COMPARATIVE GROWTH PROMOTING EFFICACY OF FETAL CALF SERUM (FCS) FOR BABY BAMSTER KIDNEY-21 (BHK-21) CELL LINE

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ABSTRACT

A study was conducted to evaluate the growth promoting effect of FCS and its proper dilution in cell culture media for persistent cultivation and maintenance of BHK-21 cell culture, by comparing it with other animal sera. Different animal sera of different species as cattle calf serum , Allanto-amniotic fluid , Sheep serum , Poultry serum and Goat serum @ 10% were added to cell culture medium (M 199, Biomedicals; USA). A fast and reliable technique, MTT (3 {4, 5-Dimethyzol-2-Yl} 2, 5-Diphenyl Tetrazolium Bromide) Assay was employed to measure the cell densities in small amount of cell culture media by measuring OD values under 570nm, with 630nm as a reference read out. It was concluded that FCS is the best growth promoting agent for BHK-21 cell line and 5-7.5 % concentration has the desired effect of persistent cultivation and maintenance of BHK-21 cell line.

Key words: BHK-21 cells, fetal calf serum, cell density, growth promoting factor.

INTRODUCTION

Baby Hamster Kindney-21 (BHK-21) fibroblast cell line is extensively used all over the world for propagation and maintenance of Foot and Mouth Disease Virus (FMDV) (Spier and Whiteside**,** 1976) for the extraction of Pseudorabies virus DNA (Wei-Wei *et al*., 1998) for production of ELISA antigen of Japanese encephalitis (JE) virus (Bundo *et al., 1989).*

Fetal calf serum (FCS) in BHK-21 cell culture medium is used as a growth promoting factor, attachment factor and nutritional and for macromolecular growth factors essential for cell growth. Although a number of synthetic media have been prepared, serum continues to be used in cell culture by many investigators. The most frequently used supplement to basal medium is fetal calf serum (FCS) for all types of cell cultures. Different cell culture laboratories have their own standards for appropriate concentration of FCS, required for maintenance of different cell lines. So this study was designed to study the growth promoting effect of FCS and its proper concentration for the maintenance and persistent cultivation of BHK-21 cell line in cell culture laboratory of our own local environment. The efficacy of sera from other sources (cattle calf serum, allanto-amniotic fluid, sheep serum and goat serum) was also compared with FCS.

MATERIALS AND METHODS

Source of BHK-21 cell line and animal sera: BHK-21Cell line was obtained from the Department of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore. FCS (local market), cattle calf serum , Allanto-amniotic fluid , Sheep serum , Poultry serum and Goat serum were obtained from local abattoir and were sterilized by gamma radiations and inactivated by heating at 56 ^{o}C through 45 mint.

Production of monolayer on flat bottom cell culture plates: BHK-21 cells were harvested from Roux flask using Trypsin (0.25%, Gibco). The cells were then washed once in serum free medium @ 1000 rpm for 3 minutes. The pellet was dissolved in serum free medium of known volume and a small aliquot was taken for cell counting by trypan blue staining technique as modified earlier (Davis *et al*., 2002) The cell count was adjusted to ($1x10^5$ cells/ml) approximately. Cell suspension of 100ul was transferred to 96 well gamma radiated flat bottom cell culture plate till monolayer formation during next 72 hrs incubation.

Comparative growth promoting effect of FCS: The BHK-21 cells from Roux flask were harvested and transferred to 96 well gamma radiated cell culture plates such that each well receive 100ul serum free medium 199 containing cells ($1x10^5$ cells/ml) .The plate was divided into six replicates such that each replicate containing 16 wells column wise. Each replicate was added with 10% cattle calf serum , Allanto-amniotic fluid , Sheep serum , Poultry serum , Goat serum and Fetal calf serum (all sera samples were gamma radiated and sterilized, heat inactivated at 65^{o}C for 45 mint), respectively. The plate was incubated at 37^{o}C for 72 hours in which a complete monolayer is formed under normal circumstances that is when we use fetal calf serum as a growth promoting agent. After 72 hours post cultivation MTT Assay was performed as Mosm**a**nn, (1983) with the modi**f**ic**a**tions suggested by Muhammad, (1993).

