

Short Research Article**The Establishment of a Fibroblastic Cell Line From Caudal Tissue of *Poecilia reticulata*, Peters 1859****ABSTRACT**

Establishment of cell lines from different tissues of different teleost fishes has remarkably increased. Development of fish cell lines has significantly contributed to recent advances in many research areas. *Poecilia reticulata* also known as guppy is a teleost fish which is widely used for ecotoxicological research. In this study to isolate fibroblast cell from caudal tissue of *P. reticulata* and to develop a cell line. For this purpose caudal tissue samples were collected from ten male *P. reticulata* under aseptic conditions. Primary culture was performed with tissue explant technique. Primary cells fed with L15 culture medium supplemented with FBS. To investigate the effects of different incubation temperature on proliferation rate of cells, seven different incubation temperatures (15°C, 20°C, 25°C, 28°C, 30°C, 32°C, 37°C) were assessed. Same experimental design was used for the effects of different FBS concentration (5%, 10%, 15%, and 20%) on proliferation rate of cells. Optimum growth rate was observed at 28°C temperature and 10% FBS concentration. Cells were subcultured successfully more than 60 times and this resulted in development of a cell line named as PSF. Cultured cells were also successfully cryopreserved. The revival rate of PSF cell line was up to %85-90 and this result could be admitted as a good when compared to other fish cell lines.

Keywords: Poecilia reticulata, cell culture, caudal fibroblast, cell line

1. INTRODUCTION

In the recent years, establishment of cell lines from different organs or tissues of teleost fishes has remarkably increased. First cell line was reported in 1952 by Wolf and Guimby [1]. Since that time, about 238 cell line have been established from teleost around the World [2]. The development of fish cell culture and cell lines has significantly contributed to recent advances in toxicology [3, 4], virology [5] and also cytogenetics [6], biomedical research [7], biotechnology and aquaculture [8], endocrinology [9], fish immunology [10, 11], ecotoxicology [12, 13, 14], disease control [15] and radiation biology [16]. Investigating of environmental pollution and effects of xenobiotics on living organisms, in vitro techniques and cell culture methods is widely used for replacement of animals [12, 17]. Many cell lines especially isolated from gonads, liver, gills and total embryo tissue of fishes has been used for investigate the effects of various chemicals in cellular level. Fibroblast cells isolated from caudal biopsies of fishes can also be used for that purpose [12].

Poecilia reticulata, also called guppy or millionfish is a world wide distributed tropical fish. Because of its small size, easy acclimation of laboratory conditions, easy maintenance and short live cycle and reproduction, it has become widely used laboratory model for ecotoxicological research [17]. A cell line named as GFT was isolated from fully formed unborn embryos of a female guppy [18], but there is no other cell line has established according to literature [18]. The aim of this study was to develop a cell line from caudal fin tissue of guppy and optimize culture conditions.

2. MATERIAL AND METHODS

2.1 Tissue Explant: Healthy juveniles (ten male) of *P. reticulata* were obtained local commercial supply and maintained at the Ege University Science Faculty Biology Department Zoology Section, Ecotoxicology laboratory. Fishes were starved for a day and allowed to swim in well-aerated

33 sterile water before the collecting of fin tissue. The specimens were anaesthetized with keeping them on
34 +4 °C cold water for 5-7 min. Then surface sterilized by wiped with %70 alcohol.

35 Primary cell cultures were initiated by collecting caudal fin tissues from the fishes under aseptic
36 conditions. The tissue samples were transferred into phosphate buffered saline supplemented with 10
37 µg/ml Gentamycin (Gibco). The samples were minced with sterile scissors at room temperature and
38 washed three times with washing buffer.

39 The minced tissue fragments were seeded into 25 cm² tissue culture flasks [TPP, Switzerland] in
40 nearly 1 ml of L15 culture media supplemented with %10 FBS. The explants allowed to attached to the
41 surface of the flask in an incubator at 28 °C for 4 h. Care was taken to avoid over drying to the tissue.
42 After observing tissue adherence, the explants were fed with L15 culture media supplemented with %10
43 FBS.

