Establishment of primary culture method for *Hyriopsis cumingii* cells in vitro

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Abstract

In order to establish a set of effective techniques and methods for isolation and culture of freshwater mussel cells in vitro, this study took *Hyriopsis cumingii*, the most important freshwater pearl mussel in China, as an experimental animal, from the key aspects of sterilization process, enzyme digestion system, proliferation medium improvement and microscopic observation. The methods of cell culture of mantle tissue, gill tissue and pallial line tissue of shellfish were discussed in order to provide support for the establishment of freshwater shellfish cells in vitro.The research showed that vitro tissues were sterilized with three resistance concentration gradients and digested in 0.25% trypsin for cold digestion and heat digestion respectively.The morphology and growth characteristics of cells were observed by inverted phase contrast microscope.The results showed that the cells are mostly round. The cytoplasm is blue-purple and the nucleus is purplish red by Modified Giemsa Staining Solution.The mantle tissue cells could be successfully cultured to 11 generations in the improved medium.The survival time is up to five months.

Methods

1、The separation of cells

Temporarily breed → Tissue samples → Cleaning and sterilizing tissue → Digest tissue → Cells

2、Culture medium preparation

①Preparation of *Hyriopsis cumingii* serum.
②The medium was proportioned.

3、Cell culture and passage culture

①Discard the original culture medium in a culture flask full of cells.
②Trypsin was added so that cells at the bottom of the dish were immersed in the solution.
③Digestion was terminated in culture medium and the adherent cells were blown into suspension into a new dish.

Results

1. The most suitable method for sterilizing tissue

In the sterilization of in vitro tissues, the addition of different concentrations of three resistance gradient sterilization(2%-10%-20%-10%-2%), in order to fully sterilize the tissues.

2、Exploration of digestive tissue methods

In terms of the method of cell acquisition, we explored the use of cold digestion and thermal digestion at the same time, expecting to obtain the maximum extent of isolated cells.

3、Cells identification

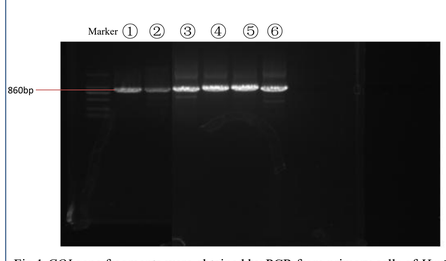

Fig.1 *COI* gene fragments were obtained by PCR from primary cells of *Hyriopsis cumingii* (①gill②gill tissue cells③mantle④mantle tissue cells⑤pallial line ⑥pallial line tissue cells)

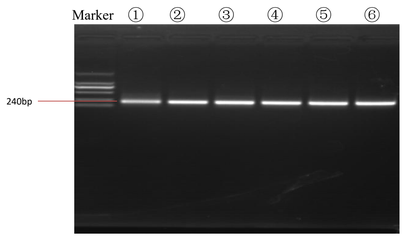

Fig.2 *cyclin D2* gene fragments were obtained by PCR from the 5th generation cells of *Hyriopsis cumingii* (①gill②gill tissue cells③mantle④mantle tissue cells⑤pallial line⑥pallial line tissue cells)

Conclusion

Through this experimental study, the most suitable method for isolating *Hyriopsis cumingii* cells was explored, and it was found that the culture system with homemade serum could successfully cultivate *Hyriopsis cumingii* cells and successfully carry out subculture.

