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Some chemical and physical properties of 18 tobacco mosaic virus isolates from tomato

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Abstract

From 18 tobacco mosaic virus isolates from tomato, which could be divided into 3 pathogenicity groups, the buoyant density, the S value, the base composition, the amino acid composition, and the behaviour of their tryptic peptides in thin-layer chromatography were compared. There were no differences in buoyant densities and S values. With respect to the other characteristics only small individual differences could be detected. Pathogenicity of the isolates was not correlated with the chemical and physical properties.

Introduction

From greenhouse tomato crops in the Netherlands Rast (1967) obtained many tobacco mosaic virus (TMV) isolates. These can be classified according to their pathogenicity to clones of *Lycopersicum peruvianum* (Rast, 1967 and 1968), as earlier described by McRitchie and Alexander (1963). These authors, however, found no correlation between this grouping and symptom expression in tomato.

Many studies were undertaken to correlate pathogenicity of TMV strains with chemical properties. Much work has been done particularly on the correlation between pathogenicity and characteristics of the coat protein (Woody and Knight, 1959; Wang and Knight, 1967; Wittmann, 1967; Dawson et al., 1969 and 1970; Rees et al., 1971). In all cases differences could be found, but there was no direct correlation between pathogenicity and amino acid composition.

The present investigation was started to determine whether or not the different pathogenic groups can be distinguished on the basis of chemical and physical properties. We therefore compared the buoyant density, S value, base composition and amino acid composition of 18 TMV isolates with those of TMV 'Vulgare'. Furthermore we performed thin-layer chromatography with tryptic peptides of the coat proteins.

Materials and methods

Virus isolates. In Table 1 origin, codes and pathogenic type (Rast, 1968) of the isolates are presented. The numbers in the first column will be used to indicate the isolates in

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Table 1. TMV isolates from tomato.

Virus isolate No.	Origin and code of the isolates	Pathogenic type
1	GM-65 Lp6	1
2	GM-65 Lp2	1a[1]
3	GM-65 Lp4 (1971)	1a
4	GM-65 Lp4 (1969)	1
5	GM-65	1
6	Ohio III	2
7	SD 69	2
8	GD 68	2
9	NH 69	2
10	GeBl	2
11	GB 68-2	2
12	SPS	2
13	MH	3
14	Ohio IV	3
15	GeRo	3
16	SG 64	3
17	VW	3
18	SK 68-2	3
19	TMV 'Vulgare'	1

[1] Isolate derived from a group 1 isolate (Rast, 1971).

Tabel 1. TMV-isolaten van tomaat.

this paper. Purified TMV 'Vulgare' was supplied by Dr A. van Kammen, Laboratory of Virology, Agricultural University at Wageningen.

Standard RNA. Ribonucleic acid from Nutritional Biochemicals Corporation, Cleveland, Ohio was used as a standard.

Virus purification. The virus isolates were purified by column chromatography using polyethylene glycol 6000 (PEG) (Venekamp et al., 1973). In order to separate the RNA of the tomato isolates from the coat protein with the acetic acid method the preparations were centrifuged for 1.5 h at 73,000 *g* to remove residual PEG.

Density-gradient centrifuging in cesium chloride. Tubes of an SW 39 rotor were filled with 2.25 ml of CsCl solution (6.23 g CsCl/10 ml water), 0.75 ml water, 0.1 ml of a virus suspension (1 mg/ml), and 2 ml paraffin oil. In each run three tubes were spun. Two contained one virus isolate each; the third a mixture of both. The tubes were spun overnight at 134,000 *g* at 5 °C. The virus bands were visualized by light scattering and their position was determined by measuring the distance from the middle of the band to the bottom of the tube.

Determination of sedimentation coefficients. Sedimentation coefficients were determined with an analytical ultracentrifuge Spinco Model E, with Schlieren optics. Per run two cells were filled, one with the virus isolate (2 mg/ml) to be tested, and the other with a

mixture of that isolate and isolate 14 (2 mg/ml in total). The latter was used as an internal standard. After measuring the displacement of the peaks in the photographs, we used the graphical method of Markham (1960) to determine S values.