44 **2.2 Subculture:** For proliferation of cells and morphological details, the flasks were observed
45 daily with using inverted microscope (Olympus Tokyo japan). After cells reach %95 confluency they were
46 trypsinized using a trypsin EDTA solution (trypsin 0.25%, EDTA 0.02%) in phosphate buffered saline. The
47 subcultured cells were grown in fresh L15 with %10 FBS.

48 **2.3 Growth Studies:** For assessment of growth characteristics of the cell line in L15 media,
49 cells were incubated different incubation temperatures (15°C, 20°C, 25°C, 28°C, 30°C, 32°C, 37°C) for 7
50 days. On the consecutive days, three flasks from different temperatures at which they were incubated
51 were withdrawn, the cell lines trypsinized and cell counting performed using a hemocytometer. Same
52 procedure were performed for the effects of various concentrations of FBS (5%, 10%, 15%, and 20%) on
53 cell growth at 28 °C for 7 days.

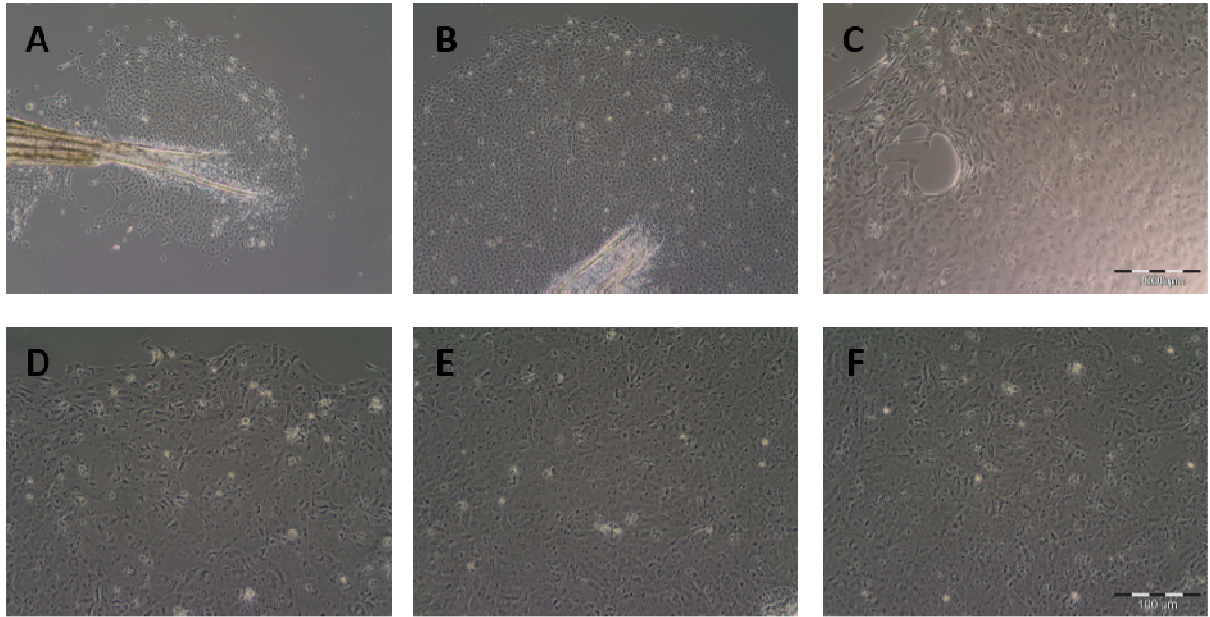
54 **2.4 Cryopreservation:** To observe the ability of cells to survive in liquid nitrogen and stability
55 of them were assessed in freezing medium using the method that described by Freshney [19].
56 Logarithmically growing cells were harvested and re-suspended at 3x10⁶ to 4x10⁶ cells ml⁻¹ densities. The
57 cell suspensions were carefully mixed with equal volume of recovery medium (Invitrogen) which is
58 suitable complete cryopreservation medium for freezing cells (Invitrogen) at 1x10⁶ cells mL⁻¹ according to
59 manufacturer instructions. The recovery medium could increase cell viabilities average of 25% in
60 cryopreservation both adherent and suspension cell lines and it is an optimized fully supplemented
61 formulation which avoids the messy mixing of DMSO. Cell suspensions were aliquoted (1ml) and
62 distributed into 1,5 sterile cryovials (Nunc) held at 4°C for 2 h, -20°C for 2h and -70°C overnight then
63 transferred into liquid nitrogen (-196°C). The frozen cells were thawed after 6 months of post storage at
64 37°C water bath. Freezing medium was removed by centrifugation and cells were suspended in L15 with
65 10% FBS. Cell viability measured by trypan blue staining and number of cells was counted using
66 hemocytometer. Cells were seeded into 25cm² tissue culture flasks for further subculturing.