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4、Morphological observation of cells

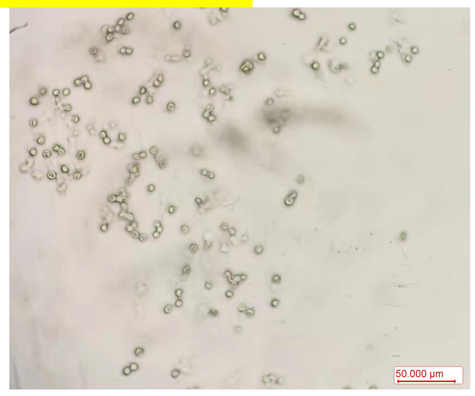

Fig.3 Primary cell smear of *Hyriopsis cumingii*

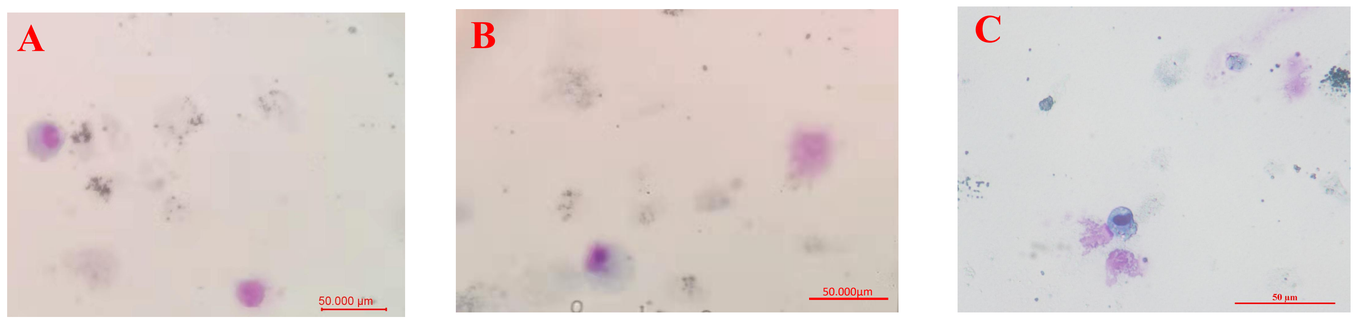

Fig.4 Results of Modified Giemsa Staining Solution of primary cells of *Hyriopsis cumingii*(A:mantle;B:gill;C:pallial line)

5、Bead nucleus enrichment

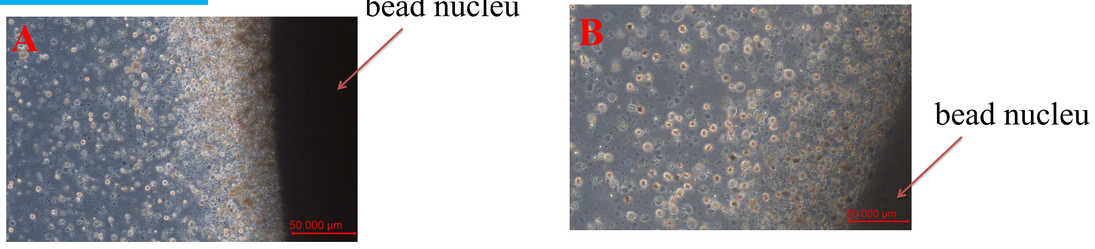

Fig.5 The bead nucleus enrichment of *Hyriopsis cumingii* cells in different tissues(A:mantle;B:pallial line)

