PVM, and this isolate was designated PVM-ID.

Early serological tests by the Idaho Crop Improvement Association (ICIA) did not detect PVM in pre-nuclear mini-tubers produced and released by the Nuclear Seed Program. PVM symptoms were not observed in greenhouse grow-out tests established for the potato certification program.

After receiving reports from Canada of PVM infection in potato line A82705-1R, symptomatic plants from the California field grow-out plots were tested by ELISA and found positive for PVM infection. Reactions were not strong, ranging from optical density (OD)₄₀₅ of 0.2 to 0.5 (*data not shown*), with positive controls containing the type culture of PVM reading 2.0.

Additional ELISA tests were run on symptomatic tissue using antisera from Agdia (Elkhart, IN), ICIA (Idaho Falls, ID), and Bioreba Inc. (South Bend, IN).

1/1	31/11
ATG GGA GAT TCA ACA AAG AAA ATT GAA GTT	GCC AAA GAA GCT GGA ACA TCG CAA GCG GCC
M G D S T K K I E V	A K E A G T S Q A A
61/21	91/31
AAG GGG AAC AGA CCA CTA CCT ACG GCG GCA	GAA TTT GAA GGT GAT GAC ACC TCT GGA GAT
K G N R P L P T A A	E F E G D D T S G D
121/41	151/51
GCT AGT GTC CGC AAT GCT GAG GCA AAT GAG	GAA GCG TCA CTG GAG CGC AGA TTG AAT AGC
A S V R N A E A N E	E A S L E R R L N S
181/61	211/71
TTG CGT GAG TTT CTA CGT GAG CGA AGA GGC	GCC ATA CGG GTA ACC AAC CCT GGT TTG GAG
L R E F L R E R R G	A I R V T N P G L E
241/81	271/91
ACT GGC CGT CCA CGA CTC AAG CTT GCA GAC	GAC ATG CGC CCG GAT CCA ACA AAT CCT TAC
T G R P R L K L A D	D M R P D P T N P Y
301/101	331/111
AAC AGG CCG TCG CTT GAG GCA CTG AGC AGA	ATT AAA CCT ATA GCT GTG TCA AAC AAT ATG
N R P S L E A L S R	I K P I A V S N N M
361/121	391/131
GCC ACA TCT GAG GAC ATG ATG CGC ATC TAT	GTC AAT CTT GAA GGT TTG GGG GTG CCA ACT
A T S E D M M R I Y	V N L E G L G V P T
421/141	451/151
GAG TAC GTG CAG CAA GTG GTG ATC CAA GCG	GTG CTT TTC TGC AAA GAT GCA AGT AGC TCG
E Y V Q Q V V I Q A	V L F C K D A S S S
481/161	511/171
GTG TAT CTC GAT CCA CGC GGC TCC TTC GAA	TGG CCA AGG GGT GCA ATC ACT GCA GAT GCG
V Y L D P R G S F E	W P R G A I T A D A
541/181	571/191
GTG CTG GCT GTG ATG AAG AAA GAC GCA GAG	ACT CTG CGC AGA GTC TGT AGG CTG TAC GCG
V L A V M K K D A E	T L R R V C R L Y A
601/201	631/211
CCC GTC ACA TGG AAT CAC ATG CTG GCG CAC	AAT TCC CCA CCA GCG GAC TGG GCG GCT ATG
P V T W N H M L A H	N S P P A D W A A M
661/221	691/231
GGT TTC CAA TAT GAA GAT AGA TTC GCA GCC	TTT GAC TGC TTT GAC TAC GTG GAG AAC ACA
G F Q Y E D R F A A	F D C F D Y V E N T
721/241	751/251
GCT GCA GTT CAA CCA CTG GAA GGG CTG ATA	CGC CGT CCT ACC CCA AGA GAG AAG GTT GCG
A A V Q P L E G L I	R R P T P R E K V A
781/261	811/271
CAT AAC ACT CAC AAG GAC ATA GCT CTG AGG	GGG GCC AAT CGC AAT CAG GTG TTT GGT TCG
H N T H K D I A L R	G A N R N Q V F G S
841/281	871/291
CTC AAT GCT GAG GTA ACT GGA GGT ATG AAT	GGT CCC GAG CTC ACT AGG GAT TTC GGC AAG
L N A E V T G G M N	G P E L T R D F G K
901/301	
TCG AAT AAC AAA TGA	
S N N K *	

Fig. 2. Nucleic acid sequence and predicted amino acid sequence of potato carlavirus M-ID coat protein region.

