J. gen. Virol. (1981), **55**, 235–239. *Printed in Great Britain*

Key words: Phialophora *sp./virus isolates/physicochemical properties*

Two Serologically Unrelated Viruses Isolated from a *Phialophora* sp.

(*Accepted 3 March 1981*)

SUMMARY

Two isometric viruses, with similar diameters (34 to 36 nm), obtained from an isolate of a *Phialophora* sp. with lobed hyphopodia were separated by salt gradient elution from DEAE-cellulose and from SP-Sephadex. Virus A had a sedimentation coefficient of 116S, three double-stranded RNA components of mol. wt. $1{\cdot}29 \times 10^6$, $1{\cdot}22 \times 10^6$ and $1{\cdot}03 \times 10^6$ and one major capsid polypeptide, mol. wt. 60000. Virus B had a sedimentation coefficient of 122S, three double-stranded RNA components of mol. wt. $1{\cdot}32 \times 10^6$, $1{\cdot}25 \times 10^6$ and $1{\cdot}03 \times 10^6$ and one major capsid polypeptide, mol. wt. 66000. The two viruses were unrelated serologically to each other and to 13 viruses obtained from a related fungus *Gaeumannomyces graminis* var. *tritici*.

Although isometric virus-like particles have been reported to occur in more than 100 species of fungi (Hollings, 1978; Buck, 1980), very few have been adequately characterized and many more data are required if progress is to be made in their taxonomy (Hollings, 1979). As part of our programme investigating possible biological effects of viruses on the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici* (hereafter called *G. graminis*) and related fungi we have detected isometric particles in several isolates of a *Phialophora* sp. with lobed hyphopodia, an avirulent cereal root parasite related to *G. graminis* (Walker, 1981). In the present paper we report on the separation and properties of two viruses obtained from one of these isolates.

A *Phialophora* sp. with lobed hyphopodia, designated as isolate 2-2, was grown in a 60 l fermenter for 3 days in a glucose–corn steep liquor medium and the filtered mycelium was suspended in three times its wet wt. of 0·03 M-sodium phosphate buffer pH 7·6 (P buffer) and disrupted by passage through a Manton-Gaulin homogenizer (The A.P.V. Co. Ltd., Crawley, Sussex, U.K.) at 8000 lb/in^2. Virus was partially purified from the homogenate by polyethylene glycol precipitation and differential centrifugation using the method of Buck *et al.* (1981) and was further purified by sucrose density-gradient sedimentation. Partially purified virus (1500 A_{260} units) was loaded on to a 20 to 50% (w/w) linear sucrose gradient (500 ml in P buffer) in an MSE B XIV zonal rotor (MSE Scientific Instruments, Crawley, Sussex, U.K.) and centrifuged at 47000 rev/min for 3 h. After centrifugation the gradient was passed through an ISCO Model 222 u.v. analyser and 5 ml fractions were collected. The material in a u.v.-absorbing peak, about halfway down the gradient, was shown by electron microscopy to comprise isometric particles of diam. 34 to 36 nm. Nucleic acid, isolated from the purified virus particles by phenol/SDS extraction, was shown to be double-stranded (ds)RNA by its differential susceptibility to ribonuclease A in buffers containing high or low salt concentrations (Bellamy *et al.*, 1967). Analysis and mol. wt. determination of viral dsRNA by polyacrylamide gel electrophoresis, using the methods described by Buck & Ratti (1977), revealed five components with mol. wt. $1{\cdot}32 \times 10^6$, $1{\cdot}29 \times 10^6$, $1{\cdot}25 \times 10^6$, $1{\cdot}22 \times 10^6$ and $1{\cdot}03 \times 10^6$.

