CHAPTER - 6
SUMMARY
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Fish cell cultures have both fundamental and practical importance. It provides an important tool for studying various aspects of cellular physiology, molecular biology, genetics, immunology, endocrinology, nutrition, comparative biology and biotechnology of the fish. Establishment of cell lines from economically important and endangered species would be of great importance for aquaculture and fisheries management. Tor tor commonly called as Tor mahseer in India, belonging to the family Cyprinidae are described as the ‘King of Indian freshwater systems’ with high commercial and recreational value. About 20 species are recognized within the genus distributed throughout Asia from the trans- Himalayan region to the Mekong river basin to Malaysia, Pakistan, Bangladesh and Indonesia. Over-exploitation of the natural stock and the deterioration of environmental condition have result in a significant decline of mahseer in the wild (Ogale, 2002). As a result of which several species of mahseer including T. tor are listed among endangered fishes (CAMP 1998, Lakra and Sarkar 2007). The development of cell line from endangered mahseer tor will facilitate invitro research in genetics and conservation of the mahsheer species. Keeping in view the importance of cell line from T. tor the present study was aimed to develop primary cell cultures and establish cell line from different tissues of T. tor. These cell lines were further characterized by various molecular and cytogenetical techniques.

Fry and fingerlings of T. tor, collected from the Narmada River, Hoshangabad (M.P.) were used to develop primary cell culture from different tissues viz. fin, heart, swim bladder, gills and eye tissues. Primary cultures were developed by explants method at 28 °C in the growth medium containing L-15 (Leibovitz) supplemented with 20% FBS. Cell culture systems developed from fin and heart were successfully subcultured to develop cell lines designated as TTCF and TTH respectively. Although the primary culture developed from eye, gills and swim bladder explants were successfully passaged up to 7, 11 & 14 but the continuous cell lines could not be developed. Both the cell lines TTCF and TTH were maintained at optimized growth conditions. The optimum temperature for the cell lines was 28 °C, which was in conformity with other fish cell lines reported earlier (Tong et al., 1997; Lakra et al., 2006a). The best growth of cells was observed in the L-15 medium with 20% FBS which showed conformity with Lakra et
al., 2006b. Morphology of TTCF and TTH cells was confirmed by immunocytochemistry. The TTCF and TTH cells both showed positive reaction for vimentin and negative reaction for cytokeratin confirming fibroblastic nature of the cells. The chromosomal analysis revealed that the number of chromosomes in TTCF cell ranged from 96–102 in TTCF and TTH cells with a model value of 100. Both heteroploidy and aneuploidy were observed in the cell line though they were small in proportion. Karyological analysis revealed that over 48% and 46% cells of the TTCF and TTH cell lines possessed a diploid chromosome number of 2n=100, which was identical to the modal chromosome number of *T. tor* (NBFR, 1998). TTCF and TTH cells showed plating efficiency of 63% and 67% respectively with no significant differences between replicates when determined at seeding density of 200, 500 and 1000 cells. Origin of cell line was authenticated with sequence analysis of 16S rRNA and COI region of mitochondrial DNA. The sequence analysis of both 16S rRNA and COI fragments from fin and heart cell lines showed 98-99% similarity with respective gene fragment of *T. tor* already submitted in NCBI.

Applicability of the cell line was determined by determining its transfection efficiency and establishing it as an *in-vitro* model for genotoxicity analysis through comet assay. The estimated transfection efficiency for TTCF and TTH cell lines was 7% and 9% respectively when transected with pEGFP-C1 plasmid using lipofectamine LTX and Plus Reagents (Invitrogen). Comet formation was observed in both TTCF and TTH cells when treated with H2O2 which indicates damage in DNA while no comet was observed in control cells.

Both the cell lines were cryopreserved in liquid nitrogen at regular intervals to minimize genetic changes in continuous cell lines, for long-term storage and to avoid aging and transformation in cell lines. TTCF and TTH cell lines were revived with 72% and 67% viability respectively after 6 month of cryopreservation without any significant morphological alteration or changes in growth rate and cell doubling times after freezing and thawing. These cryopreserved cell line will be instrumental in conserving biodiversity of this important Mahsheer species.

The success in establishing these cell cultures from the different tissues of *Tor* would definitely facilitate *in vitro* research in genetics and conservation of endangered mahsheer species in India. Ultimately leading to improved health management and conservation of aquaculture species.