ACTA BOTANICA NEERLANDICA, Vol. 4 (3), 1955

GEL DIFFUSION OF TOBACCO MOSAIC VIRUS, DEMONSTRATED BY SEROLOGICAL ANALYSIS OF ITS COMPONENTS AND BY ELECTRON-MICROSCOPY

BY

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(*received July 1st, 1955*)

INTRODUCTION

As early as 1898, BEIJERINCK concluded from his experiments that the agent causing the mosaic disease in Tobacco, diffuses into agar gel.

This property of the pathogen and its filterability through bacteria-proof filters led to his theory of a 'contagium vivum fluidum'.

It has been our aim to confirm this gel diffusion of T.M.V. with the aid of some of the newer tools of virology viz. serology and electron-microscopy.

SEROLOGICAL ANALYSIS

The gel diffusion method as developed by OUCHTERLONY (1949) was used in our experiments. This method is based on the precipitation reaction that occurs when antigens and their corresponding antibodies are diffusing towards each other in an agar gel. Specifically located lines of precipitate are formed in the gel in proportion to the diffusion rates and the concentrations of the reactants.

Agar was prepared according to the recipe of BJÖRKLUND (1952). Moreover the pH of the agar media in most experiments, was adjusted to values varying between 6 and 7, by addition of phosphate buffer (Sörensen) to make the concentration of buffer salts in the media 1/15 mol.

Diffusion plates were made in a petridish according to some technical modifications of Björklund's procedure as described in detail by VAN SLOGTEREN (1954). By means of a metal matrix 5 diffusion centres are spared in the agar after its congelation viz. one square pit in the centre of the dish and four such pits surrounding it.

EXPERIMENTS

Plate I represents a photograph of the precipitation pattern of the following system: juices pressed from the leaves of 3 different Tobacco plants (*Nicotiana tabacum, var. White Burley*), infected with T.M.V. have been pipetted separately in the pits 2, 3 and 4, whereas pit 1 contained

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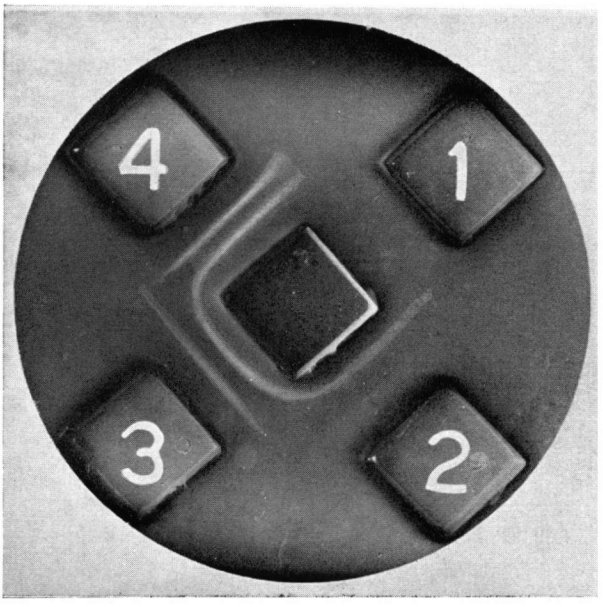

Plate I. Precipitation patterns of the separate juices pressed from the leaves of 3 Tobacco plants, infected with T.M.V., and pipetted in the pits 2, 3 and 4. Juice from virus free Tobacco leaves has been pipetted in pit 1. The centre pit contained Antiserum against T.M.V.