Purified virus preparations, denatured by boiling with 1% SDS and 1% 2-mercapto-ethanol, were subjected to electrophoresis in polyacrylamide–SDS gels, using the methods

0022-1317/81/0000-4541 $02.00

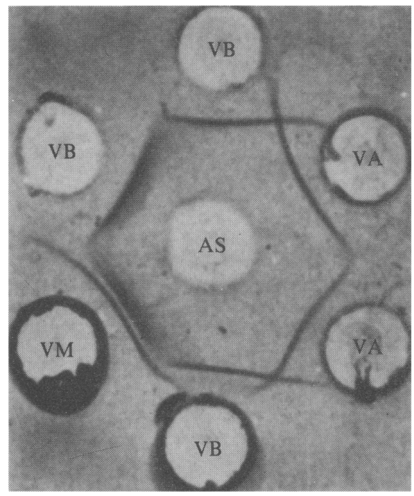

Fig. 1. Gel immunodiffusion tests with *Phialophora* viruses. AS, Antiserum to viruses A and B; VM, mixture of viruses A and B; VA, virus A; VB, virus B. The gel was stained with Coomassie Blue.

and standards described by Buck & Kempson-Jones (1974); Coomassie Blue staining revealed two polypeptide components of mol. wt. 66000 and 60000. When undenatured virus preparations were subjected to electrophoresis in 0·5% agarose tube gels, as described by Ratti & Buck (1972), two components with mobilities of about $1{\cdot}2 \times 10^{-5}$ and $3{\cdot}8 \times 10^{-5}$ $cm^2/s/V$ towards the anode were detected by staining gels with either Coomassie Blue or with toluidine blue. The fact that virus particles took up both stains indicated their nucleoprotein nature. After electrophoresis and staining the agarose gel was sliced and the gel slices corresponding to each of the two nucleoprotein components were separately boiled with 1% SDS and 1% 2-mercaptoethanol and the products were loaded on 8% polyacrylamide–SDS tube gels. Electrophoresis and staining showed that the nucleoprotein with the slower electrophoretic mobility in agarose gel electrophoresis contained the polypeptide of mol. wt. 60000, whereas the component with the faster mobility contained the mol. wt. 66000 polypeptide. These results suggested either that isolate 2-2 contained two distinct viruses with similar diameters, as has been found in several fungi (Hollings, 1978) or that the virus particles may have been partially degraded by a protease giving rise to two electrophoretic forms, as has been shown with cowpea mosaic virus (Geelen *et al.*, 1973). To help to distinguish between these two possibilities a rabbit antiserum to the purified virus particles was prepared using the method of Hollings (1962). When purified virus preparations were allowed to diffuse against this antiserum in gel immunodiffusion tests, two clear precipitin lines were formed (Fig. 1), consistent with the presence of two distinct viruses.

The two viruses were separated by chromatography on a column of an anion exchanger by an adaptation of the method of Buck & Kempson-Jones (1973). Purified virus preparation (4 ml, $A_{260} = 5$) in P buffer was run on to a column of DEAE-cellulose (Whatman DE 52, 10 ml) (anion exchanger), previously equilibrated with P buffer. The column was eluted first with P buffer (10 ml) and then with a linear gradient of 0·6 M-NaCl (50 ml) in P buffer at a flow rate of 20 ml/h; 0·5 ml fractions were collected and analysed by agarose gel electrophoresis as above. The virus with the slower electrophoretic mobility in agarose (virus A) was eluted in P buffer and was homogeneous. The virus with the faster electrophoretic mobility (virus B) was eluted with 0·4 M-NaCl, but contained a trace of virus A. For further purification virus B was dialysed against 10 mM-sodium acetate buffer pH 4·5 and adsorbed on to a column of SP-Sephadex (10 ml) (cation exchanger) previously equilibrated with the same buffer. The

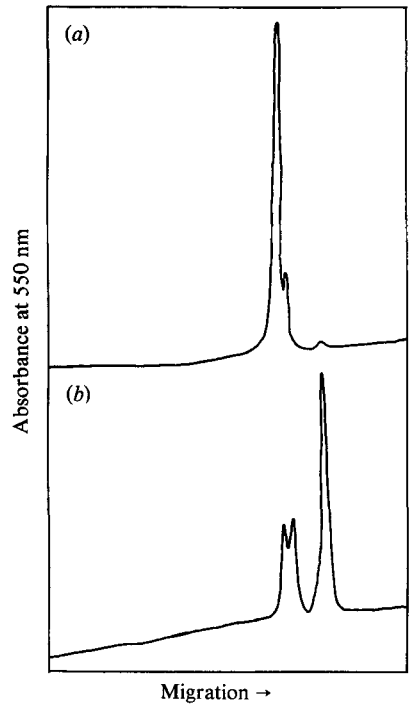

Fig. 2. Electrophoresis patterns of dsRNA isolated from *Phialophora* viruses: (*a*) virus B; (*b*) virus A.

