Isolation and Identification of a Novel Reovirus by Using a Newly Established Cell Line Derived from Kidney of Channel catfish, *Ictalurus punctatus*, Rafinesque

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Channel catfish (*Ictalurus punctatus*)

- One of the most popular species of farm-raised fish (global annual output: ~440,000 tons, FAO, 2006; ~480 million $/yr industry in USA, 2008);
- A couple of advantages for artificial culture;
- Imported to China in 1984;
- Rapid growth of a artificially cultured species in China (~180,000 tons, 2006).

Major diseases of Channel catfish

- Channel catfish virus disease (CCVD), caused by a herpesvirus 1 designated by the ICTV, but the commonly used name is channel catfish virus (CCV);
- Enteric Septicemia of Catfish, ESC , caused by *Edwardsiella ictaluri* ;
- Columnarris, caused by *Flavobacterium columnare*;
- Intestinal intussusception, caused by *Stenotrophomonas maltophilia* (??);
- Channel catfish Hemorrhage, caused by CCRV .

Cell lines from Channel catfish available

- Channel catfish ovary cell line (P R Bowser, 1980);
- Channel catfish fin cell line (Q Zhang, 1994);
- Channel catfish B cell line (N W Miller, 1998);
- In addition, BB cell line derived from Brown Bullhead was widely used.

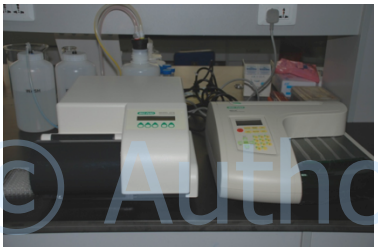
Aims of this research

- Establish a novel cell line derived from Channel catfish kidney tissue source other than that of other cell lines;
- For isolation, identification and propagation of the Channel catfish viral pathogens;
- Establish a solid foundation for developing vaccine against the viral disease in future;

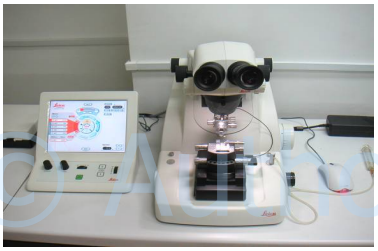
Facilities and conditions(1)

Facilities and conditions(2)

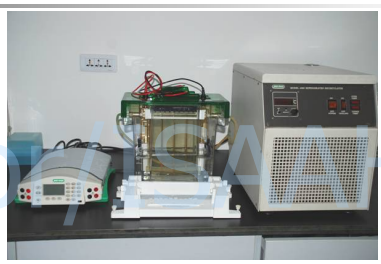


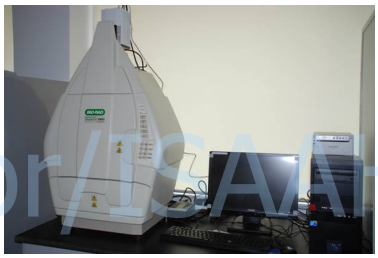



Facilities and conditions(3)

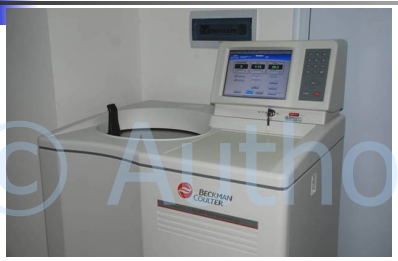




Facilities and conditions(4)



Facilities and conditions(5)



