J. Vet. Malaysia (1992) 4(2): 133-138

GROWTH AND PURIFICATION OF TWO LOCAL ISOLATES OF AVIPOX VIRUS

Yusoff, K., Shamaan, N.A., Menon, A. and *Ibrahim, A.L.

Department of Biochemistry and Microbiology,
**Department Of Veterinary Pathology and Microbiology,*
Universiti Pertanian Malaysia,
43400 UPM, Serdang.

SUMMARY: Two local isolates of the avipoxvirus group, a pigeon pox and a fowlpox, were grown on chorioallantoic membranes (CAM) of 9-12 day embryonated chicken eggs. The viruses were harvested and purified using differential and sucrose gradient centrifugations. It was found that the optimal incubation period was 3 days at 37°C. The yield for the pigeon pox virus was lower than that of the fowlpox virus suggesting that CAM is not ideally suited for its growth. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis analysis of the final viral samples showed the presence of more than 30 viral proteins. The protein profiles of the two viruses were similar. These proteins have yet to be identified.

Keywords: Fowl pox virus, pigeon pox virus, viral purification, SDS-PAGE analysis

INTRODUCTION

The Poxviridae are the largest and most complex animal virus that replicate within the cytoplasm of infected cells. The completely depend on the host cell's machinery for their protein synthesis and most other functions. Only transcription and DNA replication, which occur in the cytoplasm, use viral-coded enzymes. The avipoxvirus, in particular the fowlpox virus (FPV), has generated a lot of interest as it has the potential to be developed as a vector for the expression of foreign genes in an manner analogous to that successfully employed with the vaccinia virus (Mackett and Smith, 1986). Poultry vaccine antigens can be delivered using recombinant fowlpox virus (Coupar and Boyle, 1988; Taylor *et al.*, 1988) as host-specific live viral vectors. Thus, they would be potential vectors for veterinary vaccines (Campbell *et al.*, 1989).

The first step towards this end is the detailed genomic and biological characterisation of the virus. Many of these studies require the use of DNA that is isolated from purified virions. As a preliminary step in the biological study of the viruses, a method for the purification of the poxviruses from chorioallantoic membrane (CAM) has been developed. This method, which is a modification of the method reported by Joklik (1962), is presented in this paper.

MATERIALS AND METHODS

Fowlpox virus (virulent strain; FPV-A) isolate was a gift from the Veterinary Research Institute, Ipoh. It was twice pock-purified from the CAM of embryonated eggs. The pigeonpox virus (virulent strain; PPV-A) was isolated in the Universiti Pertanian Malaysia.

The viruses were inoculated and grown on the CAM of 9-12 day old embryonated chicken eggs at 37°C. After 3-5 days incubation, the viruses were harvested. The membranes and the allantoic fluid from approximately 200 eggs inoculated with the virus were homogenised in twice the original volume of Tris buffer (0.01 M Tris-HCl, pH 9.0) at 4°C. All subsequent work was carried out at 4°C. The homogenate was centrifuged at 750 *g* for 10 min. The supernatant was kept and the pellet was back-extracted by adding twice the volume of Tris buffer. The pellet was again homogenised and centrifuged at 750 *g* for 10 min. The supernatants were pooled followed by a final centrifugation at 750 *g* for 10 min. The resulting supernatant was then centrifuged at 15,000g for 30 min after which the pellet was resuspended in 50 mL Tris buffer. The viral suspension was then layered onto a 36% sucrose cushion and centrifuged at 13,000 *g* for 80 min. The pellet was resuspended in a minimal volume of Tris buffer and purified over a 25 to 55% (w/v) sucrose gradient centrifugation at 39,000 *g* for 80 min. The virus band (Fig. 1) which was formed approximately a quarter way up from the bottom of the centrifuge tube was carefully removed with a pasteur pipette and pelleted at 27,000 *g* for 30 min. The final pellet obtained was assessed for purity and bacterial contamination by electron microscopy. Electron microscope grids were prepared using negative staining with 2% (v/v) phosphotungstic acid (PTA).

In order to obtain the protein profiles of the two viruses, aliquots of the purified viral samples were solubilised in 62.5 mM Tris-HCl (pH 6.8), 5% (v/v) 2-mercaptoethanol, 10% (w/v) glycerol, 2.3% (w/v) sodium dodecyl sulphate, 0.001% (w/v) bromophenol blue by boiling for 20 min. before cooling on ice. The samples were then subjected to electrophoresis in 12% SDS-polyacrylamide gel according to the method of Laemmli (1970). The gel was run initially at 25 mA until the bromophenol blue dye front reached the top of the separating gel after which 18 mA constant current was applied until the dye front reached the bottom of the gel. The separated polypeptides were stained with Coomassie Brilliant Blue R-250.

