Network for Research and Development in Africa
International Journal of Pure and Applied Science Research
ISSN: 2384-5918. Volume 12, Number 5
Pages 111-117 (September, 2022)
DOI: 457277112513
www.arcnjournals.org

Application of Cell Suspension Culture in Plant & Animal Biotechnology

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Abstract: *Cell suspension culture is a type of culture in which single cells or small aggregates of cells multiply while suspended in an uptight liquid medium. The establishment of single cell cultures through suspension cultures provides an exceptional chance to investigate the characteristics and potentialities of plant cells as well as animal cells for several reasons such as growth and characteristics in different cell lines, physiology and morphological presentations of such cell undergoing finite and continuous growth, studies for industrial research as well as Agricultural purposes. Such systems contribute to our understanding of the interrelationships and corresponding influences of cells in higher organisms. In addition, free cells in cultures permit quick manipulation and withdrawal of diverse chemicals/ substances thereby making them easy targets for mutant selection. Therefore, both plants and animal cells are in direct or indirect participation in suspension cultures where different varieties of crops and cell lines are grown for research and industrial purposes.*
Keywords: Cell, Culture, Suspension, Plants, Animal

Key words: *Aspergillus flavus, AgraQuant, Aflatoxin, Kwata, ELISA*

INTRODUCTION

Suspension culture or otherwise cell suspension culture as the name implies is a type of culture in which single cells or small aggregates of cells multiply while suspended in an uptight liquid medium. The establishment of single cell cultures provides an exceptional chance to investigate the characteristics and potentialities of plant cells. Such systems contribute to our understanding of the interrelationships and corresponding influences of cells in higher organisms. Many plant biotechnologists recognized the merits of applying cell cultures over an intact organ or whole plant cultures to synthesize natural products. Generally, substantial numbers of differentiated cells obtained from diverse tissue origin lose their specialized features and dedifferentiate when grown under conventional two-dimensional cell culture conditions (Hammond; 2001). Suspension culture is introduced as the most popular way of

preventing this problem and maintaining specialized features of cells. The rich and diverse range of available culture vessels for suspension culture makes the selection of specific culture devices baffling (Hammond; 2000) First attempt to perform cell suspension culture was performed by Haberland in 1902. However the process failed to achieve divisions in free cells, but his detailed paper in 1902 stimulated further studies in this area. Similarly, Steward and Shantz; 1996, performed far-reaching work on carrots and reported tremendous success in suspension cultures from carrot root explants and obtained a very large number of plantlets from the culture. More than half a century has passed since the concept and practice of plant cell culture was rest introduced. Unlike most animal cells, plant cells can change from one differentiated state, representing a committed developmental program, to an entirely dissimilar one through a transition by a dedifferentiated state typical of callus tissue. This process is attained by changing concentrations and relative size of two major plant growth regulators (auxin and cytokinin) in the culture medium. Under proper conditions, callus cells can continue to grow in ‘‘immortalized’’ suspension cultures, which can be maintained continuously without differentiation. Plant cells grown in culture showed extraordinary levels of genetic and epigenetic instability. Through reform exhibited in gene activity, plant cells are able to counter to the challenges presented by tissue culture conditions and continue to grow according to internal and external cues. Epigenetic regulation plays an important role. For example, hormone habituation is a process during which plant cells in culture swing their needs for exogenous growth regulators. (Tanurdzic; 2008)

Importance of Cell Suspension Culture Cell suspension culture is performed to obtain single cell clones. Through cell suspension culture the morphological and biochemical changes during plant growth and development phases are studied. Free cells in cultures permit quick administration and withdrawal of diverse chemicals/ substances thereby making them easy targets for mutant selection. Single cells derived from medicinally important plants can be studied for the production of secondary metabolites like alkaloids, glycosides. Suspension is now involved in mutagenesis study. The mutagens can be added directly in the liquid medium. After the mutagen treatment, cells are plated on agar medium for the selection of mutant cell clones (Encina *et al.,* 2001). The hope is that permanent changes in the DNA patterns of some of the cells would be achieved by such treatments. Plant-suspension cells are an in vitro system that can be used for recombinant protein production under carefully controlled certified conditions. Plant-suspension cells can be grown in shake flasks or fermenters to produce secondary metabolites, like vincristine and vinblastine, and to produce recombinant proteins after transformation. Plant cell suspension cultures have been used in various applications in research and discovery, as well as commercial production of the plants by means of micropropagation. They endow with suitable in vitro studies for genetic manoeuvring, mutant initiation, and protoplast production. The cultivation of these cultures can be done at any time in a suitable laboratory environment without being affected by natural conditions such as weather or seasons. Consequently, suspension culture proved promising in the field of plant biotechnology and therefore could help in increase in food production for the rapid increasing global population (Encina et al., 2001). In an upgrading arrangement three-dimensional

suspension culture have established to be an favourable alternative to monolayer techniques for major expansion of cells, suspension methods have been widely adopted: (1) for scalable and controlled extension of stem cells as well as cancer cells ; (2) for channelling stem cell differentiation; (3) for the fabrication of cellular spheroids well as tissue-like constructs . The stipulation of a 3D suspension culture atmosphere, imitating the microenvironment of the cellular niche, has confirmed to be advantageous, encouraging cell survival and maintaining cell functional properties *in vitro* (Massai *et al*, 2016).

