ISOLATION OF A NEW AQUAREOVIRUS FROM GILTHEAD SEA BREAM CULTURED IN GALICIA (N.W. SPAIN)

BY I. BANDÍN 1, C. RIVAS 1, M. NOVA 2, J.M. CYPHER 3, J.L. BARRA 4 & C.P. DOPAZO 1

Introduction
During early autumn of 1994 moderate mortalities were observed among gilthead seabream (Sparus aurata) fry cultured in Galicia (N.W. Spain). Fish showing no external symptoms of disease were examined for the presence of bacterial and viral agents. Whereas no bacteria were recovered from the samples, the virological analysis conducted to the isolation of an apparently previous undescribed virus. In the present paper we show the preliminary results obtained in the characterisation of this viral isolate.

Materials and Methods
Cell lines
The following fish cell lines were used in this study: epithelioma papulosum cyprini (EPC), rainbow trout mesothelium (RTM), chinook salmon embryo (CHSE-214) and brown bullhead (BB). All cell lines were propagated in Eagle’s minimum essential medium (EMEM) supplemented with 10% foetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. The RTM and CHSE-214 cells were grown at 15°C and EPC and BB at 25°C.

Virus isolation
Poools of 10 fish (0.5 g average weight) were mixed 1.5 with Earle’s buffer supplemented with antibiotics and homogenised using a stomacher. After centrifugation of the homogenates at 2000 x g during 15 min, the supernatants were transferred to new tubes and incubated 4 h at 4°C. The supernatants were inoculated onto monolayers of EPC and RTM cells and incubated at 15°C. Control (non-infected cells) and infected cultures were daily examined for 3 weeks to detect development of cytopathic effect (CPE).

Physico-chemical characterisation
The sensitivity of the virus to chloroform was examined basically as previously described (Vestergard-Jørgensen 1972). Viral stability in acid and alkaline conditions was determined following the procedure of Meyers (1979). Thermostability was tested according to the method of Plumb et al. (1979). Torfot aquareovirus (TRV, a non-enveloped virus) and Infectious haematopoietic necrosis virus (IHNV, an enveloped virus) were used as controls.

To determine whether the virus contained DNA or RNA the effect of the DNA inhibitor bromo-2-deoxyuridine (BDU) on the virus was examined as described by Plumb et al. (1979). In addition, viral samples were treated with RNase, which degrades single stranded RNA, following the method of Gornats and Tamm (1963). TRV (a dsRNA virus), IHNV (a ssRNA virus) and the channel catfish virus (CCV, which is a DNA virus) were used as controls.

Viral titration was determined according to the method of Reed and Muench (1938) using RTM cells for the viral isolate and TRV, EPC for IHN virus and BB cells for CCV. Loss of infectivity greater than 1 log was evidence of susceptibility to the treatment.

Electron microscopy
To study the morphology of the viral isolate, the virus was inoculated in 150cm² flasks containing monolayers of RTM cells. After CPE was extensive, cells were scraped off and the suspension centrifuged at low speed to pellet cell debris. Virus from the supernatant was concentrated in 1 ml of SSC...
Nucleic acid analysis

Genome profile of the viral isolate was analysed by electrophoresis in SDS-polyacrylamide gels (SDS-PAGE) and the bands visualised following staining with ethidium bromide.

Results & Discussion

Processed samples were inoculated on RTM and EPC cells for virus isolation. After an incubation period of 3 weeks, when second blind passage had already been considered as negative for presence of virus, appearance of focal areas of syncytia was observed on RTM cells. The supernatant of the infected cells was again inoculated onto RTM and CHSE-214 cells, and development of CPE, which was similar in both cell lines, was observed in 1 week. As shown at Fig 1A, syncytia first appeared as plaques containing a variable number of nuclei surrounded by a clear area. As CPE was progressing, number and size of syncytia increased, and nuclei were not observed in the internal area of the plaques (Fig 1B).

The physico-chemical characterisation of the viral isolate revealed that it was resistant to the treatment with chloroform, stable at both acid and alkaline pH as well as at high temperatures (50°C). These results indicated that the virus was non-enveloped. Since most of the non-enveloped virus isolated in our area belong to Brevirubivirus, neutralisation test with IPNV specific antisera was performed. However, the incubation with these antisera failed to affect infectivity of the viral isolate, which enabled us to rule out the possibility that it was an IPNV virus. When examined by electron microscopy, the virions appeared as isometric particles with a diameter of 75nm. In some occasions and inner capsid of around 45-50nm was observed. The viral agent was resistant to treatment with BUO and RNase, indicating that the virus contained double stranded RNA. Moreover electrophoresis in SDS-PAGE gels demonstrated that the viral genome was distributed in 11 segments (Fig 2).

The characteristics determined so far for this viral isolate are shared by viruses belonging to the family Reoviridae. During the last years reoviruses have been isolated from a variety of aquatic animals (Hetrick et al. 1992) and these isolates have been grouped in the new genus Aquareovirus (Francki et al., 1991).

We believe that the present study represents the first isolation of an aquareovirus from gilthead sea bream and propose the name of Sea Bream Aquareovirus (SBA).

Summary

A viral agent was isolated from gilthead sea bream suffering moderate mortalities. The viral isolate appeared to be a reovirus, because its cytopathic effect, its morphology (75 nm isometric particles with double capsid), its resistance to lipid solvents and its genome (double stranded RNA distributed in 11 segments).

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References


