NUCLEOTIDE SEQUENCES OF COAT PROTEIN CODING REGIONS OF SIX POTATO MOP-TOP VIRUS ISOLATES

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Summary. – Coat protein (CP) coding regions of six Potato mop-top virus (PMTV) isolates from the Czech Republic and Denmark (54-10, 54-11, 54-15, 54-19, Korneta and Pacov) were sequenced. Comparison of the obtained nucleotide sequences as well as alignment of the deduced amino acid sequences were performed. The obtained results showed that the isolates from different parts of Europe seem to have highly conserved coding regions which is unexpected for a viral RNA genome known for its high mutation rate. Thus considerable differences in virulence and significant variation in biological properties of these isolates should not be attributed to CP but to some other part of the genome.

Key words: Potato mop-top virus; virus isolates; coat protein; gene; protein; amino acid sequence; nucleotide sequence

Introduction

PMTV represents the species *Potato mop-top virus*, the *Pomovirus* genus of plant viruses (van Regenmortel *et al*., 2000). It is spread by motile zoospores of the fungus *Spongospora subterranea* (Jones and Harrison, 1969; Arif *et al*., 1995). PMTV causes a wide range of symptoms in haulms and tubers which vary depending on the potato cultivar and environmental conditions, thus causing difficulties in identification of the cause of the diseases observed (Kurppa, 1989). It occurs in various regions of Europe, North and South America and Asia with cool, wet climate (Jones, 1988). In 1983 PMTV has been found for the first time in the Czech Republic (Novák *et al*., 1983). So far, there are no sources of resistance or tolerance to PMTV that could be used in breeding programs and an effective and environmentally acceptable chemical control of the fungal vector is not commercially available (Sokmen *et al.*, 1998).

PMTV is characteristic by tubular particles consisting of one type of viral protein subunit (CP) that encapsidate three genomic RNA molecules of approximately 6.5, 3.2, and 2.4 kb. Genome RNA sequences of several different PMTV isolates have been determined completely or partially (Kashiwazaki *et al*., 1995; Reavy *et al.,* 1998; Savenkov *et al.*, 1999; Torrance *et al.,* 1999; Sandgren *et al.*, 2001). The CP coding region is located on the middle-sized RNA referred to as RNA2 (Sandgren *et al.,* 2001). The available sequence data of different PMTV isolates show that RNA2 is highly conserved, which property is quite rare in RNA virus genomes (Kashiwazaki *et al.*, 1995; Mayo *et al*., 1996).

The error-prone replication and short generation time of RNA viruses result in enormously high speed of evolution and diversification as compared to those of nuclear genomes of eukaryotic organisms (Gojobori *et al*., 1990; Drake and Holland, 1999). Sometimes recombination and segmentation can occur, producing defective and chimeric genotypes, thus further increasing population diversity (Simon and Bujarski, 1994). On the other hand, selection pressure and repeated bottlenecks may reduce that diversity (Domingo and Holland, 1997).

The aim of this study was to find the relationship between biological properties and genome sequence. We determined nucleotide sequences of CP gene regions of several different field isolates of PMTV from the Czech Republic and Denmark with well-characterized biological properties.

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Abbreviations: CP = coat protein; nt = nucleotide; PBS = phosphate-buffered saline; PBS+T = PBS+Tween 20; PMTV = Potato mop-top virus; RT-PCR = reverse transcription–polymerase chain reaction

Materials and Methods

Virus isolates. We analyzed two PMTV isolates from the Czech Republic, namely Pacov and Korneta, isolated by Dr. P. Dědič, Potato Research Institute, Havlíčkův Brod, Czech Republic, and four Danish isolates, namely 54-10, 54-11, 54-15, 54-19, kindly provided by Dr. S.L. Nielsen, the Danish Institute of Agricultural Sciences, Flakkebjerg, Denmark. The viruses were propagated in *Nicotiana debneyi* by mechanical inoculation using the sap extracted from symptomatic leaves.

Immunocapture reverse transcription–polymerase chain reaction (RT-PCR). cDNAs of PMTV RNAs were obtained by immunocapture RT-PCR. Tubes were coated with an anti-PMTV IgG (Adgen, 0.1 µg/100 µl per tube) dissolved in a coating buffer at 37°C for 3 hrs. The tubes were then washed 3 times with 150 µl of phosphate-buffered saline (PBS) containing Tween 20 (PBS+T) per tube and homogenates of leaves infected with PMTV isolates diluted 1:10 in a conjugate buffer were added (100 µl per tube). The tubes were incubated at 4°C overnight and washed again 3 times with PBS+T. After the last wash RT-PCR using the Superscript II Kit (Gibco) and Taq DNA polymerase (Fermentas) under manufacturer´s recommendations was performed. The following PMTV-specific primers were used: the forward primer MT-CP5A (5'-AAGAATTCCATGGCTGAAAACAGAGGTGA-3') and reverse primer MT-CP3A (5'-ATTACTCGAGTGCACCAGCCCAGCGTAACC-3') corresponded to nucleotides (nt) 313–333 and 822–841, respectively, of the sequence of the Sw isolate available at the GenBank database under Acc. No. AJ243719 (Sandgren *et al*., 2001). The primers introduced restriction sites needed for further manipulation. PCR consisted of 30 cycles of 94°C/30 secs (denaturation), 55°C/30 secs (annealing) and 72°C/1 min (elongation)