67 3. RESULTS AND DISCUSSION

68 The cell line named as PSF from caudal fin tissue of *P. reticulata* was established by explant
69 technique. The explant technique has many advantages such as speed, easy maintenance of cellular
70 interactions and no enzymatic damage of cell surface proteins over the trypsinization method [20].

71 For morphological and other examinations, cells were observed by inverted microscope every day
72 during subsequent passages (figure 1). Culture medium was changed every 3 day. Initially, fibroblastic
73 and epithelial cells were found at the beginning of the culture, but after 4th passage, fibroblastic cells
74 dominated over the epithelial cells (figure 1). PSF cells were predominantly observed as fibroblastic cells
75 at 10th passages (figure 1). During early passages, the heterogeneity nature of cells during early
76 passages was also reported by other researchers [21, 22, 23]. The predomination of fibroblastic cells
77 over epithelial cells in cell cultures from fin tissue of fishes also has been reported before [24, 25, 26]. A
78 fibroblast like cell line [LJH-2] was developed from *Lateolabrax japonicus* [27]. In contrast cells isolated

79 from heart tissue of sea perch, have been reported to be epithelial morphology with no change during
 80 subsequent culture [28]. Cells started to spread after 4-5 days of explant preparation from fins. Confluent
 81 monolayer cells were observed 10-12 days of culture (figure 1) Cells were subcultured in L15 with %10
 82 FBS at 5-7 day of interval and split at ratio of 1:2. Cells were subcultured more than 60 times regularly
 83 and this resulted in development of a cell line named as PSF.



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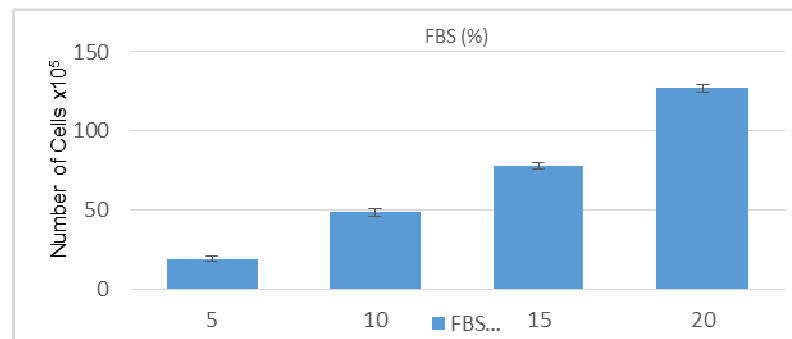
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Figure 1: microscopic observation of cells, A: 3th day of culture, B: 5th day of culture, C: 10 day of culture, D: 4th passage of cells, E: 10th passage of cells, F: 60th day of culture.

87 The effects of different FBS concentration on proliferation rate have been examined and shown in
 88 fig 2 . For this aim, four different (5%, 10%,15%,20%) FBS concentration have been examined.
 89 Relatively, growth rate of cells increased as the FBS portion increased from %5 to %20 at 28 °C
 90 incubation temperature. However, growth rate was observed as minimum at %5 concentration of FBS
 91 and maximum at %10 and 15 FBS. Comparatively fish cell lines have been exhibited good growth at %10
 92 FBS but maximum growth at %15 and %20 FBS concentrations [29, 30, 27].