The concentration of viral antigens was assessed by determining the haemagglutinating (HA) activity of the purified samples obtained.

RESULTS

The optimal incubation period for the growth of the viruses was three days at 37°C. After this period, the membranes began to disintegrate due to the confluent growth. The confluent membranes were characterised by thickening and increased opacity (Fig. 2) as opposed to a clear and thin membrane of a healthy, normal egg.

The yield for PPV-A (32 HA units) was lower than that of FPV-A (1024 HA units).

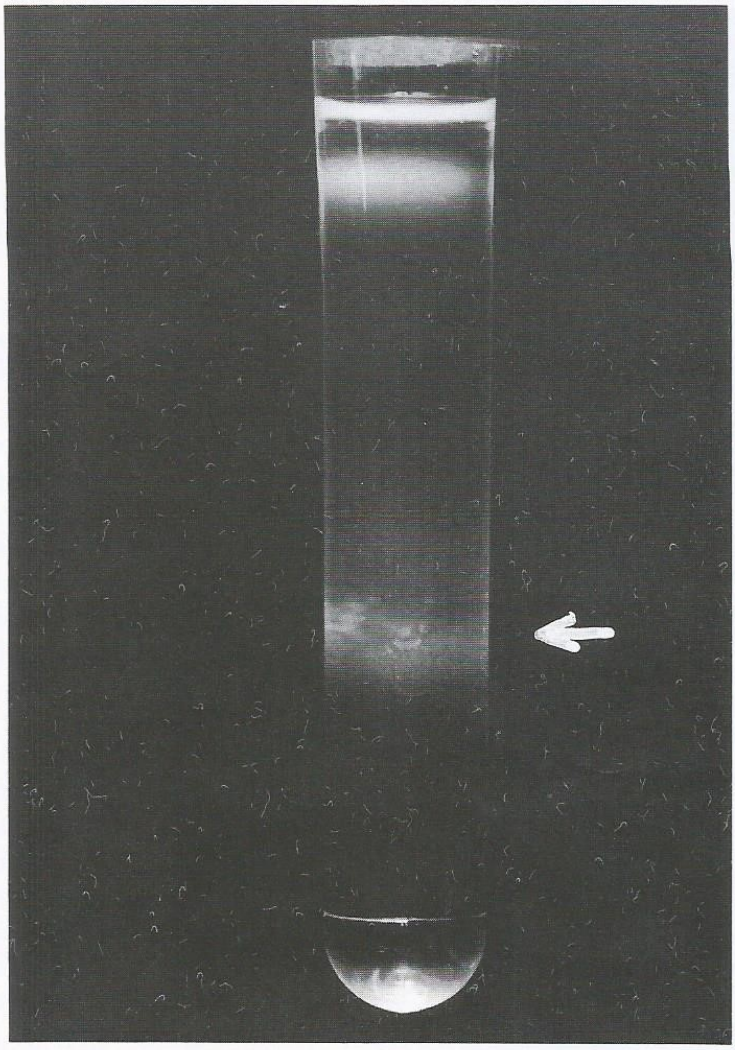

Figure 1. The appearance of the virus FPV-A band (as shown by the arrow) after being subjected 25-55% (w/v) sucrose gradient centrifugation at 39,000g for 80 min.

The viral preparations were centrifuged twice in sucrose gradients; the first was carried out in a 36% cushion while the second step involved a gradient of 25 to 55% (w/v) sucrose. During the course of the experiment, it was found that the use of a 0.5 ml step gradient of 25-55% (w/v) sucrose gave a much sharper band (Fig. 2) than a 1.0 ml step gradient.

The electron micrograph obtained showed that the virions ranged in size from 258 to 354 nm. It was evident that the samples obtained by the procedure were homogeneous and free from bacterial contamination.

Up to 28-30 polypeptides (Fig 3.) were observed when the viruses were subjected to SDS-PAGE according to the method of Laemmli (1970). The relative molecular mass (rmm) of the polypeptides ranged from greater than 45 kDa to less than 14 kDa. The most abundant polypeptides were heavily stained with two of these greater than 45 kDa rmm and one 24 kDa rmm.