Derivation, characterization and cryostorage of continuous cell lines from threatened species of groupers (Serranidae) *Cromileptes altivelis* and *Epinephelus bleekeri*

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Introduction

Globally aquatic ecosystems experience serious threats to both biodiversity and ecosystem stability. Overfishing, pollution and climate changes are the major factors impacting marine fish biodiversity. Several conservation strategies have been developed to overcome the crisis. Cryobanking of fish cell lines is recognized as an important tool for conservation of fish germplasm. The advantage of preserving cell lines lies in their ability to provide a source of renewable material that can be cultured for long periods.

The present study envisaged to develop continuous cell lines from two species of groupers (Family: Serranidae) *viz.*, *Cromileptes altivelis* and *Epinephelus bleekeri* which are categorized as vulnerable and near threatened respectively, in the IUCN Red List.

Methods

Initiation of primary culture and passaging: Attempts were made to initiate cell culture systems from various tissues *viz.*, fin, gill, caudal peduncle, brain, heart, liver and spleen, by explanation and trypsinisation methods as per the methods described by Flano *et al.* (1998). Leibovitz L-15 medium supplemented with FBS (2 - 20% v/v), antibiotics (penicillin and streptomycin 100 IU ml^{-1} and 100 µg ml^{-1} respectively) and the fungizone, amphotericin B (0.25 µg ml^{-1}) was used as growth medium. Confluent monolayers developed were passaged by subculturing using 0.05% trypsin-EDTA, to develop continuous cell lines.

Characterisation: The established cell lines were characterized by karyotyping, immunofluorescence labelling for fibroblast/epithelial markers and authenticated by sequencing mitochondrial CO1 gene as described by Fernandez *et al*. (1993).

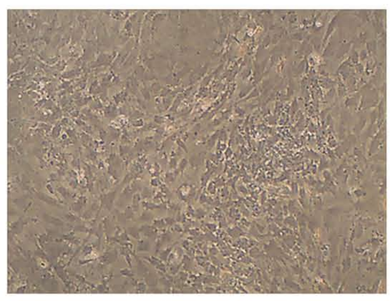
Cryopreservation: The cells were stored in liquid nitrogen (-196ºC) at various passage levels in Leibovitz L-15 medium supplemented with 20% FBS and 10% DMSO.

Results and discussion

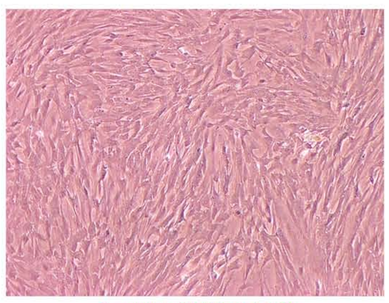
Cromileptes altivelis

Humpback grouper *Cromileptes altivelis* (Valenciennes, 1828)

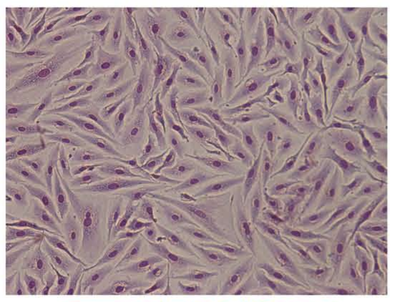
Two successful cell lines (CA1F2Tr and CA1Br2Tr) have been developed from trypsinised brain and fin tissues respectively. Clusters of cell monolayers formed in primary culture comprised both epithelioid and fibroblast-like cells. However as passaging progressed, fibroblast-like cells predominated. Giemsa stained cells of both the cell lines revealed fibroblast morphology and the cell lines were found positive for the fibroblast marker vimentin.



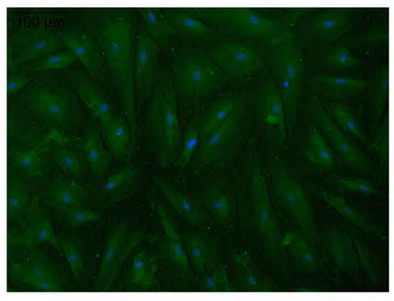
Fin (CA1F1Tr) primary culture showing mix of epithelioid and fibroblast-like cells (x100)



Fin cell line (CA1F1Tr) (x100)