Animal Suspension Cultures

As practice in the field of agriculture in particular plant propagation, suspension cultures is also applicable in field of animal as well as microbiological cell propagation. In animal tissue culture three major ways of cultivation were adopted: i. Organic culture ii. Explants plant culture iii. Organotypic culture In organic culture, the architectural properties are of the tissue is maintained in the culture, although not complete. Liquid gas interface are utilised for this purpose which enabled the maintenance of three dimensional structures. On the other hand, explants culture involved the utilization fragments of cell aggregates are placed at the glass – interface in which subsequent attachment enhances passage unto the solid substrate. Similarly, cell culture exhibits that the tissue extrusion from initial explants is dispersed by means of mechanical process or chemical process in to a cell suspension which are usually grown as adherent monolayer on growth suspension media (Lan, 2006)

Applications of pluripotent stem cells in therapeutic and industrial require outsized cell number produce in defined conditions. Single cell-inoculated suspension cultures of human pluripotent stem cells (hPSCs) counting human induced pluripotent stem cells (hips) and human embryonic stem cells (hESC) to stirred tank reactors . These systems allow uncomplicated increase and monitoring of input process. (Haverich *et al*, 2012)

Characteristics and Distribution of Suspension Cultures

The suspension cultures could be categorised into two (2) each containing subdivision: A) Batch Culture: a. Slowly rotating culture b. Shake culture c. Spinning culture d. Stirred culture B) Continuous Culture: a. Chemostats b. Turbidostats. Batch culture Batch culture is a type of suspension culture where the cell components separate in a finite volume of agitated liquid containing medium, such as cell material in quantity range of 10 ml or 20 ml or 30 ml liquid medium in each course constitute a batch culture. Batch suspension cultures are us in most cases maintained in conical flasks incubated on orbital platform shakers at the speed range of 80-120 revolutions per minute (rpm). Single cells and cell aggregates are grown in a specially designed flask; each flask contains about eight nipples like projections. The volume of each flask measured 250 ml. Ten flasks were loaded in a spherical manner on the big horizontal disc of a vertical shaker. The rotation of the flat disc at the speed of 1-2 rpm, the cell within each nipple of the flask are alternately covered in cultured medium and exposed to the air environment. The older medium is constantly replaced by the fresh liquid medium to stabilize the

physiological conditions of the multiplying cells (Biodiscussion, 2015). Under normally circumstances, the liquid medium is not replaced until the exhaustion of some nutrients available in the medium and the cells are kept in the medium for some time. As a result active growth phase of the cell declines the depletion of nutrient. In continuous culture system, nutrient depletion does not occur due to continuous flow of nutrient medium and the cells always remain in the steady state of active growth phase. In this system, culture vessels are generally cylindrical or circular in shape and possess inlet and outlet pores for aeration and the introduction of and removal of cells and medium (Biodiscussion, 2015)

Shake Culture: It is very straightforward and efficient system of suspension culture. This method involves single cells and cell aggregates in preset volume of liquid culture medium are positioned in conical flask. Conical flasks are mounted with the aid of clip on a horizontal or parallel large square plate of an orbital platform shaker. The plate moves by a circular motion at about180 rpm. Spinning Culture: Large volume of cell suspension may be cultured in 10L,bottles which are rotated in a culture spinner at 120 rpm at an angle of 45 0.

Stirred Culture: This system is also used for large scale batch culture. In this method, the large culture vessel is not rotated but the cell suspension inside the vessel is kept dispersed continuously by bubbling sterile air through culture medium. The use of an internal magnetic stirrer is the most convenient way to agitate the culture medium safely. Magnetic stirrer revolves at 200-600 rpm. The culture vessel is a 5-10 litres round bottom flask (Gregory and Susan, 2016).

Continuous Culture System: In this system, the old liquid medium is continuously replaced by the fresh liquid medium to stabilize the physiological stage of the growing cells. Normally, the liquid medium is not changed until the depletion of some

nutrients in the medium and the cells are kept in the same medium for a certain period. As a result, the active growth phase of the cell declines the depletion of nutrient. The cells passing through out flowing medium are separated mechanically and reintroduced in the culture. **Chemo stats**: In this system, cultures vessels are generally cylindrical or circular in shape and posse's inlet and outlet pores for aeration and for introduction of and removal of cells and medium. The liquid medium containing the cell is stirred by a magnetic stirrer. The introduction of fresh sterile medium, which is pumped in at a constant rate into the vessel is balanced by the displacement of an equal volume of spent or old medium and cells. Such a system can be maintained in a steady state so that new cells are produced by division at a rate which compensate the number lost in outflow of spent medium (Narges *et al*, 2015) Turbostats: In this system, the input of medium is intermittent as it is mainly required to control the rise in turbidity due to cell growth. The turbidity of a suspension culture medium changes rapidly when cells increase in number due to their steady state growth. The changes in turbidity of the culture medium can be measured by the changes of optical density of the medium. In Turbostats an automatic monitoring unit is connected with the culture vessel and such unit

adjusts the medium flow in such a way as to maintain the optical density or PH at chosen, present level (Astrid Catalina et al, 2016).