column was eluted first with the acetate buffer (10 ml) and then with a linear gradient of 0 to 1 M-KCl in the same buffer (50 ml). From this column, virus B was eluted first with 0·07 M-KCl, whereas virus A eluted with 0·3 M-KCl.

Virus A consisted of isometric particles of diam. 34 to 36 nm and $s^0_{20} = 116S$ (in P buffer). It contained one major polypeptide of mol. wt. 60000 and three dsRNA components of mol. wt. $1{\cdot}29 \times 10^6$, $1{\cdot}22 \times 10^6$ and $1{\cdot}03 \times 10^6$. Virus B consisted of isometric particles also of diam. 34 to 36 nm and $s^0_{22} = 122S$ (in P buffer). It contained one major polypeptide of mol. wt. 66000 and three dsRNA components of mol. wt. $1{\cdot}32 \times 10^6$, $1{\cdot}25 \times 10^6$ and $1{\cdot}03 \times 10^6$. In gel immunodiffusion tests using the antiserum described above, each virus gave rise to a single precipitin line. When the two viruses were placed in adjacent wells, crossing precipitin lines were obtained (Fig. 1), indicating that the two viruses were serologically unrelated. When the antiserum was titrated in twofold serial dilutions against each virus, the antiserum titre was found to be 1:2048 against virus A and 1:512 against virus B.

The proportions of the dsRNA components in each virus, as measured from the area of the peaks in toluidine blue-stained polyacrylamide gels after electrophoresis (Fig. 2), were not equal, suggesting that the three dsRNA components were not enclosed in a single particle, but were probably encapsidated separately as has been found for several other dsRNA mycoviruses (Hollings, 1978). Further evidence to support this view was obtained by polyacrylamide gel electrophoretic analysis of dsRNA prepared from virus fractions obtained from the sucrose density gradients used for virus purification. Some fractions on the slower sedimenting side of the peak contained only the $1{\cdot}03 \times 10^6$ mol. wt. RNA, whereas fractions on the faster sedimenting side were enriched in the higher mol. wt. RNAs.

Both viruses contained a dsRNA of mol. wt. $1{\cdot}03 \times 10^6$. The small amount of RNA of this mol. wt. found in virus B preparations was not due to contamination with virus A. Traces of virus A which remained in virus B after separation on the anion exchanger DEAE-cellulose

were removed by separation on the cation exchanger SP-Sephadex from which virus B was eluted first. After this second column stage, no virus A could be detected in virus B preparations by immunodiffusion analysis, by agarose gel electrophoresis of intact virions or by polyacrylamide gel electrophoresis of virus polypeptides, and neither of the other two dsRNAs of virus A could be detected in virus B. The occurrence of a dsRNA of the same mol. wt. in the two viruses could be due to genomic masking (encapsidation of the RNA of one virus by the capsid of another) or, to the necessity of having this gene for say replication in *Phialophora* sp. However, identity of mol. wt. does not necessarily indicate identity of nucleotide sequence and the two RNAs could be unrelated.