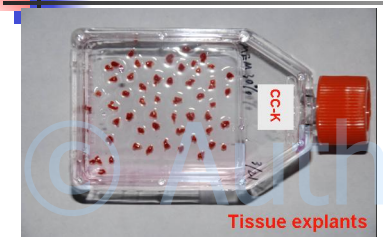


Chemicals, media and supplies

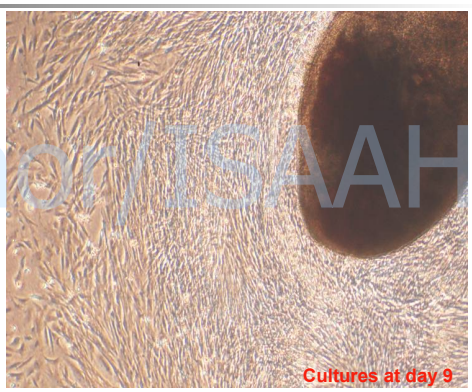
Protocol for primary tissue explant

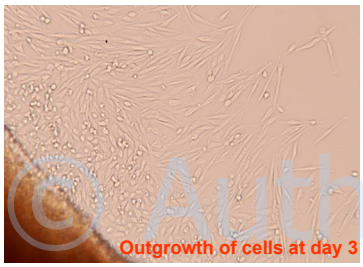
- Isolation and collection of the targeted organ or tissue;
- Transfer the tissue to a dish containing fresh, sterile DPBS, and rinse;
- Transfer the tissue to another dish, dissect off unwanted tissue, such as fat or connective materials, and chopped finely with scissors or scalpels to 1 mm cubes;
- Wash the tissue pieces 2-3 times with DPBS, supplemented with high Conc. Antibiotics;
- Transfer the tissue pieces to a flask with pre-wetted pipette;
- Remove most of the fluid and evenly spread the tissue pieces in flask with pipette;
- Completely remove the fluid and add 1 ml growth medium per flask(25cm square), tilt the flask gently and let the medium wet the culture surface and tissue pieces;
- Cap the flask and place it in a incubator for culture at proper temperature;
- If the pieces have adhered, then the medium volume may be made up gradually to 3-5 ml per flask till a substantial outgrowth of cells were observed;
- Remove the tissue pieces by tapping the flask strongly or picking off with tools;
- Replace the medium for continuous culture to the cell monolayer covered at least 50-70% of the growth surface;
- Subculture the primary cells by trypsin method to more flasks.
- *According to R Ian Freshney,2000 with modifications*

Primary culture of Channel catfish kidney cell

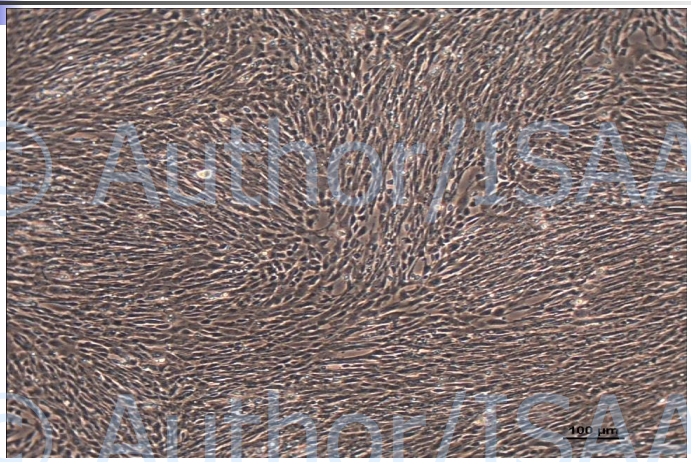


Tissue explants


Cultures at day 9

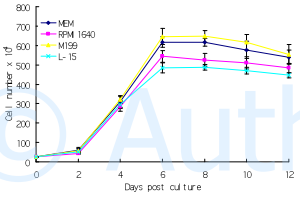

Outgrowth of cells at day 3

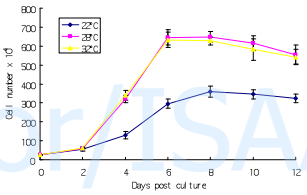
Subculture of CCK cell line

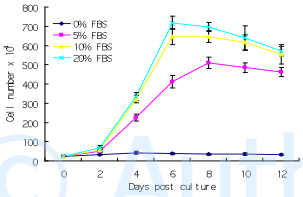


CCK cell at 75th passage

Growth Characterization—optimal medium,Temperature, FBS conc. and PE

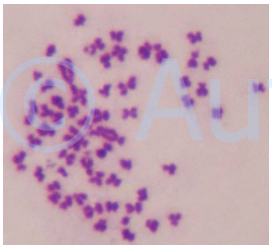




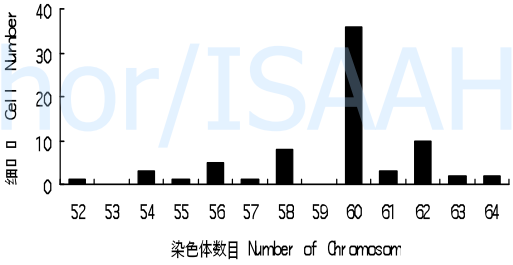


Cell density	Plating efficiency	
200	75%	70%
500	74%	68%
1000	81%	77%
Means±STDEV	74.16±3.5%	

