J Biotechnol Biomater 2012, 2:6
http://dx.doi.org/10.4172/2155-952X.S1.020

OMICS Group Conferences
Accelerating Scientific Discovery

3rd World Congress on
Biotechnology

September 13-15, 2012 Hyderabad International Convention Centre, Hyderabad, India

Isolation and characterization of ovine embryonic stem cells

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Embryonic stem (ES) cells have become very important resources in basic medical researches. ES cells are derived from explanted culture of the inner cell mass (ICM) of blastocyst stage embryos and has definite explicative potential and differentiate into derivatives of all three germ layers. Initially invitro fertilized embryos were cultured using TCM 199 +20% Fetal bovine serum + L–Glutamin 2mMol and Penicillin/Streptomycin 100mg/100IU/ml for 3 days. These cell lines were generated by removing ICM from preimplantation embryos using 0.75% pronase. Es cells are grown on a layer of mitotically inactivated primary mouse embryonic fibroblast (MEF) feeder layer to promote growth and prevent differentiation using mitomycin – c, primary embryonic fibroblast were isolated from 13 to 14 days old fetuses. The cells were disaggregated and trypsinized every 3 days until the appearance of the colonies of ES cells. The colony positive cells were fixed and stained for alkaline phosphatase. The ES cells were cultured in suspension state for 5 days; at the same time Leukaemia Inhibitory Factor (LIF) was removed from media to form embryoid bodies (EBs). Undifferentiated cells were seen as intensely small cells attached together compactly with high nucleus/cytoplasm (N/C) ratio. The cells of colonies tend to differentiate by separation from each other and became larger and diffused on substrate by attaching to dish. The positive alkaline phosphatase cells were seen in typical morphology of ES colonies. The EBs cells were seen in culture after 5 days in suspension and began to spontaneously differentiate into various types of cells such as nerve and hematopoitic lineages.

Keywords: Embryonic stem cells, Embryoid bodies, Differentiation, ovine.

Biography

P. Iyya Shankar completed her Post graduation in M.Sc Biotechnology from University of Madras in the year 2007. He is currently working as Senior Executive – cell culture department in Life cell International Private ltd, Chennai, Tamil Nadu, previously working as Senior Research Fellow in Department of Animal Biotechnology, MVC, Tanuvas, Chennai – 600053, Tamil Nadu, India.

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Molecular evidence for association of a begomovirus and betasatellite with a recently emerged leaf deformity disease of Sunn-hemp in eastern India

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Sunn-hemp (Crotalaria juncea L.) is cultivated as a multipurpose legume especially for its bast fiber in many parts of the world including India. It produces high organic matter yields, potential to fix biological nitrogen, and could reduce the build-up of root-knot nematode populations. A leaf deformity disease of the crop not hitherto known was observed recently at different farmers field of West Bengal and experimental farm of Central Research Institute for Jute and Allied Fibres (CRIJAF), Barrackpore, Kolkata, West Bengal. Diseased plants showed various symptoms like up- and down-ward leaf curling, puckering and leaf crumpling along with severe height reduction. These plants were collected and used as initial source for viral inoculum in transmission experiment. Minimum 10 whiteflies were found to transmit the virus with only 20% transmission efficiency. Increase in the number of whiteflies increased the transmission efficiency. After whitefly transmission, typical symptoms appeared after at least 16–20 days under glasshouse conditions. Total genomic DNA was extracted from the plants obtained from field (symptomatic and asymptomatic) and glasshouse (whitefly inoculated and healthy) by CTAB method. The presence of begomovirus genome (DNA-A and DNA-B) and the associated betasatellite was detected by PCR using primers PALIc1960/PARIv722, PBL1v2040/PCRc1 and β01/β02 respectively. PCR with DNA-A and betasatellite primers yielded 1.2 kb and 1.3 kb amplicon where as PCR with DNA-B primers failed repetitively indicating the presence of a monopartite begomovirus and a betasatellite with the disease.

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