Procedure for MTT Assay: When a complete monolayer was formed, 10 ul MTT salt (3{4,5-Dimethyzol-2-yl} 2,5-Diphenyl Tetrazolium Bromide) was added to each well. The plate was incubated for 16 hrs at 37°C. 100 ul Isopropanol, acidic (0.04N HCl was added to isopropanol) was added to each well. The violet color precipitate was dissolved through vigorous pipetting. ELISA reader (Trinity Biotech) recorded the absorbance values (OD) under 570nm wavelength with 630nm as a refernce read out.

Effect of different concentration of fetal calf serum on the growth of BHK-21 cells: The BHK-21 cells from Roux flask were harvested and transferred to 96 well gamma radiated cell culture plates such that each well receive 100ul serum free medium 199 containing cells ($1x10^5$ cells/ml) .The plate was divided into six replicates such that each replicate containing 16 wells column wise. Each well was added with 0, 2.5, 5, 7.5, 10 and 12 % fetal calf serum and incubated at 37°C. MTT Assay was performed 72 hours post incubation as discussed above.

RESULTS AND DISCUSSION

Comparative growth promoting effect of FCS

The OD values of Cattle calf serum, 0.406, 0.423, 0.467, 0.446, 0.428 and 0.326 with a mean value of 0.41± 0.05, of Allanto-amniotic fluid were 0.132, 0.128, 0.133, 0.112, 0.117 and 0.086 with a mean value of 0.14± 0.06, of Sheep serum, 0.457, 0.519, 0.464, 0.503, 0.468 and 0.503 with a mean value 0.49± 0.06, of Fetal calf serum, 0.590 , 0.5820, 0.536, 0.553, 0.502 and 0.554 with a mean value 0.55± 0.03, of Goat serum, 0.556, 0.472, 0.520, 0.511, 0.523 and 0.598 with a mean value 0.55± 0.05 and of Poultry serum, 0.141, 0.119, 0.130, 0.137, 0.126 and 0.115 with a mean value 0.13± 0.009. A graph was plotted and the values were compared which showed that the growth promoting effect of Fetal calf serum was more than the all sera, anyhow the goat serum was observed to have all most equal potential to support the growth of the cells and Allanto-amniotic fluid does not effect the growth (Fig. I).

Effect of different concentration of Fetal Calf Serum (FCS) on the growth of BHK-21

cells line : The OD values with 0% FCS was 0.077, 0.085, 0.081, 0.078, 0.071 and 0.073 with a mean value of 0.07± 0.008, with 2.5% FCS were 0.161, 0.141, 0.150, 0.141, 0.112 and 0.102 with a mean value 0.13± 0.02,with 5% FCS 0.336, 0.423, 0.389, 0.388, 0.377 and 0.372 with a mean value 0.35± 0.05, with 7.5% FCS 0.420, 0.559, 0.523, 0.534, 0.553 and 0.529 with a mean value 0.35± 0.05,with 10% FCS 0.469, 0.430, 0.556, 0.511, 0.524 and 0.521 with a mean value 0.45± 0.12 and with 12% FCS 0.411, 0.620, 0.607, 0.536, 0.601 and0.682 with a mean value 0.48± 0.10 were recorded and put in (Table-3).

