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Some biological properties of a rhabdovirus isolated from penaeid shrimps

Brief Report

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Summary. Some of the relevant biological properties of a rhabdovirus isolated from penaeid shrimps (RPS) were examined. The virus replicated in an established fish cell line, epithelioma papulosum cyprini (EPC) which allowed for the development of a quantitative plaque assay protocol. Virus replication was not inhibited by the DNA antagonist, 5-bromo-2′-deoxyuridine (20 μg/ml). Virus infectivity was sensitive to 20% ethyl ether, low pH, and to 37 °C. The virus showed marked lability to repeated freezing and thawing and storage at −10 °C, but was stable at −70 °C for several weeks. The virus particle to infectious unit ratio in EPC was found to be 30.

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We recently reported the isolation of a rhabdovirus from infectious hypodermal hematopoietic necrosis virus (IHHNV) infected penaeid shrimps and which replicated in an established fish cell line, epithelioma papulosum cyprini (EPC) [1]. Although rhabdoviruses have been found in invertebrates [2], the recent isolate represents the first rhabdovirus to be isolated from penaeid shrimps (RPS) and also to replicate in cell culture. Initial studies indicated that the RPS could be distinguished from some of the fish rhabdoviruses on the basis of serum neutralization kinetics, plaque reduction and Western blot analysis of the viral proteins (manuscript submitted). The present report summarizes some of the relevant elemental biological properties of the RPS and its efficiency of plating (EOP) in EPC cells.

The procedures for the recovery of RPS from infected penaeid shrimps, its purification and growth in EPC cells have been described [1]. Stocks of poliovirus type 1, vaccinia virus and rhabdovirus carpio were prepared as previously reported [3, 4].

The following cell cultures: EPC, grass carp fin cells (GCF), grass carp swim bladder cells (GCSB), grass carp snout cells (GCS-2), fathead minnow cells (FHM), chinook salmon embryo cells (CHSE-214), rainbow trout gonad cells (RTG-2), and brown bullhead caudal trunk cells (BB) were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (10FBS) at 20 °C [4].

For the plaque assay monolayers of EPC cells grown in 24-well plastic plates were inoculated with varying 10-fold dilutions of RPS. After adsorption for 1.5 h at room temperature (rt) on a rocking platform to ensure uniform distribution of the inoculum, the cultures were washed with MEM, thoroughly drained, and a semi-solid overlay medium consisting of 0.75% methylcellulose plus MEM4FBS was introduced. After incubation for 4–5 days at 20 °C, the cultures were fixed and stained with 0.6% crystal violet-formalin for 0.5 to 1 h. The semi-solid medium was then carefully removed, the cultures washed with tap water and the number of plaques formed were counted.

The number of viral particles to the number of infectious units ratio was determined according to the method previously reported by this laboratory [5].

The plaques induced in EPC by RPS were distinct and easily visible as early as 3 days post-infection (p.i.) (Fig. 1). Their size and number increased up to the 5th day after which time the number of plaques did not significantly alter. The plaques were 0.65 to 0.95 mm in diameter. The number of plaques formed was found to have a linear relationship with dilution which was reproducible within ± 10%. Under similar conditions the other fish cell lines examined produced markedly fewer plaques or no plaques (Fig. 2).

The adsorption of RPS to EPC cells at rt was determined to be maximal by 90 min p.i. The optimal temperature for virus replication was 20 °C and under single cycle of growth conditions cell-associated virus (CAV) was detected after an eclipse period of 3 h with released virus not detected until the 10th hour p.i. Released virus continued to increase until at 50 h p.i. when a yield of 10^8 PFU/ml was obtained (Table 1).

Some of the relevant biological characteristics of RPS were analyzed (Table 1). Virus replication in EPC cells was not inhibited by the DNA antagonist, 5-bromo-2′-deoxyuridine (BUDR, 20 μg/ml). The virus was sensitive to 20% ethyl ether, low pH and to temperature at 37 °C. In this latter aspect the RPS showed marked lability, readily losing infectivity after repeated freezing and thawing and storage at − 10 °C; in contrast, the virus was stable at − 70 °C for several weeks.