The results for PVM-ID varied widely. The Agdia antiserum did not react. The ICIA antiserum reacted but provided low readings. The Bioreba antiserum gave a more clear-cut reaction than was observed with

the ICIA antiserum (Table 1). PVM-ID polyclonal antibody, cross-absorbed with healthy potato extract and conjugated to alkaline phosphatase, detected PVM-ID and did not cross-react with the type strain

of PVM or PVS in subsequent ELISA tests (Table 1).

Western analysis of total protein samples paralleled ELISA data and showed that the source of antibody was critical for detec-

PVM-ID	1	M	G	D	S	T	K	K	I	E	V	A	K	E	A	G	T	S	Q	A	A	K	G	N	R	P	L	P	T	A	A	E	F	E	G	D	T	S	G	40		
PVM-G	1	M	G	D	S	T	K	K	A	E	T	A	K	D	E	G	T	S	Q	E	K	R	E	A	R	P	L	P	T	A	A	D	F	E	G	K	D	T	S	E	40	
PVM-R	1	M	G	D	S	T	K	K	A	E	T	A	K	D	E	G	T	S	Q	E	R	R	E	A	R	P	L	P	T	A	A	D	F	E	G	K	D	T	S	E	40	
PVM-Z	1	M	G	D	S	T	K	K	A	E	T	A	K	D	E	G	T	S	Q	E	R	R	E	A	R	P	L	P	T	A	A	D	F	E	G	K	D	T	S	E	40	
PVS-An	1	M	-	-	-	P	P	K	P	D	P	S	S	S	G	E	A	P	Q	A	M	Q	P	A	P	P	P	P	R	A	-	E	G	-	-	-	-	-	H	M	Y	30
PVS-O	1	M	-	-	-	P	P	K	P	D	P	T	S	S	G	E	T	P	Q	A	I	P	L	A	P	P	P	R	N	V	E	E	-	-	-	-	-	-	H	R	I	31
PVM-ID	41	A	S	V	R	N	A	E	A	N	E	E	A	S	L	E	R	R	L	N	S	L	R	E	F	L	R	E	R	R	G	A	I	R	V	T	N	P	G	L	E	80
PVM-G	41	T	D	G	R	A	A	D	A	D	G	E	M	S	L	E	R	R	L	D	S	L	R	E	F	L	R	E	R	R	G	A	I	R	V	T	N	P	G	L	E	80
PVM-R	41	T	D	G	R	A	A	D	A	D	G	E	M	S	L	E	R	R	L	D	S	L	R	E	F	L	R	E	R	R	G	A	I	R	V	T	N	P	G	L	E	80
PVM-Z	41	T	D	G	R	A	A	D	A	D	G	E	M	S	L	E	R	R	L	D	S	L	R	E	F	L	R	E	R	R	G	A	I	R	V	T	N	P	G	L	E	80
PVS-An	31	A	Q	P	E	G	P	G	Q	N	E	E	A	M	L	E	Q	R	L	I	R	L	I	E	L	M	A	T	K	R	H	N	S	T	L	S	N	I	S	F	E	70
PVS-O	32	G	P	N	Q	G	H	G	Q	N	E	E	A	M	L	E	Q	R	L	I	R	L	I	E	L	M	A	S	K	R	H	N	S	T	L	S	N	I	A	F	E	71