Table-1. Comparative growth promoting effect of FCS

S.#	Growth Factor (10%)	Optical Density at 570 & 630 nm						MTT Assay MEAN ± S.D
1	Cattle calf serum	0.406	0.423	0.467	0.546	0.428	0.326	0.41± 0.05
2	Allanto-amniotic fluid	0.132	0.128	0.133	0.112	0.117	0.086	0.14± 0.06
3	Sheep serum	0.457	0.519	0.464	0.503	0.468	0.503	0.49± 0.06
4	Fetal calf serum	0.590	0.5820	0.536	0.553	0.502	0.554	0.55± 0.03
5	Goat serum	0.556	0.472	0.520	0.511	0.523	0.598	0.55± 0.05
6	Poultry serum	0.141	0.119	0.130	0.137	0.126	0.115	0.13± 0.009

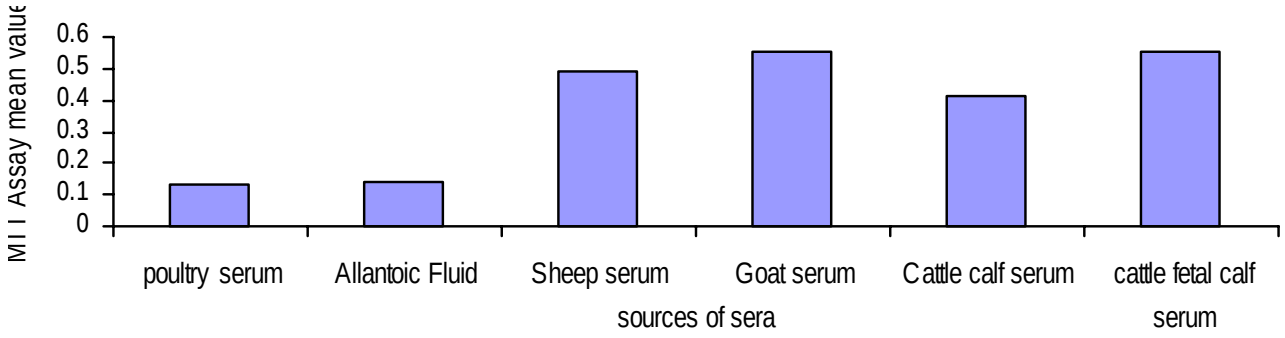

Figure-1: Comparative growth promoting efficacy of FCS

Table- 3: Effect of FCS the growth of BHK-21 cells.

Serum conc.*	Optical Density at 570 & 630 nm**						MTT Assay Mean ± S.D
0	0.077	0.085	0.081	0.078	0.071	0.073	0.07+0.008
2.5	0.161	0.141	0.150	0.141	0.112	0.102	0.13+0.02
5	0.336	0.423	0.389	0.388	0.377	0.372	0.35+0.05
7.5	0.420	0.559	0.523	0.534	0.553	0.529	0.35+0.05
10	0.469	0.430	0.556	0.511	0.524	0.521	0.45+0.12
12.5	0.411	0.620	0.607	0.536	0.601	0.682	0.48+0.10

*= Percentage level of FCS. It was used in varying level of percentage in cell culture medium M199. **= Optical density value was measured under double wavelength using filters of 570& 630 nm.

Different animal sera were provided @ 10% in cell culture media and the quantitative analysis of BHK-21 is carried out through a new and modified technique to measure the cell densities. Similar studies were perofremed by (Freimoser *et al.*, 1999, Weichert ., *et al* 1991, Ciapetti *et al* 1993, Kruman and Arkhipov., 1985) for the measurements of cells densities.

BHK-21 cells are harvested from Roux flask using 0.25% v/v trypsin and transfer to a cell culture plate containing medium 199 and different concentration of FCS at 37 ^{o}C for the production of monolayer (Hussain *et al.*, 2003). Seventy two hrs post incubation, the old media was discarded and a fresh media was added. MTT salt (MTT salt 0.5mg/ml as a stock solution was placed at 4^{o}C), 100 ul/well was added and incubated at 37^{o}C and was incubated for 16 hrs. These findings are going parrallal with those of Freimoser, (1999).