To obtain the ratio of the number of viral particles per infectious unit or the EOP of the RPS in EPC cells, the virus titer and counts of viral particles were made on the same virus sample. To determine the precision of the counting method, several counts were made on the same RPS sample and the maximum deviation in ten counts was 18% and the average deviation was 7.2% (Table 2). The counts shown in the table refer to the number of viral particles found in 4.3×10^{-8} cm^2 area. Since the column height was 0.6 cm, the dilution was 100,

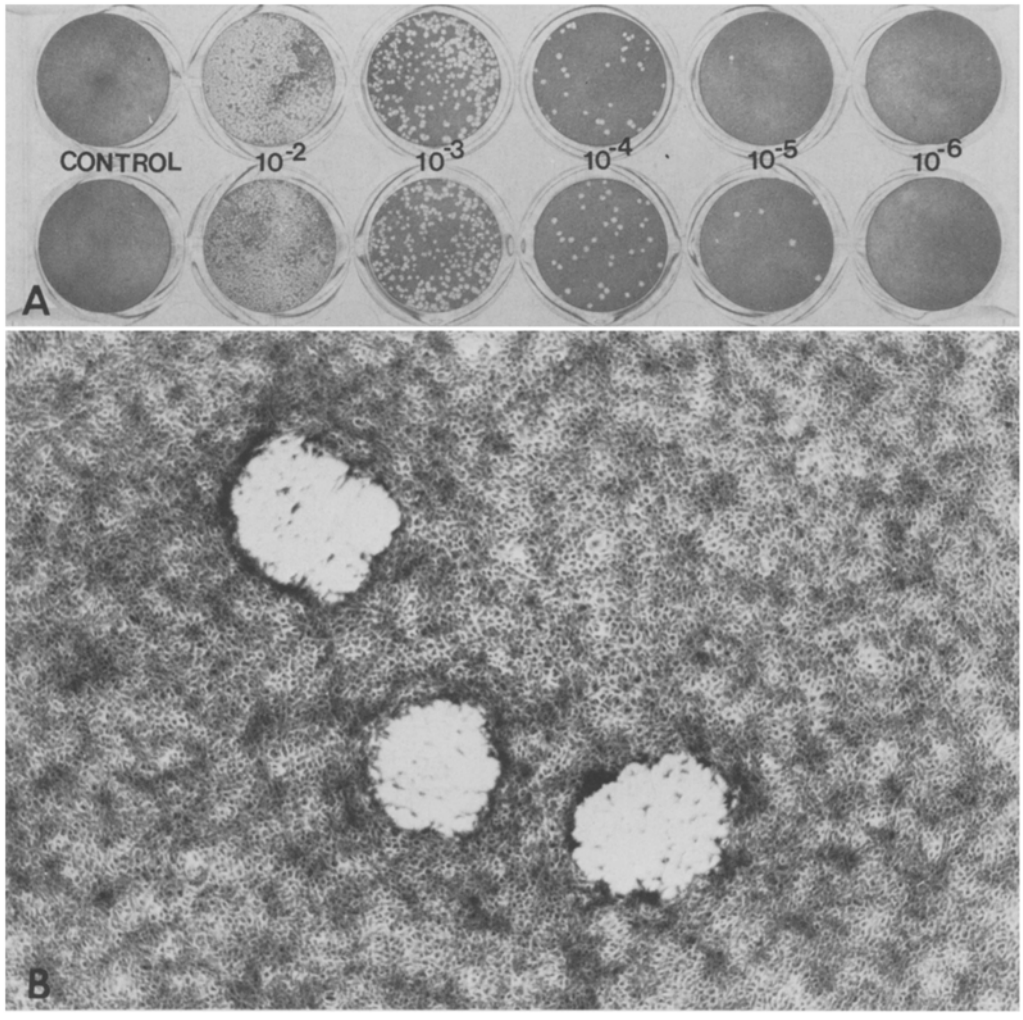

Fig. 1. **A** RPS-induced plaques in EPC cells stained with 0.6% crystal violet-formalin. **B** Enlargement of plaques

and the average count was 46.5, the average number of virus particles per milliliter was calculated to be 1.8×10^{11} particles/ml. Since the virus sample had a virus infectivity titer of 6.0×10^{9} PFU/ml the EOP was calculated to be 30 particles per infectious unit.