Cloning and sequencing. The PCR products were cloned in the vector pUC57T/A (Fermentas) using the 3'-A overhangs generated by Taq polymerase. The cloned fragments were sequenced using an ALFexpressII Sequencer and the AutoRead Sequencing Kit (AP Life Science). The obtained nucleotide sequences were deposited at the GenBank database (Table 1).

The sequence analysis was carried out using the Genescan software (Burset *et al*., 1996). The homology search was done with sequences available in GenBank using BLAST program (Altschul *et al*., 1990). A multiple sequence alignment was done using Clusal W program (Thompson *et al*., 1994). The CP sequences of other isolates used for comparison were retrieved from GenBank.

Results and Discussion

Nucleotide sequences of the complete CP coding region or parts of it were obtained for PMTV isolates Korneta, Pacov, 54-10, 54-11, 54-15 and 54-19. The fidelity of obtained results was confirmed by sequencing several clones from both strands. The obtained nucleotide sequences were analyzed for the presence of CP open reading frames based on homology with known sequences of other PMTV isolates. The CP nucleotide sequences of the isolates Pacov and 54-11 were partial, missing an amino-terminal region (73 and 93 amino acids, respectively). The CP nucleotide sequences are presented without codons for 7 amino-terminal amino acids and 7 carboxy-terminal amino acids since they were parts of the primers used.

Table 1. Characteristics of PMTV isolates subjected to sequencing and comparison of their CP coding regions

PMTV isolate	Acc. No.	Reference	Country of origin	Symptoms
54-10	AF487408	Nielsen and Nicolaisen, 2001	Denmark	Strong
54-11 (partial cds)	AY208160	Nielsen and Nicolaisen, 2001	Denmark	Medium
54-15	AF487409	Nielsen and Nicolaisen, 2001	Denmark	Very strong
54-19	AF487407	Nielsen and Nicolaisen, 2001	Denmark	Strong
Korneta	AF393507	/	Czech Republic	Medium
Pacov (partial cds)	AY208159	/	Czech Republic	Weak
Sw	AJ243719	Sandgren *et al*., 2001	Sweeden	/
T	D16193	Kashiwazaki *et al*., 1995	Scotland	/

cds = coding DNA strand.

Comparison of amino acids sequences of CPs of the isolates Korneta, Pacov, 54-10, 54-11, 54-15, 54-19, Sw and T (Kasiwazaki *et al*., 1995; Sandgren *et al.*, 2001) showed a high homology, ranging from 97 to 100% despite the fact that some of these isolates originated from geographically different parts of Europe (Fig. 1). The alignment revealed not only a high homology of the deduced amino acid sequences but also a high identity (99%) of the nucleotide sequences. Surprisingly only 4 of 14 nucleotide changes found were silent, located at the third position of codon, thus not resulting in a changed amino acid.

It is known that RNA viruses are capable of rapid evolution, however, there are also examples of genetic stability of RNA populations of some viruses, e.g. Tobacco mild green mosaic virus, Turnip yellow mosaic virus and Beet mosaic virus; it has been proposed that the time period of evolution of these viruses was similar to that of the host plant (Skotnicki *et al.*, 1993; Gibbs, 1999; Z. Šubr, personal communication). The genetic stability is explained as a consequence of strong selection and competition between the mutants that arise in each replication cycle (Domingo and Holland, 1994). However, viral populations are dynamic and founder effects or bottlenecks can allow newly arising mutants to shift the distribution of mutants and to promote rapid evolution (Domingo and Holland, 1997). On the basis of the example of Citrus tristeza virus (the family *Closteroviridae*) it was suggested that a bottleneck could arise by their spreading and

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                    1                                                  50
54-10               .......RRA AVENRYDAWD HEQAMKAAVR KFISYDQFSA QLRNWREARL
54-11               ......... ......... ......... ......... .........
54-15               .......RRA AVENRYDAWD HEQAMKAAVR KFISYDQFSA QLRNWREARL
54-19               .......RRA AVENRYDAWD HEQAMKAAVR KFISYDQFSA QLGNWREARL
Korneta             .......RRA AVENRYDAWD HEQAMKAAVR KFISYDQFSA QLRNWREARL
Pacov               ......... ......... ......... ......... .........
Sw                  MAENRGERRA AVENRYDAWD HEQAMKAAVR KFISYDQFSA QLRNWREARL
T                   MAEIRGERKA AVENRYDSWD HEQAMKAAVR KFISYDQFSA QLRNWREARL