Determination of base composition. The virus preparations were degraded by a modified acetic acid method (Fraenkel-Conrat, 1957). To 2 ml of each virus preparation, containing about 40 mg of virus, 4 ml of ice-cold acetic acid were added. The precipitated RNA was collected by centrifuging for 15 min at 2500 *g*. The residual acetic acid in the pellet was evaporated and 1 ml 0.3 N NaOH added. Subsequently the mixture was incubated during 16 h at 37 °C. The hydrolyzate was applied on a 1 × 1 cm column of Dowex 1 × 8 formate without neutralization (Bautz and Hall, 1962). The column was washed with 30 ml water and then with 30 ml of 0.005 N formic acid. The nucleoside phosphates were successively eluted from the column with the following solutions: 50 ml of 0.025 N formic acid for cytidine monophosphate (CMP), 50 ml of 0.2 N formic acid for adenosine monophosphate (AMP), 75 ml of 0.01 N formic acid + 0.05 N ammonium formate for uridine monophosphate (UMP) and 80 ml of 0.1 N formic acid + 0.2 N ammonium formate for guanosine monophosphate (GMP). The amount of each was determined spectrophotometrically.

Determination of the amino acid composition. The supernatant fluid remaining after RNA sedimentation was dialyzed against distilled water during 3–4 days at 5 °C. After the protein had precipitated at its iso-electric point, it was collected by centrifuging for 10 min at 3000 *g*. The pellets were resuspended in water. The pH was raised to 8 and then the mixture was centrifuged for 1 h at 110,000 *g* to remove undegraded virus. The supernatant fluid was lyophilized. To aliquots of 10 mg of protein each 10 ml of 6 N HCl were added and incubation during 24 h at 110 °C in a sealed tube permitted the hydrolysis. The hydrolyzates were evaporated, the residues dissolved in water, and the mixture evaporated again. The final residues were dissolved in a citrate starting buffer, pH 2.2. The analyses were done with a Beckman Multichrom amino acid analyzer, equipped with an automatic sample injector.

Thin-layer chromatography. The protein was digested into tryptic peptides according to a modified method of Woody and Knight (1959). To 1 ml of virus suspension (5mg/ml) 1 ml of 0.01 M phosphate buffer pH 8.5 was added. After heating for 10 min at 100 °C, to each sample 5 mg of trypsin (free of chymotrypsin) were added. This mixture was then incubated for 16 h at 38 °C. The pH was adjusted to 4.5 and the resulting precipitate removed by centrifuging. The supernatant fluid was lyophilized and stored at −22 °C. After resolving in water, 10 µl samples were transferred to Merck precoated silicagel plates and chromatographed with a solvent, consisting of butanol, acetic acid and water (4:1:1) for about 8 h. After drying of the plates (1.5 h at 90 °C) the spots were visualized by spraying with a Merck ninhydrin aerosol (0.1 %) and subsequent heating at 110 °C for at least 10 min.

Results

Buoyant density. All virus preparations reached equilibrium in one narrow opalescent zone, indicating that the preparations were homogeneous with respect to density. All

isolates from tomato reached equilibrium at 18 mm from the bottom of the tube. The 'Vulgare' strain appeared to have a somewhat lower buoyant density. Its band was found at 20 mm from the bottom of the tube.

S value. In the analytical ultracentrifuge most of the isolates had one peak, indicating that the preparations consisted of particles of uniform size. In some preparations a small peak appeared of faster sedimenting material (193 S). This may be attributed to aggregation of TMV to dimeric particles.

The S values of the isolates from tomato and of the 'Vulgare' strain all were 177 S in 0.01 M phosphate pH at 20 °C.

Base composition. First 6 samples of a standard RNA preparation were analyzed with 2 columns, three samples each. The results (Table 2) showed that reproducibility was very good.

The base compositions of 17 TMV isolates are presented in Table 3. The standard deviations of these data are higher than those of the standard RNA preparation.

Amino acid composition. The results of the analyses are given in Table 4. It shows that within the group of the type 1 isolates (1–5) there are only minor differences in the amino acid composition of the coat protein. Somewhat more variation is found in the group of the type 3 isolates (13–18). Most variation is present in the group of the type 2 isolates (6–12). In this group isolate 6 has a remarkably high alanine content.

Table 2. Base composition of 6 samples of a standard RNA preparation as analysed with two columns.

Nucleoside phosphate	Column					
	1			2		
	moles (%)	main value	σ	moles (%)	main value	σ
CMP	24.0			24.1		
	23.9	23.8	0.2	23.8	23.8	0.3
	23.6			23.5		
AMP	19.8			19.0		
	19.6	19.6	0.2	19.8	19.5	0.5
	19.5			19.8		
UMP	18.1			18.4		
	18.8	18.7	0.6	18.2	18.6	0.5
	19.2			19.1		
GMP	38.1			38.4		
	37.7	37.8	0.5	38.2	38.0	0.5
	37.7			37.5		

Tabel 2. Basesamenstelling van 6 monsters van een standaard-RNA-preparaat, geanalyseerd met twee kolommen.