The present studies of the RPS isolated from penaeid shrimps have demonstrated that it has many of the biological properties characteristic of the enveloped rhabdoviruses [6]. Although this penaeid shrimp virus replicated in an aneuploid fish cell line, it did not grow well in the three normal grass carp cell lines and four other fish cell lines tested. The mechanism for the relatively greater resistance of these cell lines to RPS remains to be analyzed.

Little is known regarding the disease-causing capacity of RPS in the penaeid shrimp. Although the virus was originally isolated from three different sources

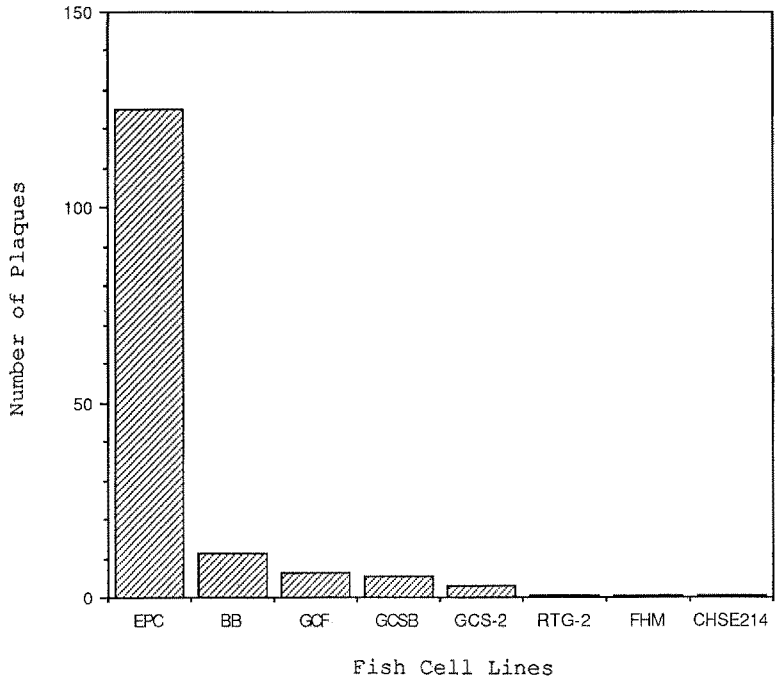

Fig. 2. Plaque forming efficiency of RPS in different fish cell cell lines: *EPC* epithelioma papulosum cyprini, *BB* brown bullhead, *GCF* grass carp fin, *GCSB* grass carp swim bladder, *GCS-2* grass carp snout, *RTG-2* rainbow trout gonad, *FHM* fathead minnow, *CHSE-214* chinook salmon embryo

Table 1. Some biological characteristics of the rhabdovirus of penaeid shrimp (RPS)

Replication affected by	
5-bromo-2′-deoxyuridine (20 μg/ml)	−
Single cycle growth	
Eclipse period	3–5 h
Maximum virus production	50 h
Sensitivity to	
20% ethyl ether (2 h, rt)	+
pH 3 (3 h, rt)	+
37 °C (12 h)	+
−10 °C (4 weeks)	+
−70 °C (4 weeks)	−
Freezing-thawing (3 ×)	+

of penaeid shrimps histopathologically diagnosed with IHHN disease [1], its capacity to infect and cause disease in the penaeid host is not known. Our preliminary studies suggest that the RPS can infect a subadult species of penaeid shrimp, *Penaeus stylirostris* causing cytopathic alterations in their lymphoid

Table 2. Precision of rhabdovirus of penaeid shrimp particle counting

Replicates[a]	Particels/unit area[b]	Deviation[c] (%)
1	40	14
2	49	5
3	51	10
4	45	3
5	46	1
6	49	5
7	47	1
8	38	18
9	53	14
10	47	1
average	46.5	7.2

[a] Replicate counts taken from different area of the same grid
[b] Number of particles counted within a 4.3×10^{-8} cm^2 area
[c] Deviation from average computed as follows:
(Difference between actual and average count)/(Average count) $\times 100$

Oka organs (manuscript submitted). Although no overt symptoms were observed, additional comprehensive studies on the disease causing capacity of the RPS is required.

Acknowledgements

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