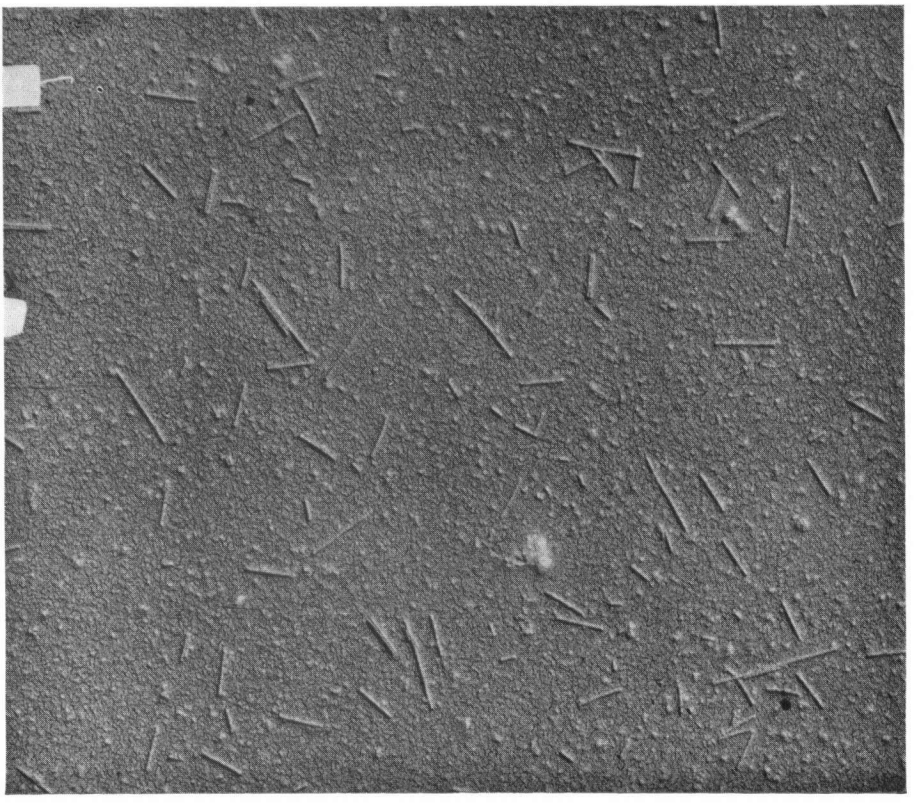

Plate II. Electron micrograph of a suspension obtained by freezing of pieces from agar diffusion medium, into which T.M.V. particles were made to diffuse from juice of infected Tobacco leaves. The distance between the white marks at the top left corner of the micrograph represents 1 micron. Magnification 25000 ×. Shadow-cast Palladium.

the sap of virus-free Tobacco. Antiserum prepared against T.M.V., diluted 1:5 with saline, has been pipetted in the centre pit.

Between the centre pit and the pits 2, 3 and 4, after five days a pattern has been formed consisting of double lines of precipitate. The inner lines nearest to the centre pit have fused into an U-shaped configuration. Furthermore distinct lines have been formed nearer to the top left and bottom left pit, while of the bottom right pit this outer line is too faint to be photographed. Towards the top right pit, filled with sap of virus-free Tobacco, no line of precipitate whatsoever has appeared.

Similar patterns of precipitation have been obtained in a great number of such experiments with Tobacco Mosaic Virus.

Thus it becomes evident that at least 2 components are present in the relevant suspensions.

It may be assumed that the lines of precipitate represent virus particles of different size and shape.

TAKAHASHI and ISHII (1953) have demonstrated a macromolecular protein to be associated with T.M.V., consisting of particles smaller than the rods of T.M.V. when observed by electronmicroscopy. This 'X protein', first demonstrated by electrophoresis, and Tobacco Mosaic Virus were subject to a cross reaction with the relevant antisera prepared to both.

Whether in our experiments the inner and outer lines of precipitate correspond resp. to 'X protein' and rod-shaped T.M.V. particles will have to be decided by a further study.

GEL DIFFUSION OF TOBACCO MOSAIC VIRUS AS EVIDENCED BY ELECTRON-MICROSCOPY

Sap of Tobacco plants, infected with T.M.V., was pipetted in the pits of a similar diffusion plate as was used in the above experiments. Great care was taken that no sap was spilled over the edge of the pits in order to make sure that it did not contaminate the upper surface of the agar.

3 days afterwards small pieces of agar were cut out of the medium at a distance of about 0,3 cm from the edge of the pits.

From these pieces the upper halves, including the top surface of the agar medium, were discarded. The remaining halves were rinsed in distilled water for 15 minutes whereafter they were put in small glass tubes. In order to obtain the solution present within the intermicellar spaces in the gel, the pieces of agar were then frozen by placing the tubes for about 16 hours at 18° C. below zero.

The suspension set free by contraction of the agar, after thawing was dialysed against distilled water for 16 hours, whereafter droplets were put on the grid of the electronmicroscope to dry by the usual method.

Plate II shows an electron micrograph of such a preparation at 25000 times magnification.

The rodshaped particles of T.M.V. are seen to be present, the greater part of which have a rather uniform length of about 250 mμ.

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