Fig. 1. Thin-layer chromatograms of 12 TMV isolates.

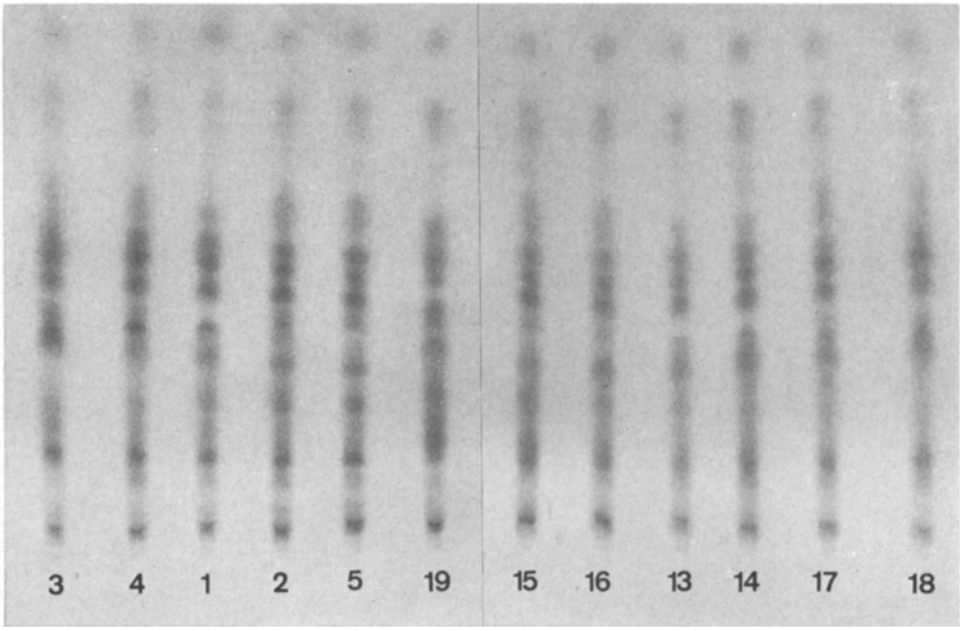

Fig. 1. Dunne-laagchromatogrammen van 12 TMV-isolaten.

Thin-layer chromatography. Thirteen separate peptides could be detected. Fig. 1 presents the chromatograms of 12 isolates. It can be seen that the small and big peptides are identical in all isolates. The medium size peptides are different, because the places of their appearance is variable. However, their number and their concentration remained quite constant throughout the series of isolates.

Conclusions and discussion

From the results presented in Table 2 it can be concluded that the accuracy of the method for the determination of the base composition is high, especially with respect to CMP and AMP. This agrees with the results of van Kammen and van Griensven (1970). We consider this method more accurate than paper chromatography as described by Knight (1963). With the latter standard deviations up to 4.0 were found (van Kammen, 1967).

There are two explanations for the high standard deviations we found in the base composition of the TMV isolates. The first is that the RNA preparations all have the same base composition but that they were less pure than the standard RNA; perhaps some protein or PEG remained attached to it, which may have interfered in the separation of the nucleoside monophosphates. The second is that a few RNA's (nrs. 5, 10, 15, 17, and 19) are different from the others. The standard deviations for the latter ones, to be calculated from the data of Table 3, are 0.7, 0.7, 0.8, and 0.6 for CMP,

Table 3. Base compositions of 17 TMV isolates.

Virus isolate No	Moles (%)			
	CMP	AMP	UMP	GMP
1	18.8	27.8	23.5	29.9
2	18.9	28.0	23.1	30.0
3	18.6	28.6	22.7	30.1
4	18.9	27.3	24.3	29.4
5	18.6	28.4	25.1	27.9
6	20.2	27.4	23.0	29.4
7	18.3	27.7	24.2	29.8
8	18.6	27.5	23.3	30.6
9	18.6	27.6	24.0	29.7
10	16.8	26.9	23.9	32.3
12	19.0	27.4	24.3	29.3
14	18.2	28.6	21.7	31.3
15	18.3	28.2	19.9	33.4
16	18.3	28.8	22.3	30.5
17	19.3	27.7	24.5	28.3
18	18.5	29.4	22.8	29.2
19	19.0	26.9	21.6	32.5
Main value	18.6	27.9	23.2	30.2
σ	0.7	0.7	1.3	1.5

Tabel 3. Basesamenstellingen van 17 TMV-isolaten.

