Fish & Shellfish Immunology 55 (2016) 444–451

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Establishment and characterization of a skin epidermal cell line from mud loach, *Misgurnus anguillicaudatus*, (MASE) and its interaction with three bacterial pathogens

Xiaohui Xu, Elayaraja Sivaramasamy, Songjun Jin, Fuhua Li, Jianhai Xiang*

Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Rd., Qingdao, 266071, China

ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form
1 June 2016
Accepted 6 June 2016
Available online 7 June 2016

Keywords:
Misgurnus anguillicaudatus
Skin epidermal cell line
Fish skin bacteria
Aeromonas veronii
Vibrio parahaemolyticus

ABSTRACT

A continuous skin epidermal cell line from mud Loach (*Misgurnus anguillicaudatus*) (MASE cell line) was established with its application in bacteria infection demonstrated in this study. Primary MASE cell culture was initiated at 26 °C in Dulbecco's modified Eagle medium/F12 medium (1:1; pH7.2) supplemented with 20% fetal bovine serum (FBS). The primary MASE cells in spindle morphology proliferated into a confluent monolayer within 2 weeks, and were continuously subcultured even in 10% FBS- DMEM/F12 after 10 passages. Impacts of medium and temperature on the growth of the cells were examined. The optimum growth was found in DMEM/F12 with 20% FBS and at 26 °C. The MASE cells have been subcultured steadily over Passage 90 with a population doubling time of 53.3 h at Passage 60. Chromosome analysis revealed that 60.5% of MASE cells at Passage 60 maintained the normal diploid chromosome number (50) with a normal karyotype of 10m+4sm + 36t. Bacteria from the three species (*Aeromonas veronii*, *Vibrio parahaemolyticus* and *Escherichia coli*) were used to investigate the interactions between bacteria and cellular hosts. The three strains could be attached to the MASE cells and replicate at different levels. *A. veronii* could induce apoptosis in the MASE cells, with highest adherence rate among the three strains, whereas *V. parahaemolyticus* could cause highest cell death rate through a non-apoptotic cell death pathway, with high level of replication. The results revealed that different bacteria could interact with the MASE cells in different manners, and divergent pathways might lie in mediating cell death when cellular hosts confronted with pathogen infection. Therefore, the MASE cell line may serve as a useful tool for studying the interaction between skin bacteria and fish cells.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The cyprinid loach, *Misgurnus anguillicaudatus*, is a freshwater fish species of the loach family Cobitidae, widely distributed in East Asia and introduced to Europe and North America [1]. As a traditional and popular food, loach has a high nutritional and medical value, therefore it is called 'Ginseng in water' [2] and consequently become commercially important species in aquaculture. Like other aquaculture animals, some disease occurred frequently due to the intensive farming of loach. Numerous diseases such as skin ulcer and intestinal hemorrhage, probably caused by bacteria, fungi or parasites, posed serious threats in the loach farming. However, up today it is not clear about the main pathogens and possible pathological mechanisms, which need to further explore.

Fish skin as immunologically active tissue is an important first line of defense against the attachment and penetration of various invading pathogens. In general, fish skin is composed of three layers namely the epidermis, dermis and hypodermis [3]. The epidermal layer are made up of epithelial cells, mucus cells, club cells and numerous other cell types, including neutrophils and macrophages in inflammatory reactions of the superficial tissue layers, and each of them plays important functions on the adaptation of the organism to its environment [4,5]. So the epithelial cells are good model for studying the immune function of fish skin. However, only few papers [6,7] have discussed the isolation and culture of this kind of cells and their use for understanding specific responses in the presence of biological and physical stimuli. One of the reasons is the lack of skin epidermal cell lines. There are a few obstacles in the development of a skin epidermal cell line, including microbial contamination of primary culture, the mixing of dermis fibroblasts

* Corresponding author.
E-mail address: jhxiang@qdio.ac.cn (J. Xiang).

http://dx.doi.org/10.1016/j.fsi.2016.06.009
1050-4648/© 2016 Elsevier Ltd. All rights reserved.

and culture conditions. In this study, we overcame these problems and established a skin epidermal cell line from *M. anguillicaudatus*.

The life style and surroundings of *M. anguillicaudatus* make its skin in close contact with many kinds of bacteria. In addition, due to snake-like movement and lack of protection from scale, its skin is more likely to get hurt and infection. There was no pathogen except *Aeromonas veronii* that had been identified and characterized in the culture of *M. anguillicaudatus* [8]. In addition, genes involved in immunity and epidermal mucus secretion from *M. anguillicaudatus* skin transcriptome had been identified [9]. Up to date, the mechanisms on how pathogens influence the host cells were fragmentary and least explored. Here we had previously isolated and identified two strains (*Aeromonas veronii* and *Vibrio parahaemolyticus*) from the skin of diseased loach. *Aeromonas veronii* has been reported to cause wound infection and digestive tract diseases in human, and also cause high mortality in farmed freshwater/marine fish [10,11]. It has been described as an important fish pathogen of *Aeromonas* spp. bacteria. *Vibrio parahaemolyticus* was first isolated in 1950 from explosive food poisoning, also an important zoonotic pathogen that caused vibriosis in many freshwater/marine fish, and sepsis, gastroenteritis and wound infection in humans [12,13]. For exploring possible pathological mechanisms on how they interact with the loach cells, using the skin epithelial cell line developed and the two strains isolated, we investigated the relationship between infection and cell death, the interation mechanisms through multiple optical and biochemical features.