PVM-ID	81	T	G	R	P	R	L	K	L	A	D	D	M	R	P	D	P	T	N	P	Y	N	R	P	S	L	E	A	L	S	R	I	K	P	I	A	V	S	N	N	M	120
PVM-G	81	T	G	R	P	R	L	Q	L	A	E	N	M	R	P	D	P	T	N	P	Y	N	R	P	S	T	E	A	L	S	R	I	K	P	I	A	I	S	N	N	M	120
PVM-R	81	T	G	R	P	R	L	Q	L	A	E	N	M	R	P	D	P	T	N	P	Y	N	R	P	S	I	E	A	L	S	R	I	K	P	I	A	I	S	N	N	M	120
PVM-Z	81	T	G	R	P	R	L	Q	L	A	E	N	M	R	P	D	P	T	N	P	Y	N	R	P	S	I	E	A	L	S	R	I	K	P	I	A	I	S	N	N	M	120
PVS-An	71	T	G	R	P	S	L	E	P	T	P	E	M	R	R	N	P	E	N	P	Y	S	R	F	S	I	D	E	L	F	K	M	E	I	R	S	V	S	N	N	M	110
PVS-O	72	I	G	R	P	S	L	E	P	T	P	E	M	R	R	N	P	E	N	P	Y	S	R	F	S	I	D	E	L	F	K	M	E	I	R	S	V	S	N	N	M	111
PVM-ID	121	A	T	S	E	D	M	M	R	I	Y	V	N	L	E	G	L	G	V	P	T	E	Y	V	Q	Q	V	V	I	Q	A	V	L	F	C	K	D	A	S	S	S	160
PVM-G	121	A	T	S	E	D	M	M	R	I	Y	V	N	L	E	G	L	G	V	P	T	E	H	V	Q	Q	V	V	I	Q	A	V	L	F	C	K	D	A	S	S	S	160
PVM-R	121	A	T	S	E	D	M	M	R	I	Y	V	N	L	E	G	L	G	V	P	T	E	H	V	Q	Q	V	V	I	Q	A	V	L	F	C	K	D	A	S	S	S	160
PVM-Z	121	A	T	S	E	D	M	M	R	I	Y	V	N	L	E	G	L	G	V	P	T	E	H	V	Q	Q	V	V	I	Q	A	V	L	F	C	K	D	A	S	S	S	160
PVS-An	111	A	N	T	E	Q	M	A	Q	I	T	A	D	I	A	G	L	G	V	P	T	E	H	V	A	G	V	I	L	K	V	V	I	M	C	A	S	V	S	S	S	150
PVS-O	112	A	N	T	E	Q	M	A	Q	I	T	A	D	I	A	G	L	G	V	P	T	E	H	V	A	G	V	I	L	K	V	V	I	M	C	A	S	V	D	S	S	151
PVM-ID	161	V	Y	L	D	P	R	G	S	F	E	W	P	R	G	A	I	T	A	D	A	V	L	A	V	M	K	K	D	A	E	T	L	R	R	V	C	R	L	Y	A	200
PVM-G	161	V	F	L	D	P	R	G	S	F	E	W	P	R	G	A	I	T	A	D	A	V	L	A	V	L	K	K	D	A	E	T	L	R	R	V	C	R	L	Y	A	200
PVM-R	161	V	F	L	D	P	R	G	S	F	E	W	P	R	G	A	I	T	A	D	A	V	L	A	V	L	K	K	D	A	E	T	L	R	R	V	C	R	L	Y	A	200
PVM-Z	161	V	F	L	D	P	R	G	S	F	E	W	P	R	G	A	I	T	A	D	A	V	L	A	V	L	K	K	D	A	E	T	L	R	R	V	C	R	L	Y	A	200
PVS-An	151	V	Y	L	D	P	A	G	T	V	E	F	P	T	G	A	V	P	L	D	S	I	A	I	M	K	N	R	A	G	-	L	R	K	V	C	R	L	Y	A	189	
PVS-O	152	V	Y	L	D	P	A	G	T	V	E	F	P	T	G	A	V	P	L	D	S	I	A	I	M	K	N	R	A	G	-	L	R	K	V	C	R	L	Y	A	190	