                    51                                                 100
54-10               NIIEHATSVL SQVSNSGRTH FYSRTERFGG SSLVGDKLYV CLNETRMKTA
54-11               ......... ......... ......... ......... ...ETRMKTA
54-15               NIIEHATSVL SQVSNLGRTH FYSRTERFGG SSIVGDKLYV CLNETRMKTA
54-19               NIIEHATSVL SQVSNLGRTH FYSRTERFGG SSLVGDKLYV CLNETRMKTA
Korneta             NIIEHATSVL SQVSNLGRTH FYSRTERFGG SSLVGDKLYV CLNETRMKTA
Pacov               ......... ......... ...RTERFGG SSLVGDKLYV CLNETRMKTA
Sw                  NIIEHATSVL SQVSNLGRTH FYSRTERFGG SSLVGDKLYV CLNETRMKTA
T                   NIIEHATSVL SQVSNLGRTH FYSGTERFGG SSLVGDKLYV CLNETRMKTA

                    101                                                150
54-10               LNNIIVALQT VNGEGRARRL GPREASANTG GEDSALNVAH QLAEVDDLLT
54-11               LNNIIVALQT VNGEGRARRL GPREASANTG GEDSALNVAH QLAEVDDLLT
54-15               LNNIIVALQT VNGEGRARRL CPREASANTG GEDSALNVAH QLAEVDDLLT
54-19               LNNIIVALQT VNGEGRARRL GPREASANTG GEDSALNVAH QLAEVDDLLT
Korneta             LNNIIVALQT VNGEGRARRL GPREASANTG GEDSALNVAH QLAEVDDLLT
Pacov               LNNIIVALQT VNGEGRARRL GPREASANTG GEDSALNVAH QLAEVDDLLT
Sw                  LNNIIVALQT VNGEGRARRL GPREASANTG GEDSALNVAH QLAEVDDLLT
T                   LNNIIVALQT VNGEGRARRL GPREASANTG GEDSALNVAH QLAEVDDLLT

                    151                          177
54-10               DESFLREAVF TQDKYELVN. .......
54-11               DESFFREAVF TQDKYELVN. .......
54-15               DESFLREAVF TQDKYELVN. .......
54-19               DESFLREAVF TQDKYELGN. .......
Korneta             DESFLREAVF TQDKYELVN. .......
Pacov               DESFLREAVF TQDKYELVN. .......
Sw                  DESFLREAVF TQDKYELVNG LRWAGA.
T                   DESFLREAVF TQDKYELVNG LRWAGA.
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Fig. 1
Alignment of deduced amino acid sequences of CPs of the PMTV isolates under comparison
The changed amino acids are in bold.

mixing by graft and aphid transmission, allowing minor viral variants to become predominant (Albiach-Marti *et al*., 2000).

However, the very strong conservation of CP sequences of PMTV shown here cannot be explained by strong conservative selection only. Since even silent mutations are extremely rare, it could be possible that replication of PMTV is not, in fact, error-prone, implying some proofreading mechanisms intrinsic to either viral or host cell component of replicative machinery. Since this observation is based on the sequences of a small number of isolates only we expect that more sequence data are needed for elucidation of the basis of such a high sequence conservation. Our present work is directed to this aim.

The obtained results showed that the origin of significant variation of symptoms of virus infection with many PMTV isolates should be attributed to some genome part other than CP. Only knowledge of complete genome sequences of various PMTV isolates could show whether such an evolutionary stasis is typical for the whole genome or its particular part. In fact such sequence stability could allow more success in some biotechnological strategies, e. g. production of efficient polyclonal antibodies or manipulating

the resistance of a plant infected with a mild strain to superinfection with a more severe strain.

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References

Albiach-Marti MR, Mawassi M, Gowda S, Satyanarayana T, Hilf ME, Shanker S, Almira EC, Vives MC, Lopez C, Guerri J, Flores R, Moreno P, Garnsey SM, Dawson WO (2000): Sequences of *Citrus tristeza* virus separated in time and space are essentially identical. *J. Virol.* **74**, 6856–6865.

Altscul SF, Stephen F, Gish W, Miller W, Myers EW, Lipman DJ (1990): Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410.

Arif M, Torrance L, Reavy B (1995): Aquisition and transmission of potato mop-top furovirus by a culture of *Spongospora subterranea* f. sp. *subterranea* derived from a single cytosorus. *Ann. Appl. Biol.* **126**, 493–503.