2. Materials and methods

2.1. Primary culture and subculture

The adult loach (*M. anguillicaudatus*) about 15 cm in length obtained from Nanshan Fish Market (Qingdao, China) was reared in fresh water containing 1000 IU mL^{-1} penicillin and 1000 μg mL^{-1} streptomycin (Lukang) for 24 h before experiment. After euthanized by etherification, the loach was sterilized with 75% alcohol disinfectant, meanwhile the mucus on the surface of skin were wiped off with gauze. The skin sample from the dorsal region was dissected using scalpel and checked free of muscle and connective tissue under stereo microscope. The sample was washed three times with PBS, minced into small pieces (about 1 mm^3 in size) with ophthalmic scissors, and digested with 0.2% collagenase IV in PBS for 20 min. After digestion, the enzyme was removed by centrifuging at 1000 *g* for 5 min three times. The tissue pieces were resuspended in 1 mL DMEM/F12 (pH 7.2) medium containing 15% fetal bovine serum (FBS, Gibco), 100 IU mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (Gibco), and were seeded into 25 cm^2 culture flasks for attachment at 26 °C, finally added 4 mL the same medium to the flasks after two days. At the first few days, the morphology of migrating cells should be carefully observed. Once fibroblastic cells found, the cells and the tissue pieces should be removed with cell scraper to prevent contamination. Every three days half of the medium was changed until the cells grew into a confluent monolayer.

Monolayer cells were dispersed to single cells by trypsinization and half of cells were transferred to another flask to subculture. After 10 passages, the medium was changed to 10% FBS- DMEM/F12 until the loach *M. anguillicaudatus* skin epithelial cell line was established.

2.2. Cryopreservation and thawing

Full monolayer cells were collected and adjusted to 6.0×10^6 cells mL^{-1} with cell freezing medium consisting of DMEM/F12, FBS, dimethyl sulphoxide, at a ratio of 3: 1: 1. The cell suspension was pooled into a 2 mL sterile cryovial (Corning), placed into a gradient-cooling box (Thermal) at −80 °C overnight and finally stored in liquid nitrogen (−196 °C). When thawed, the cryovial was put in 40 °C water bath to reach the optimal temperature. After centrifugation at 1000 *g* for 10 min, the cell pellets were suspended with fresh medium and seeded into the flasks.

2.3. Cell viability assay

Trypan blue staining was used to count the alive cells when determining the survival rate of the thawed cells. Specifically, 0.4% trypan blue (Gibco) was added to cell suspension and got the mixture with 0.04% trypan blue. The number of the death (blue) and alive cells was counted by a hemocytometer and the cell survival rate was calculated.

2.4. Growth studies

The effects of different medium and culture temperatures were studied on Passage 10. The cells were added to 24-well culture plates with 1.0×10^5 cells per well. Six hours later the medium of every well was removed and washed with PBS three times. The cells were added with either DMEM/F12, DMEM, Leibovitz's L-15 containing 20% FBS and incubated at 26 °C. In the next 6 days, cells of triplicate cells were harvested by trypsinization and counted using a hemocytometer every day. The average numbers were used to plot the growth curve of cells on different conditions of three kinds of medium. In a similar way, the growth curves of cells at different culture temperatures (18 °C, 22 °C, 26 °C, 30 °C) were graphed out.

The cells continued to be subcultured on the optimal culture condition and were used to graph the growth curve at Passage 60. The number of cells at an interval of 12 h was counted and plotted the curve. The population doubling time of cells was calculated by the formula of $T = t \times \lg 2/\lg (N_t/N_0)$ [14], where T stands for population doubling time, t for inoculation time, and N_0, N_t for cell number.

2.5. Chromosome analysis

Cells at logarithmic phase at Passage 10, 30, 60 were used for chromosome analysis according to [14] with modifications. Briefly, these cells were treated with 20 μg mL^{-1} colchicine for 10 h, and harvested by trypsinization. The single cells were suspended in 0.3% KCl hypotonic solution for 30 min, fixed with Carnoy's solution for 20 min, stained with Giemsa for 60 min and finally air dried. Three hundred metaphase cells were collected under microscope (Nikon, Japan) and chromosome numbers and karyotype was statistically analyzed.

2.6. Bacterial culture

Bacteria were isolated from skin of diseased mud loach (*M. anguillicaudatus*), which had abdomen congestion and skin ulcerative symptoms (Fig. 1) and was sampled from the farm in Zhejiang province of China. The skin tissues were aseptically removed and homogenized in 10 mL sterile saline. The homogenates were serially diluted in sterile saline water and plated on Nutrient Broth and Tryptic Soy Broth separately, and then incubated at 37 °C for 24 h for appropriate colony formation. After the incubation, the single colony of each plate was selected for re-isolation to a pure culture and was identified by biochemical methods. The results were cross-checked with Bergey's Manual of systematic bacteriology and were confirmed through molecular identification [15].

Among many kinds of strains isolated, *Aeromonas veronii* and *Vibrio parahaemolyticus* srains were chosen to infect cells. They

Download English Version:

https://daneshyari.com/en/article/2430799

Download Persian Version:

https://daneshyari.com/article/2430799

Daneshyari.com