PVM-ID	201	P	V	T	W	N	H	M	L	A	H	N	S	P	P	A	D	W	A	A	M	G	F	Q	Y	E	D	R	F	A	A	F	D	C	F	D	Y	V	E	N	T	240
PVM-G	201	P	V	T	W	N	H	M	L	T	H	N	A	P	P	A	E	W	A	A	M	G	F	Q	Y	E	D	R	F	A	P	F	D	C	F	D	Y	V	E	N	T	240
PVM-R	201	P	V	T	W	N	H	M	L	T	H	N	A	P	P	A	D	W	A	A	M	G	F	Q	Y	E	D	R	F	A	A	F	D	C	F	D	Y	V	E	N	T	240
PVM-Z	201	P	V	T	W	N	H	M	L	T	H	N	A	P	P	A	D	W	A	A	M	G	F	Q	Y	E	D	R	F	A	A	F	D	C	F	D	Y	V	E	N	T	240
PVS-An	190	P	V	V	W	N	Y	M	L	V	Q	N	R	P	P	S	D	W	Q	A	M	G	F	Q	W	N	A	R	F	A	A	F	D	T	F	D	Y	V	T	N	G	229
PVS-O	191	P	V	V	W	N	Y	M	L	V	Q	N	R	P	P	S	D	W	Q	A	M	G	F	Q	W	N	A	R	C	A	A	F	D	T	F	D	Y	V	T	N	G	230
PVM-ID	241	A	A	V	Q	P	L	E	G	L	I	R	R	P	T	P	R	E	K	V	A	H	N	T	H	-	-	-	K	D	I	A	L	R	G	A	N	R	N	Q	V	277
PVM-G	241	A	A	V	Q	P	L	E	G	L	I	R	R	P	T	P	R	E	K	V	A	H	N	T	H	-	-	-	K	D	I	A	V	R	G	A	N	R	N	Q	V	277
PVM-R	241	A	A	V	Q	P	L	E	G	L	I	R	R	P	T	P	R	E	K	V	A	H	N	T	H	-	-	-	K	D	I	A	V	R	G	A	N	R	N	Q	V	277
PVM-Z	241	A	A	V	Q	P	L	E	G	L	I	R	R	P	T	P	R	E	K	V	A	H	N	T	H	-	-	-	K	D	I	A	V	R	G	A	N	R	N	Q	V	277
PVS-An	230	A	A	I	Q	P	V	E	G	L	I	R	R	P	T	P	E	E	T	I	A	H	N	A	H	-	-	-	K	S	M	A	I	D	K	S	N	R	N	E	R	266
PVS-O	231	A	A	V	Q	P	V	E	G	L	I	R	R	P	T	P	E	E	T	I	A	H	N	A	H	N	A	H	K	S	M	A	I	D	K	S	N	R	N	E	R	270
PVM-ID	278	F	G	S	L	N	A	E	V	T	G	G	M	N	G	P	E	L	T	R	D	F	G	K	S	N	N	K	304													
PVM-G	278	F	S	S	L	N	A	E	V	T	G	G	M	N	G	P	E	L	T	R	D	Y	V	K	S	N	R	K	304													
PVM-R	278	F	S	S	L	N	A	E	V	T	G	G	M	N	G	P	E	L	T	R	D	Y	V	K	S	N	R	K	304													
PVM-Z	278	F	S	S	L	N	A	E	V	T	G	G	M	N	G	P	E	L	T	R	D	Y	V	K	S	N	R	K	304													
PVS-An	267	L	A	N	T	N	V	E	Y	T	G	G	M	L	G	A	E	I	V	R	N	H	R	N	A	I	N	Q	293													
PVS-O	271	L	A	N	T	N	V	E	Y	T	G	G	M	L	G	A	E	I	V	R	N	H	R	N	A	I	N	Q	297													