The dsRNA mycoviruses are transmitted only intracellularly, during fungal growth, in spores or via hyphal anastomosis (cell fusion). They, therefore, may be expected to have a limited natural host range and the occurrence of serologically related viruses has, in general, been confined to related fungi (Hollings, 1978). Recently, 13 isometric viruses from *G. graminis*, a fungus related to *Phialophora* sp. with lobed hyphopodia (Walker, 1981), have been characterized and classified into three groups on the basis of their physicochemical and serological properties (Buck *et al.*, 1981). On the basis of its physicochemical properties virus A from *Phialophora* sp. with lobed hyphopodia isolate 2-2 could be classified with the *G. graminis* group I viruses. Virus B, on the other hand, has a sedimentation coefficient and dsRNA mol. wt. similar to the group I viruses, but its polypeptide mol. wt. is closer to that of the group II viruses. In gel immunodiffusion tests none of the 13 *G. graminis* viruses, 11 of which were in groups I and II, reacted with the antiserum to the *Phialophora* viruses A and B described here, nor did viruses A and B react with any of eight antisera containing antibodies to a total of 11 of the *G. graminis* viruses. However, the absence of serological cross-reactions does not preclude other relationships between these viruses. The antigenic determinants of a virus are encoded by a comparatively small portion of its genome and a fairly small number of mutations may lead to marked changes in its serological properties. Studies of nucleotide sequence homology of the viral dsRNAs, preferably carried out by direct comparison of sequences, may provide a greater insight into possible relationships between the viruses of these two fungal species.

We thank Mr R. D. Woods for electron microscopy and the Science Research Council for the award of a C.A.S.E. studentship (to R.M.M.).

Department of Biochemistry
Imperial College of Science and Technology
London SW7 2AZ, U.K.

K. W. BUCK*
R. M. MCGINTY†

[1] *Plant Pathology Department*
Rothamsted Experimental Station
Harpenden, Herts AL5 2JQ, U.K.

C. J. RAWLINSON[1]

† Present address: Department of Medical Oncology, Southampton General Hospital, Southampton, U.K.

REFERENCES

BELLAMY, A. R., SHAPIRO, L., AUGUST, J. T. & JOKLIK, W. K. (1967). Studies on reovirus RNA. I. Characterisation of reovirus genome RNA. *Journal of Molecular Biology* **29**, 1–17.

BUCK, K. W. (1980). Viruses and killer factors of fungi. In *The Eukaryotic Microbial Cell. Society for General Microbiology Symposium*, vol. 30, pp. 329–375. Edited by G. W. Gooday, D. Lloyd and A. P. J. Trinci. Cambridge: Cambridge University Press.

BUCK, K. W. & KEMPSON-JONES, G. F. (1973). Biophysical properties of *Penicillium stoloniferum* virus S. *Journal of General Virology* **18**, 223–235.

BUCK, K. W. & KEMPSON-JONES, G. F. (1974). Capsid polypeptides of two viruses isolated from *Penicillium stoloniferum*. *Journal of General Virology* **22**, 441–445.

BUCK, K. W. & RATTI, G. (1977). Molecular weight of double-stranded RNA: a re-examination of *Aspergillus foetidus* virus S RNA components. *Journal of General Virology* **37**, 215–219.

BUCK, K. W., ALMOND, M. R., McFADDEN, J. J. P., ROMANOS, M. A. & RAWLINSON, C. J. (1981). Properties of thirteen viruses and virus variants obtained from eight isolates of the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici*. *Journal of General Virology* **53**, 235–245.

GEELEN, J. L. M. C., REZELMAN, G. & VAN KAMMEN, A. (1973). The infectivity of the two electrophoretic forms of cowpea mosaic virus. *Virology* **51**, 279–286.

HOLLINGS, M. (1962). Viruses associated with a die-back disease of cultivated mushroom. *Nature, London* **196**, 962–965.

HOLLINGS, M. (1978). Mycoviruses – viruses that infect fungi. *Advances in Virus Research* **22**, 3–53.

HOLLINGS, M. (1979). Taxonomy of Fungal Viruses. In *Fungal Viruses*, pp. 165–175. Edited by H. P. Molitoris, M. Hollings and H. A. Wood. Berlin, Heidelberg and New York: Springer-Verlag.

RATTI, G. & BUCK, K. W. (1972). Virus particles in *Aspergillus foetidus:* a multicomponent system. *Journal of General Virology* **14**, 165–175.

WALKER, J. (1981). Taxonomy of take-all fungi and related genera and species. In *Biology and Control of Take-all*. Edited by M. J. C. Asher and P. J. Shipton. New York and London: Academic Press (in press).

(*Received 12 December 1980*)