6、Microscopic observation of cells culture

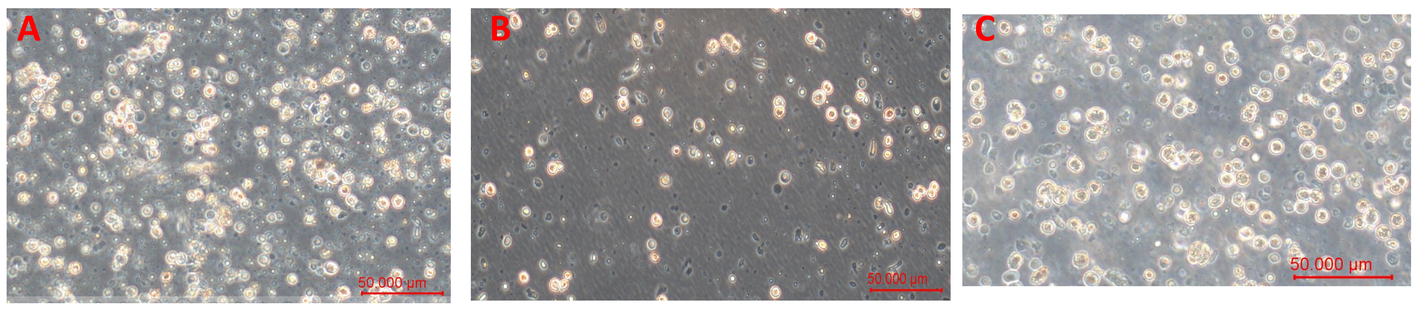

Fig.6 Primary cells of *Hyriopsis cumingii* cultured in vitro day 1(A:mantle; B:gill;C:pallial line)

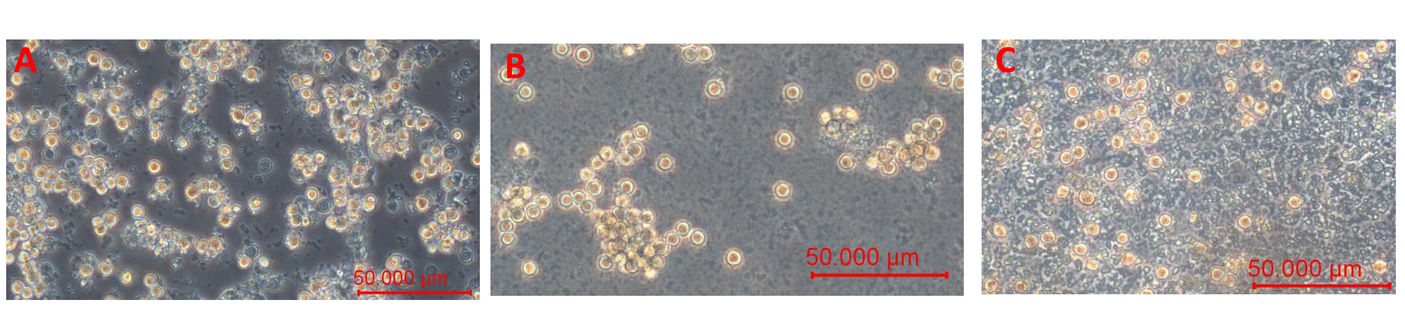

Fig.7 The primary cells of *Hyriopsis cumingii* were cultured in vitro and passed through the 1st generation(A:mantle; B:gill;C:pallial line)

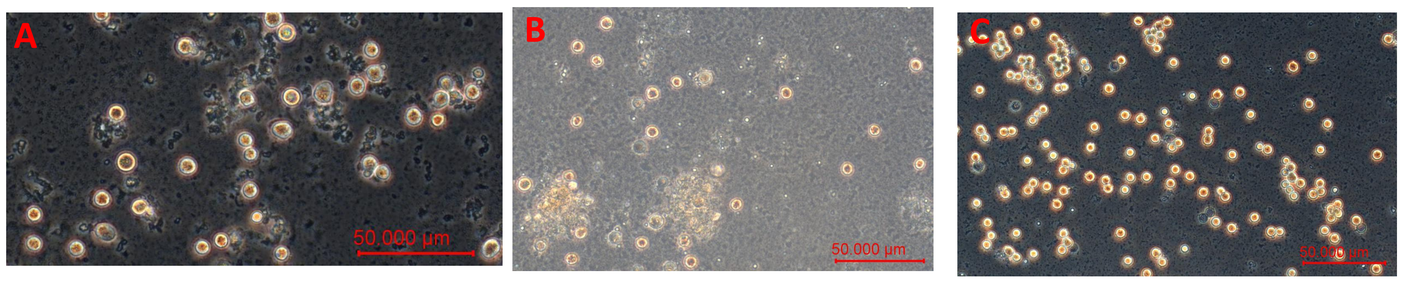

Fig.8 The primary cells of *Hyriopsis cumingii* were cultured in vitro and passed through the 6th generation(A:mantle; B:gill;C:pallial line)