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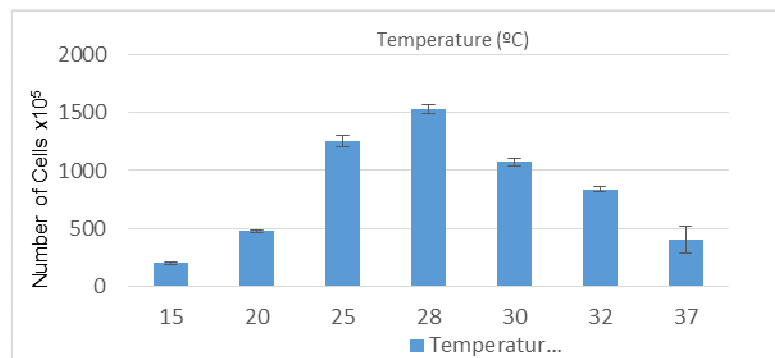


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Fig. 2. proliferation of cells on different FBS concentration

97 For observation of different growth patterns of PSF cell line at different incubation temperatures
 98 between 15°C and 37°C was examined and shown in Fig. 2.1 . The minimum growth rate was observed
 99 at 15 °C incubation temperature. The monolayer detachment was observed 37 °C incubated cells.
 100 However optimum culture temperature was found to be 28 °C. This temperature also reported for other
 101 fish cell lines in the literature before (21, 31, 32, 33, 34, 35, 23).



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Fig. 2.1: proliferation of cells on different incubation temperatures

104 As described before, ideal growth temperature for cultured fish cells is a few degrees above
 105 environmental living temperature of fish. [37], because of selection or adaptation of cells to growth of
 106 higher than normal temperature [38]. For tong et al [39], temperatures between 35-37°C has been found
 107 lethal for many fish cells. As seen in the literature, fish cells could be grown at over wide temperature
 108 range. This could be an advantage for isolating both warm water and coldwater fish viruses [40].

109 The cells that grow in medium containing %20 FBS has showed higher proliferation rate than
 110 cells in medium containing %5-15 FBS (Fig. 2). The growth rate of the cells increased parallel with FBS
 111 concentration from %5 to %20. Nevertheless %10-15 concentration of FBS also provided good growth.
 112 So it would be an advantage to use %10 FBS to maintain the cell line because of low cost. In the
 113 literature, relatively good growth of the fish cell observed at %10 FBS but maximum growth also observed
 114 at the concentration of %15 to %20 (29,26, 27).

115 Protection of cell lines from genetic change and avoid aging and transformation, cryopreservation
 116 is needed in long term storage [19]. The PSF cell line frozen in liquid nitrogen and storage during 6
 117 months. When cells were thawed, they showed %85-90 viability and continued to grow and became
 118 confluency within 7 days. The revival rate of the PSF cell line was up to %85-90. This result could be
 119 admitted as a good when compared to that of other fish cell lines reported by Parameswaran et al. [41]
 120 who could revive SISE cell successfully after cryopreservation with the survival rate of %70-80. The GFM
 121 and GSFb cell lines showed %93 and %92 survival rate after cryopreservation according to Roughee et
 122 al [42]. The cell line named as MFF-1 from mandarin fish, *Siniperca chuatsi* revived %80-90 after
 123 cryopreservation [43]. Cells were cryopreserved and successfully revived with the survival rates of %75,
 124 %70 and %72 for RHi RF and RSB cell lines [2]. Swaminthan et al. [32] successfully cryopreserved and
 125 revived with the survival rate of %70. Cell line named as PSF successfully cryopreserved and revived with
 126 the survival rate of %75 by Lakra and Goswami [30].

127 4. CONCLUSION

128 In conclusion, the present study results in the development of cell line PSF from the caudal fin of
 129 *P. reticulata*. The development of cell line PSF from *P. reticulata* has not been reported before. Therefore,
 130 development of a cell line from tissues of guppy (*P. reticulata*) could open new horizons of in vitro
 131 research in fish genetics, conservation and fish biotechnology. Also it could further be used for developing
 132 cellular models for toxicological, ecotoxicological and genotoxicological studies to replace whole animals.

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