The results of this study revealed that the transformation of the tetrazolium salt to formazan and its quantification could serve as a measure for cell densities. (Muhammad, 1993 and Freimoser *et al*., 1999) The removal of the medium prior to the measurement make all measurements transparent and uniform because all the measurements were made in isopropol and different complex medias did not interrupt the OD values. (Freimoser *et al.*, 1999). This study reveals that FCS has growth stimulating effects on this cell line and increase in serum level in the growth media, increases cells density by stimulating its growth. These findings are in line with Kruman *et al*. (1984) who, compared the growth-stimulating effect of two calf and adult animal serum ultrafiltrates (with molecular weight of the components up to 100 000 daltons) on BHK-21 cells. The growth stimulating effect of FCS is due to its certain nutritional and macromolecular growth factors essential for cell growth (Paranjape, 2004). MTT assay has been employed to evaluate the growth stimulating effects of FCS for measurement of cell densities and it has been shown that the color optical density (OD) is directly proportional to the density of viable cells. The activity of MTT Assay depends on the mitochondrial dehydrogenase activity which transforms the MTT salt, (3{4, 5-dimethyzol-2-yl} 2, 5-diphenyl tetrazolium bromide), into violet water soluble formazan in living cells. The quantity of formazan is directly proportional to the number of living cells (Muhammad, 1993). The results of the present study are congruent with the findings of Gomez *et al*. (1997), who used the colorimetric MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide)] assay to assess cell viability in isolated quiescent adult guinea-pig ventricular myocytes exposed to different insults or cardioprotective conditions, including adenosine and hyperkalemic-cardioplegia. Similarly, present findings are in line with Ahne *et al.* (2005) who identified Coxsackievirus B3 (CVB3) as a major causative agent of acute and chronic myocarditis. Morphological abnormalities were examined on primary cardiomycytes by both light and fluorescence microscopy after Hoechst 33342 staining, and loss of cell viability was estimated by MTT assay.

The results of MTT assay in present study shows that 5-10% FCS can be used for the maintenance and persistent cultivation of BHK-21 cells. These findings are congruent with Davis (2002) and Freshney (1998). Both studies recommended the use of 5-10% FCS for maintenance and persistent cultivation of all types of adherent cell cultures. Similar observations also recorded by Ryan (1997), who studied the effect of Eagle's minimal essential medium, containing different fetal bovine serum (FBS) concentrations, on the proliferation and replicative life span of cultured chick cells. Their results showed that the rate of chick cell proliferation and the cell density at stationary phase increased as a function of serum concentration between 5 and 30% FBS. He showed that the kinetics of cell population aging can be affected by the culture medium. Other animal sera have been tested for its growth supporting ability to BHK-21 cell line, the results show that FCS has better growth supporting ability than other source of sera like bovine, sheep, goat serum and poultry serum. These findings are parallel with Padamraj *et al.* (1991) and Kruman *et al*. (1984). However, Padamaraj *et al*. (1991) has also shown that PEG treated bovine serum has similar effect to FCS and better than goat serum. It has been thought that the enhancement in growth stimulating effect of bovine sera may be due to PEG treatment.

Goat serum (GS) was seemed to be comparably with FCR or the maintenance of BHK-21 cells to in cell culuture laboratories. This finding couples with findings of Paranjape *et al*., (2004) and Castillo, 1991). They found that the Goat serum (GS) was suitable for most of the cell lines and primary cultures. They prepared the primary cultures from guinea pig embryo, monkey kidney, chick embryo, mouse peritoneal macrophages, and established cell lines were prepared and grown in growth media supplemented with GS. Organ cultures from mammalian, reptile and avian hosts; successfully grown in GS supplemented growth media.

CONCLUSION

It may be concluded that FCS is the best growth promoting agent for BHK-21 cell line and 5-7.5 % concentration has the desired effect of persistent cultivation and maintenance of BHK-21 cell line in our own local conditions. It is further added that goat serum (G.S.) can be used for the maintenance of BHK-21 cells to in cell culture laboratories this needs further investigations.

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