Characterization—chromosome analysis



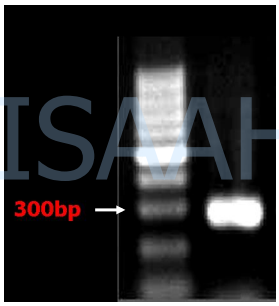
Before 20th passages



After 33th passages

Characterization—origin identification

- Genomic DNA was extracted from CC-K cells;
- Partial fragment of 28S rRNA gene was amplified by PCR with proper primers;
  PF：5'- AAATCTGGTGGAGGTCCGTA -3'
  PR：5'- GCTCTTACAAAAGTGGCCCA -3';
- Amplified fragment was sequenced ,blasted and aligned with the sequence from Channel catfish posted in Gene Bank (AF056008) ;
- 94.0% identities to the Channel catfish 28S rRNA gene (287bp) .



Reovirus infected Channel catfish

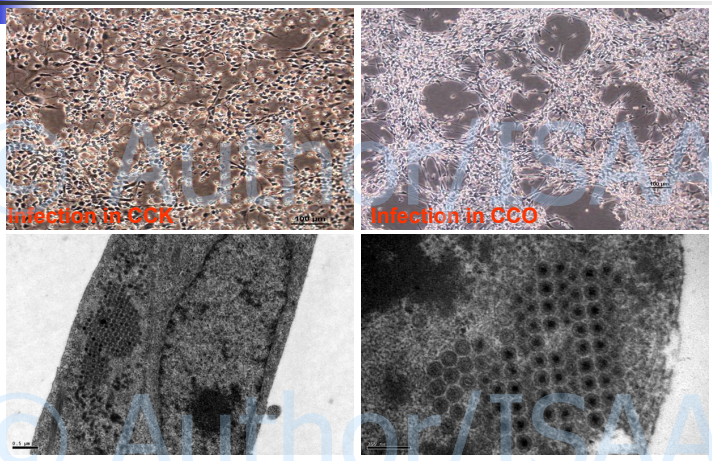
CCV infected fish (Alan C Camus, MSU, 2004)

Abdominal swelling, hemorrhage, bulging eyeballs

CCRV naturally infected fish

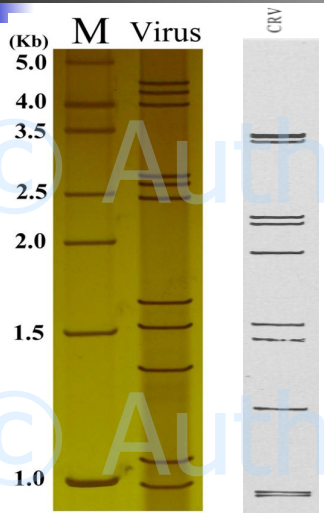
CCRV experimentally infected fish

CCRV infected cells and ultra-thin section



Ultrasection of infected CCK cells CCRV in CCK cells

SDS-PAGE Analysis of CCRV genome



- M: Fermentas 1KB DNA ladder;
- Virus: Channel catfish Reovirus RNA genome;
- CRV: Channel catfish reovirus isolated by R P Hedrick, 1984.

Similarity in sequences of CCRV S7-11 segments with the other aquareoviruses

CCRV / Aquareovirus	S7		S8		S9		S10		S11	
	Query coverage	Max Ident	Query coverage	Max ident	Query coverage	Max ident	Query coverage	Max ident	Query coverage	Max ident
GCRV-873	99%	99%	100%	99%	100%	99%	100%	99%	100%	100%
GSRV	99%	95%	100%	94%	100%	92%	100%	91%	100%	97%
GCRV-876	-	-	85%	100%	-	-	84%	99%	-	-
GCRV-875	-	-	85%	100%	-	-	84%	90%	-	-
GCRV-991	-	-	85%	99%	-	-	84%	99%	-	-
AGCRV_PB01-155	2%	85%	68%	66%	63%	72%	-	-	-	-
CSV	-	-	7%	73%	-	-	-	-	-	-

Summary

- A new cell line derived from the kidney of Channel catfish designated CCK has been fully established and well characterized;
- Susceptibility of CCK cells to the infection of Channel catfish reovirus has been revealed;
- A Channel catfish Reovirus has been isolated and identified;

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Thanks for your attention !