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RESEARCH ARTICLE

Development of a Primary Cell Culture from Hepatopancreas of the Common Fresh Water Crab, *Paratelphusa hydrodromus* Using Serum Free Medium

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Abstract

A primary cell culture from hepatopancreas of the common fresh water crab, *Paratelphusa hydrodromus* was developed using serum free medium. The cells were grown at 25ºC in L-15 medium with glutamine and antibiotics. The cells of the hepatopancreas were round and showed a strong tendency to attach to the surface of the flask. The cells became confluent after 84 hours of post seeding and seen attached to the surface of the flask.

Introduction

Studies on the cell culture of crabs are very few, despite its economic importance [1-5]. Cooke et al. [2] developed the primary cell culture of crustacean neurons, taken from the peptidergic neurosecretory system of the eyestalk of crab *Cardisoma carnifex* using crab saline supplemented L-15 medium. Sashikumar and Desai [3] developed the primary cell culture of neurons and hepatopancreas from edible crab *Scylla serrata* using L-15 medium supplemented with crab saline or citrate buffer. The development of cell culture of hepatopancreas from *Scylla paramamosain* using commercially available culture media supplemented with salts was reported by Zeng et al. [4]. They have also reported that, FBS and crab muscle extract as supplement stimulate growth but crab haemolymph inhibit cell growth. Xu et al. [5] cultured crustacean neurons obtained from mud crab *S. paramamosain* using FBS, muscle extract and haemolymph.

Paratelphusa hydrodromus is a common fresh water crab found in paddy fields, which is widely distributed in India. This species is used as a model organism for developmental, biochemical, endocrinological and toxicological studies. Availability of cell line of *P. hydrodromus* may accelerate studies on its biochemical and molecular characterization. Here we report the development of an *in vitro* cell culture of the hepatopancreas of *P. hydrodromus* using serum free L-15 medium with glutamine and antibiotics.

Materials and Methods

The crabs were anesthetized using chloroform and washed with sterile water. The body surface of the crab was sterilized by successive washing with 2% tincture iodine, 70% ethanol and sterile distilled water. The hepatopancreas was aseptically dissected out and transferred to PBS antibiotic solution (PBS with Ampicillin, 100µg/ml, Chloramphenicol, 30µg/ml and Amphotericin B, 10µg/ml) followed by a brief washing with 10% ethanol and finally transferred to PBS antibiotic solution. The tissue was disrupted with a sterile glass rod and resuspended in PBS antibiotic solution.

The cell suspension in PBS antibiotic solution was centrifuged at 2000 rpm for two minutes and the supernatant was aspirated out. The pelleted cells were resuspended again in PBS antibiotic solution and pelleted repeatedly two times. Finally the pelleted cells were resuspended in filter sterilized L-15 medium with glutamine and an antibiotic cocktail (Ampicillin, 100µg/ml, Chloramphenicol, 20µg/ml, and Amphotericin B, 10µg/ml, Nystatin, 10µg/ml). The suspended cells were seeded onto a 5x5cm tissue culture flask and grown at 27˚C. The medium was changed every 24 hours.

Results and Discussion

The cells of the hepatopancreas of *P. hydrodromus* were attached to the surface of the culture flask within 10 hours of post-seeding. Groups of cell colonies were found after 52 hours of seeding. A layer of cells was observed after 72 hours which became a confluent monolayer after 84 hours of post seeding (Fig.1)

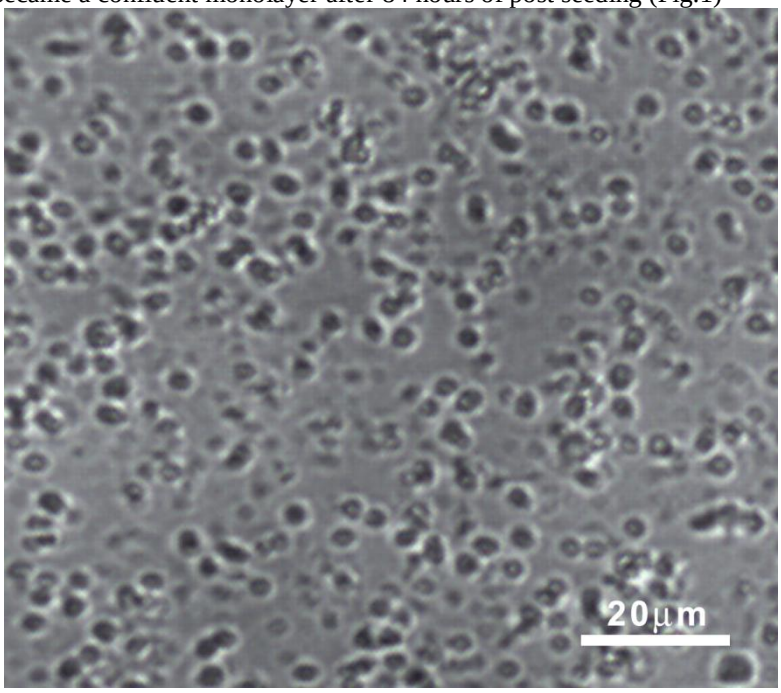

Figure 1. Primary cell culture hepatopancreas of *Paratelphusa hydrodromus*: confluent monolayer of cells formed after 84 hours of post seeding

In the past, primary culture from hepatopancreas, lymphoid and ovarian tissues of a few Crustaceans were developed using culture medium containing serum. In the present study an *in vitro* primary culture from hepatopancreas of *P. hydrodromus* was developed using a serum free L-15 medium with glutamine. The cells of hepatopancreas of *P. hydrodromus* were round and showing attachment to the surface of the culture flask. The L-15 medium with glutamine in the presence of antibiotics supported cell growth without the supplementation of any growth factor in an open system at 25ºC. The bacterial and fungal contamination in the culture was eliminated by antibiotics whereas protozoan contamination was eliminated by repeated washing. After 24 hours no floating cells were observed in the culture, indicating their strong tendency of attachment to the surface of the culture flask.

The crustaceans are known for their efficiency of organ regeneration. In the fiddler crab *Uca pugilator*, limbs that are lost due to injury as a result of the reflexive autotomy response can be regenerated completely during a single intermolt cycle [6, 7]. The rapid growth of cells from the hepatopancreas of the *P. hydrodromus* in a serum free culture medium is in turn with their regenerative abilities. The results demonstrate a primary cell culture of the hepatopancreas of *P. hydrodromus* in a serum free L-15 medium with glutamine.

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