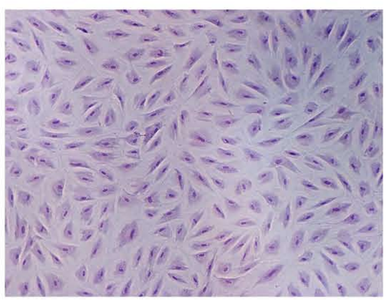
Giemsa stained cells of CA1F1Tr showing fibroblast morphology (x100)



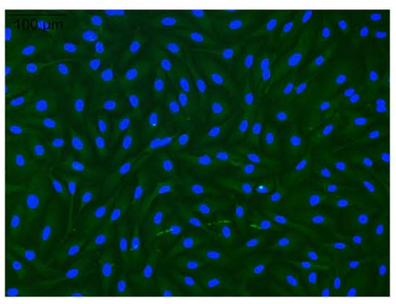
Vimentin (fibroblast marker) positive cells of CA1F1Tr (x100) with DAPI stained nuclei

Brain (CA1Br2Tr) primary culture showing mix of epithelioid and fibroblast-like cells (x100)

Brain cell line (CA1Br2Tr) (x100)



Giemsa stained cells of CA1Br2Tr showing fibroblast morphology (x100)

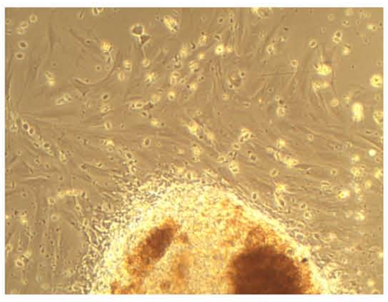


Vimentin (fibroblast marker) positive cells of CA1Br2Tr (x100) with DAPI stained nuclei

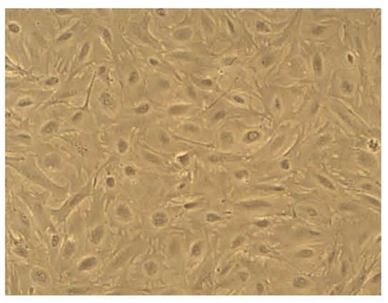
Epinephelus bleekeri

Duskytail grouper *Epinephelus bleekeri* (Vaillant, 1878)

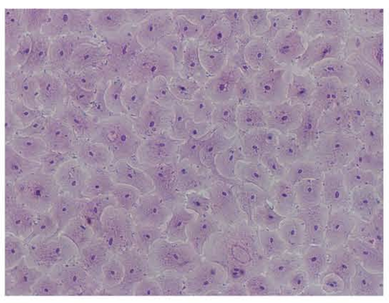
Successful cell line, EB2SpEx was developed from spleen explants. Confluent monolayers in primary culture comprised both epithelioid and fibroblast-like cells. As subculturing progressed, epithelioid cells predominated. Giemsa stained cells of EB2SpEx cell line revealed epithelioid morphology and the cells were found positive for the epithelial marker pancytokeratin.



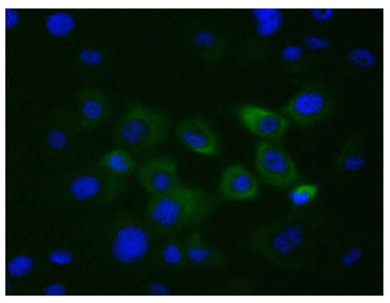
Spleen (EB2SpEx) primary culture (x100) showing the explant along with spreading and attaching cells



Spleen cell line EB2SpEx (x100)



Giemsa stained cells of EB2SpEx showing epithelial morphology (x100)



Pancytokeratin (epithelial marker) positive cells of EB2SpEx (x200) with DAPI stained nuclei

Chromosome analyses revealed that the three cell lines developed are aneuploid. PCR amplification and sequencing of mitochondrial CO1 gene followed by comparative analysis of the identified sequences revealed match with the corresponding species of origin. Cryostorage of the cell lines and their subsequent revival showed >80% viability.

Conclusion

The continuous cell lines developed from the two threatened species of groupers have been successfully cryopreserved which can serve as genetic as well as cellular resource material for scientific study and can aid in biodiversity conservation of the declining species.

Acknowledgements: Study was supported by grants from DBT, New Delhi. We thank the Director, ICAR-CMFRI, Kochi for the research facilities provided.

References

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