Characteristics and Features of Suspension cultures in Plants

The concept of plant suspension cultures has soon started for about half a century when the idea and practice of animal cell suspension cultures was also introduced (Tanurdzic et al., 2008). The most striking and fascinating about the plant suspension culture is its ability to change from one differentiated state to an entirely different one through a transition via a dedifferentiated state typical of callus tissue. This process is attained by different concentrations as well as relative proportions of two most important classes of plant growth regulatory hormones (auxin and cytokinin) in the growth medium (Osborne and Mcmanus, 1986). Establishment of suspension cultures of plant cells in liquid medium, similar to microbes, in the mid-1950s prompted scientists to apply this system for the production of natural plant products as an alternative to whole plant. The first attempt for the industrial production of secondary metabolites in vitro was made during 1950-1960 by the Pfizer Company and the first patent was obtained in 1956 by Routien and Nickell. However, not much progress in this area was made for many years. Apparently, the industrial production of secondary metabolites required large scale culture of cells (Bhojwani and Razdan,1996).

Flow diagram showing cell suspension culture and regeneration of plant by embryogenesis

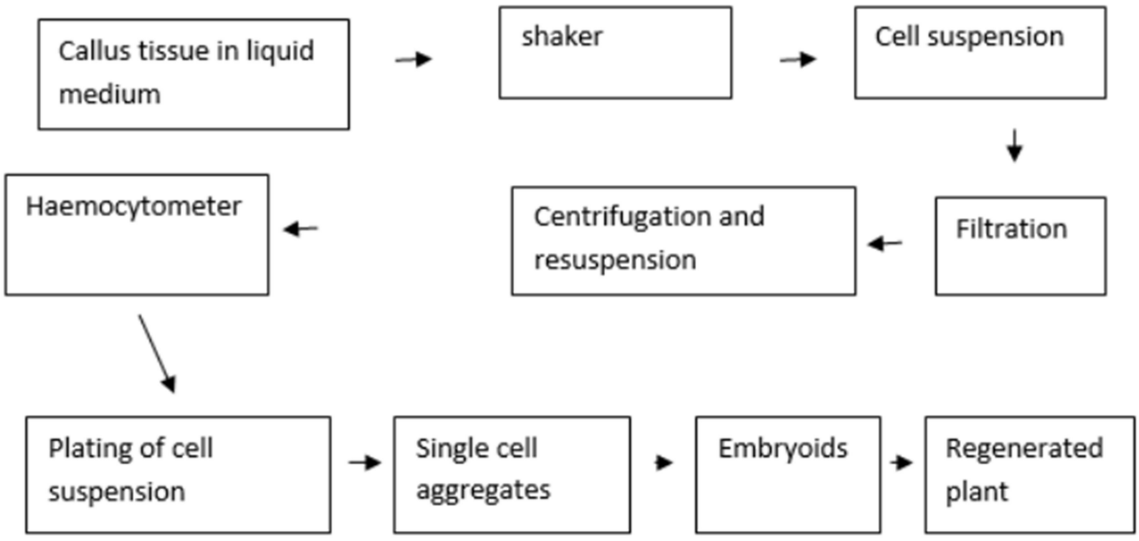

Figure 1; Flow diagram showing cell suspension culture and regeneration of plant by embryogenesis

Culture medium for suspensions

The medium used for raising fast growing friable callus should generally prove suitable for initiating suspension cultures of that species provided, of course, agar is omitted from it. Manipulation of the auxin/ cytokinin ratio to achieve better cell dispersion is desirable. For tobacco, increasing the concentration of 2,4-D from 0.3 mg 1-1 to 2 mg 1-1 and supplementing the callus medium with additional vitamins and casein hydrolysate have been recommended In actively growing suspension cultures the inorganic phosphate is rapidly utilized and, consequently, it soon becomes a limiting factor have demonstrated that in tobacco suspension cultures maintained in a medium with standard MS salts the phosphate concentration declines to almost zero within 3 days of the initiation of culture. When the phosphate concentration in the medium was raised three times the original level, it was completely utilized by the cells within 5 days. B5 and ER media given in Table 3.1 were developed for suspension cultures of higher plants. These and other synthetic media are normally suitable only if the initial population density is around 5 • 104 cells m1-1 or higher. With a lower cell density the medium needs to be enriched with various other components (Bhojwani and Razdan,1996)

In a nutshell, both plants and cells are in direct or indirect participation in suspension cultures where different varieties of crops and cell lines are grown for research and industrial purposes.

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www.biologydiscussion.com , October 20th, 2015