Fig. 3. Alignment of coat protein sequence of potato carlavirus M (PVM)-ID with three sequences of PVM and two strains of potato virus S. Homologous amino acids are identified by shading; boxed amino acids indicate identity for each position in the amino acid chain.

tion of this virus. When protein samples were probed with antibodies raised against the type strain of PVM, detection was sporadic, and cross-reaction with proteins from PVS often was observed (Fig. 1A and 1C, respectively). Antibodies raised to purified PVM-ID (Fig 1B) detected protein of the expected size (~34 kDa) and only faintly cross-reacted with PVS.

Initial cloning efforts from cDNA from intact virions using custom primers yielded a fragment of 804 base pairs (bp). Sequence data confirmed that the fragment corresponded to the 5'-terminus and core of the coat protein region of PVM, but lacked the 3'-terminus expected from published sequences. Subsequent clones generated from oligo d(T)-primed cDNA revealed the entire coat protein sequence (Fig. 2) of 928 bp coding for 304 amino acids, the sequence encompassing the triple block genes, and the 3' open reading frame normally associated with carlaviruses (*data not shown*). Sequence alignment of the putative coat protein translation product (33.6 kDa) show that PVM-ID is more closely related to other PVM sequences (92% similarity, 88% identity) than to PVS sequences (57-58% similarity, 46% identity; Fig. 3). Within the first 50 a.a. of the coat protein region, similarity to other PVM sequences drops to 70 to 72%.

DISCUSSION

PVM-ID is typical of many latent potato viruses, in that symptoms range from very slight to severe depending on the potato variety and environmental conditions. Symptom expression of PVM-ID was not noticeably different from that of ordinary PVM. Typical symptoms included mottling, crinkling, and leafrolling, and stunting of shoots.

Antisera from Agdia, ICIA, and Bioreba varied widely in ELISA and Western blot tests of PVM-ID in potatoes. These tests suggested that there was a new strain of PVM in the released material. It also became clear that the problem of detection of PVM-ID was rooted in the latency of the virus and in the source of antisera, with varying levels of detection correlating with

the source of antisera. Because of this variability in reaction to antisera raised against type PVM antigen, we produced antiserum to PVM-ID. This new antiserum can reliably detect PVM-ID and distinguish it from the type strain of PVM.

Sequence data revealed a greater homology between published PVM sequences and PVM-ID than with PVS, clearly indicating that it is an isolate of PVM. Genomic structure is typical of carlaviruses genome organization, with evidence of the replicase motif in the RNA-dependent RNA polymerase (RdRp) gene, an overlapping triple block gene set followed by the coat protein gene, and ending with a 3'-end open reading frame. Protein alignments of published carlavirus coat protein sequences place most of the heterogeneity within the amino terminus (*data not shown*). This region of heterogeneity falls within the range normally associated with plant virus coat protein antigenic sites (4,8), and may explain why antisera to the ordinary strain of PVM failed or only marginally detected PVM-ID using ELISA.

Virus speciation and strain delineation is difficult given the polythetic nature of viruses. However, while there is no criterion concerning classification of virus species and strains, it is widely accepted that delineation based on a combination of molecular, serological, and biological properties can be both useful and convenient. Properties such as the degree of coat protein sequence identity (approximately 90 to 99% for strains) and serological relationships have been used in the past to establish membership in virus species groups and to distinguish strains (1,6,10). Discounting the normally variable and latent nature of PVM symptom expression in general, we believe it is appropriate to use the serological and molecular properties of this isolate to designate PVM-ID as a new strain of PVM.

We have presented evidence that potato breeding selection A82705-1R contained PVM, that there is a difference in coat protein sequence from published PVM isolates, especially within the first 50 a.a. normally associated with major antigenic

sites, and that there are significant differences among various PVM antisera. We believe these factors indicate that PVM-ID is a distinct strain.

The origin of this new strain has not been established, but subsequent testing of breeding germplasm detected the virus in two introduced potato lines. They probably became the initial source of inoculum, which spread to the breeding selection A82705-1R. The fact that this strain is normally latent under summer growing conditions made visual identification unlikely.

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