Burset M, Guigo R (1996): Evaluation of gene structure prediction programs. *Genomics* **34**, 353–367.

Domingo E, Holland JJ (1994): Mutation rates and rapid evolution of RNA viruses. In SS Morse (Ed.): *The Evolutionary Biology of Viruses*. Raven Press, Ltd., New York, N.Y, pp. 161–184.

Domingo E, Holland JJ (1997): RNA virus mutations and fitness for survival. *Annu. Rev. Microbiol*. **51,** 151–178.

Drake JW, Holland JJ (1999): Mutation rates among RNA viruses. *Proc. Natl. Acad. Sci. USA* **96**, 13910–13913.

Gibbs A (1999): Evolution and origins of tobamoviruses. *Philos. Trans. R. Soc. London B.* **354**, 517–685.

Gojobori T, Moriyama EN, Kimura M (1990): Molecular clock of viral evolution, and the neutral theory. *Proc. Natl. Acad. Sci. USA* **87**, 10015–10018.

Jones RAC (1988): Epidemiology and control of potato mop-top virus. In JI Cooper, MJC Asher (Eds): *Developments in Applied Biology II. Viruses with Fungal Vectors*. Wellesbourne, UK: Association of Applied Biologists, pp. 250–270.

Jones RAC, Harrison BD (1969): The behaviour of potato mop-top in soil, and evidence for its transmission by Spongospora subterranea (Wallr.) *Lagerh. Ann. Apppl. Biol.* **63**, 1–17.

Kurppa A (1989): The distribution and incidence of potato mop-top virus in Finland as determined in 1987 and on the variation of disease symptoms in infected potatoes. *Ann. Agric. Fenn.* **28**, 285–295.

Kashiwazaki S, Scott KP, Reavy B, Harrison BD (1995): Sequence analysis and gene content of potato mop-top virus RNA 3: further evidence of heterogeneity in the genome organization of furoviruses. *Virology* **206**, 701–706.

Mayo MA, Torrance L, Cowan G, Jolly CA, Macintosh SM, Orrega R, Barrera C, Salazar LF (1996): Conservation of coat protein among isolates potato mop-top virus from Scotland and Peru. *Arch. Virol.* **141**, 1115–121.

Novak JB, Rasocha V, Lanzova J (1983): Detection of Potato mop-top virus in Czechoslovakia, *Ochr. Rostl.* **19**, 161–167 (in Czech).

Nielsen SL, Nicolaisen M (2001): Variation in Danish isolates of potato mop-top virus. *Proceedings of the 11th Virology Section Meeting*, Havlíčkův Brod-Třešť, October 2001.

Reavy B, Arif M, Cowan GH, Torrance L (1998): Association of sequences in the coat protein/readthrough domain of potato mop-top virus with transmission by *Spongospora subterranea. J. Gen. Virol.* **79**, 2343–2437.

Sandgren M, Savenkov EI, Valkonen JP (2001): The readthrough region of Potato mop-top virus (PMTV) coat protein encoding RNA, the second largest RNA of PMTV genome, undergoes structural changes in naturally infected and experimentally inoculated plants. *Arch. Virol.* **146**, 467–477.

Savenkov EI, Sandgren M, Valkonen JP (1999): Complete sequence of RNA 1 and the presence of tRNA-like structures in all RNAs of Potato mop-top virus, genus Pomovirus. *J. Gen. Virol.* **80**, 2779–2784.

Simon AE and Bujarski JJ (1994): RNA-RNA recombination and evolution in infected plants. *Annu. Rev. Phytopatol.* **32**, 337–362.

Skotnicki ML, Mackenzie AM, Ding SW, Mo JQ, Gibbs AJ (1993): RNA hybrid mismatch polymorphisms in Australian populations of turnip yellow mosaic tymovirus. *Arch. Virol.* **132**, 83–99.

Sokmen MA, Barker H, Torrance L (1998): Factors affecting the detection of potato mop-top virus in potato tubers and improvement of test procedures for more reliable assays *Ann. Appl. Biol*. **133**, 55–63.

Thompson JD, Higgins DG, Gibson TJ (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680.

Torrance L, Cowan GH, Sokmen MA, Reavy B (1999): A naturally occurring deleted form of RNA 2 of Potato mop-top virus. *J. Gen. Virol.* **80**, 2211–2215.

Torrance L, Mayo MA (1997): Proposed re-classification of furoviruses. Virology Division News. *Arch. Virol.* **142**, 435–439.

Van Regenmortel MHV, Fauquet CM, Bishop DHL (Eds): *Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses.* Academic Press, San Diego-San Francisco-New York-Boston-London-Sydney-Tokyo, 2000.