AMP, UMP, and GMP, respectively, values well in agreement with those obtained with the standard RNA preparation. Thus the isolates may be considered to be identical with respect to base composition. It must be noted that aberrant base composition is not correlated with a special type of pathogenicity.

Small differences were found between the amino acid compositions of the different TMV isolates. These differences, however, should be interpreted with reservation, because the specificity of the trypsin digestion is not complete. There is no indication of correlation between amino acid composition of the isolates and their pathogenic type.

We could not detect any correlation between the type of pathogenicity and the pattern of the peptides of the coat protein in the thin-layer chromatograms. All differences were individual. The same was found with respect to the base composition. Here too, only slight individual differences were observed.

The fact that isolates of different pathogenic types have similar amino acid compositions and peptide patterns, conforms to the findings of Alexander et al. (1963) that no serological distinction could be made between different pathogenic types. Similar results were obtained by Tremaine and Stace-Smith (1969) and Silber and Heggestad (1965), studying amino acid composition and serology, respectively, of strains of alfalfa mosaic virus.

All 18 isolates from tomato had the same buoyant density and the same S value. The 'Vulgare' strain differed from the others only in buoyant density.

Table 4. Amino acid composition of 18 TMV isolates (moles amino acid per mole protein).

Amino acid[1]	Virus isolate No																	
	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	19
Asp	18	19	19	19	18	17	19	18	17	18	18	19	18	17	18	17	18	18
Thr	16	15	16	16	16	16	14	14	14	16	16	16	14	15	16	16	16	14
Ser	15	15	14	14	15	14	14	13	14	15	16	14	14	14	14	15	14	13
Glu	20	20	20	21	21	18	21	18	18	20	19	20	20	21	20	20	21	18
Pro	8	8	8	8	8	8	8	9	8	8	8	9	8	9	8	8	8	9
Gly	7	7	7	6	6	6	7	6	6	6	6	7	6	6	6	6	6	6
Ala	11	11	11	11	11	15	11	11	11	11	11	12	11	12	11	12	12	14
Cys	1	1	1	1	1	1	1	–	–	1	1	–	1	1	1	–	1	1
Val	15	15	15	15	14	14	16	17	18	15	15	15	16	15	15	15	15	15
Met	1	1	1	1	1	–	1	1	1	1	1	1	1	1	1	1	1	–
Iso	6	6	6	6	6	8	7	7	7	7	7	7	7	7	7	7	7	8
Leu	14	14	14	14	14	14	12	14	14	14	14	14	14	14	14	13	13	13
Tyr	5	5	5	5	5	4	5	6	6	5	5	4	5	5	5	5	5	5
Phe	8	8	8	8	8	8	9	10	9	8	8	7	9	8	8	8	8	9
Lys	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Arg	8	8	8	8	9	10	8	9	10	8	8	8	9	8	9	10	8	10

[1] Listed in sequence of elution

Tabel 4. Aminozuursamenstelling van 18 TMV-isolaten (moleculen aminozuur per eiwitmolecuul).

The similarity of buoyant densities and the S values indicates that no big differences in nucleic acid content occur. The base compositions in almost all cases were identical too; hence the differences in pathogenicity of the isolates are the results of differences in the base composition so small that they escaped detection or of differences in base sequences only. The absence of correlation between coat protein composition and pathogenicity indicates that the genetic codes for pathogenicity and coat protein composition have a different location in the polycistronic TMV-RNA. Thus determination of coat protein composition and of base composition of the RNA does not help in differentiating pathogenic types of TMV isolates from tomato.

Samenvatting

Enige chemische en fysische eigenschappen van 18 tabaksmozaïekvirusisolaten van tomaat

Van 18 tabaksmozaïekvirusisolaten van tomaat, die konden worden onderscheiden in 3 pathogeniteitsgroepen, werden de zweefdichtheid, de sedimentatiecoëfficiënt, de basesamenstelling, de aminozuursamenstelling en het gedrag van peptiden van het manteleiwit in dunne-laagchromatografie vergeleken. Er was geen verschil in zweefdichtheid en sedimentatiecoëfficiënt. Met betrekking tot de andere kenmerken konden alleen individuele verschillen worden aangetoond. Er kon geen verband worden gevonden tussen de pathogeniteit van de isolaten en hun chemische en fysische eigenschappen.

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