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Development of a Primary Cell Culture from the Caudal Fin of an Indian Major Carp, *Labeo rohita* (Ham.)

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Abstract - A simple and reproducible short-term fish cell culture technique with minimum or no stress to the specimens is described. Gill, scale and fin tissues from eight fish species viz., *Labeo rohita, Catla catla, Cirrhinus mrigala, Cyprinus carpio, Clarias batrachus, Heteropneustes fossilis, Anabas testudineus* and *Oreochromis mossambicus,* were used to develop primary cell cultures. A primary culture from the caudal fin of *L. rohita* was successfully obtained on the fourth post-culture day using L-15 medium supplemented with 20% Fetal Calf Serum (FCS) and 20% Fish Muscle Extract (FME) at an incubation temperature of 28-29°C. This experiment could not obtain primary cultures from other fish species and tissues, either due to non-attachment of explants or fungal contamination.

Eversince the first cell line was developed from rainbow trout over 25 years ago (Wolf and Quimby 1969; Wolf and Ahne 1982), several short-term and continuous cell cultures from a variety of fish species have been developed (Nicholson 1988). Though the major applications of fish cell culture are in virological and biomedical studies (Wolf and Mann 1980; Hightower and Renfro 1988), it also offers the best potential source of chromosome prepara-tion for the application of banding techniques (Blaxhall 1983; Hartley and
Horne 1983; Amemiya et al. 1984; Alvarez et al. 1991). To the best of our knowledge, there is no published document on cell cultures from tropical fishes. Therefore, the development of a simple and reproducible short-term monolayer cell culture technique for obtaining high quality metaphase spreads from Indian fish species was highly desirable.

Eight fish species viz., *Labeo rohita, Catla catla, Cirrhinus mrigala, Cyprinus carpio, Clarias batrachus, Heteropneustes fossilis, Anabas testudineus* and *Oreochromis mossambicus*, were used as the source of tissue. The fish were brought to the laboratory of the National Facility for Animal Cell and Tissue Culture, Pune, India. The fish were swabbed with alcohol and betadine,

then the caudal fins, gills and scales were removed. The tissues were cut into fine pieces and washed twice in Phosphate Buttered Saline (PBS) at room temperature for 10 minutes. All these operations were done under sterile conditions in the laminar hood. The PBS was then removed and the cut pieces of fin, gill and scales were kept in a china dish for 1 h at room temperature in the laminar hood. Then the tissues in each china dish were provided with 0.5 ml 20% Fetal Calf Serum (FCS) and 1 ml L-15 medium. Care was taken that the tissues did not float. Excess medium was sucked back. The culture dishes were incubated at 28-29°C overnight. After ascertaining tissue attachment, fresh medium, FCS and FME were added in the ratio of 3:1:1, and the explants were allowed to grow at 28-29°C in the incubator. To prepare FME, 20 g fish muscle was macerated in 200 ml PBS (1X) and centrifuged to remove debris. The supernatant was inactivated in a water bath at 56°C for 30 minutes. It was again centrifuged to remove the coagulated protein, and then filtered through 0.45 u and 0.22 u porosity filters for sterilization.

In the present investigation, several experiments were conducted using various tissues, such as fin, scale and gill, to determine the best tissue, its optimum growth conditions and potential (Table 1). The results have proven that fin tissue is the best in terms of attachment and growth as would be expected from its regenerative ability. The growth potential of scales was found to be extremely weak. However, fibroblasts were obtained in fin tissue of *L. rohita* where L-15 medium with 20% FCS and 20% FME of the same species was used. Fibroblast cells outgrowing from primary explants of *L. rohita* are shown in Figs. 1 and 2. Alvarez et al. (1991) have also reported successful growth from fin tissue in several marine and freshwater fish species.

The first 4 d after the explants were planted proved crucial to successful growth. While fin and gill tissues appeared better attached, the explants from scales did not attach at all in the present experiment. The china dishes were incubated at 20, 25, 28 and 30°C to determine optimum growth temperature. The optimum growth temperature was found to be 29°C. This corroborates the

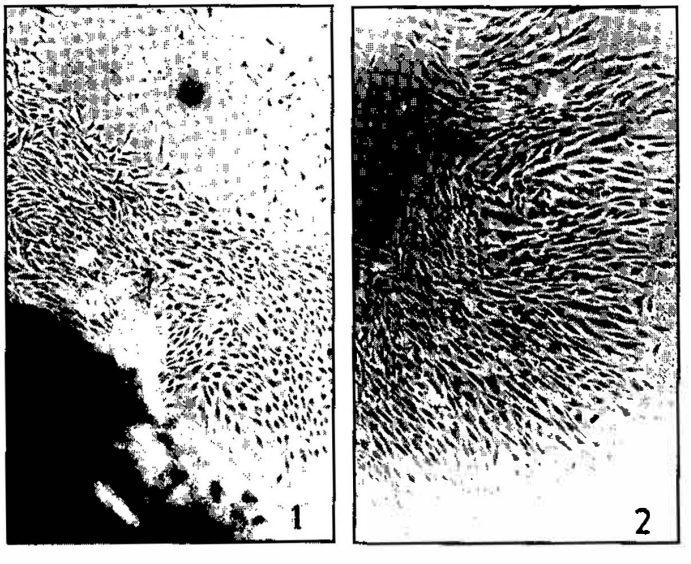

Fig. 1. Primary culture from caudal fin explant showing spindle-shaped fibroblasts in *Labeo rohita* (200 X).

Fig. 2. Primary culture from caudal fin explant showing spindle-shaped fibroblasts in *Labeo rohita* (400 x).

Table 1. Summary of results in developing primary cell cultures from various fish species at an incubation temperature of 28-29°C.

Fish species	Tissues used	Number of tissue samples	Medium employed	Observations and remarks
Labeo rohita	Caudal fin, scale	6	L-15 with 20% FCS and 20% FME	Attachment of explants from caudal fin; multiplication started on 4th post-culture day which resulted in the formation of spindle-shaped fibroblasts.
Catla catla	Caudal fin, scale	6	L-15 with 20% FCS and 20% FME	Attachment of explants from fin tissue but no growth. No attachment of explants from scales.
Cirrhinus mrigala	Caudal fin, scale	6	L-15 with 20% FCS and 20% FME	Attachment of explants from fin tissue but no growth. No attachment of explants from scales.
Cyprinus carpio	Caudal fin, gill	8	L-15 with 20% FCS	Attachment of tissue but no growth.
Clarias batrachus	Caudal fin, gill	10	L-15 with 20% FCS	Attachment of tissue but no growth.
Heteropneustes fossilis	Caudal fin, gill	10	L-15 with 20% FCS	Attachment of tissue but no growth.
Anabas testudineus	Caudal fin, gill	4	L-15 with 20% FCS	Attachment of tissue but no growth.
Oreochromis mossambicus	Caudal fin, gill	4	L-15 with 20% FCS	Attachment of tissue but no growth.

observations of Alvarez et al. (1991) who also reported the optimum in-vitro growth temperature a few degrees above the preferred environmental level for live fish. Wolf and Ahne (1982) suggested that the difference between in-vivo and in-vitro temperature can be due to selection or adaptation of cells to growth of higher than normal temperature.

It is inferred from this study that the use of FME of the same species led to the successful development of primary cultures in *L. rohita*. We failed to obtain primary cultures from other fish species and tissues either due to non-attachment of explants or fungal contamination.

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