

A Review of Insect Cell Culture: Establishment, Maintenance and Applications in Entomological Research¹

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Abstract A great number of cell lines from hemipteran, lepidopteran, and dipteran insects have been developed and characterized. Using the advent of new techniques and instruments in molecular biology as well as the advancement in biotechnology, the indigenous cell lines might prove useful in the development of alternatives to conventional chemical pesticides for agriculture and in the creation of vaccines and life-saving pharmaceuticals for human and animal diseases. Cell cultures of insects are used for the generation of vaccines, viral pesticides, and recombinant proteins, and in basic research in virology, endocrinology, molecular biology, genetics, and biochemistry. This paper summarizes information collected regarding the different insect cell lines developed and characterized thus far and also describes different applications in entomological research.

Key Words insect cell lines, tissue culture, cell culture

At present, according to American Type Culture Collection records, there are more than 4,000 cell lines from more than 150 distinct species including approximately 1,000 cancer cell lines and other collections, such as stem cells (https://www.atcc.org/en/Products/Cellsand_Microorganisms/Cell_Lines.aspx). Newly established cell lines are to be authorized by the International Cell Line Authentication Committee. Regularly used immortalized cell lines are HeLa (human epithelial cells), 3T3 (mouse fibroblast), S2 (*Drosophila* macrophage-like cells), and Sf9 (fall armyworm ovary cells) (Alberts et al. 2002). Although immortalized cells are suitable for conducting research in controlled environments, proteome profiling reveals that immortalized cell lines can have significant rearrangement of metabolic pathways and up-regulation in cell cycle-assoc (Craven et al. 2006, Merkley et al. 2009, Pan et al. 2009). Our objective in this report was to review currently available information on the types, historical uses, and potential uses of cell cultures related to entomological research.

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Definitions and Types of Cell Cultures

The isolation of cells from animal tissues and their subsequent and successful growth in artificial culture situation is referred as cell culture (Phelan and May 2016, Reid et al. 2016). Cells for cell culture might be removed directly from the tissue and separated by mechanical or enzymatic means, or they might be collected from a previously established cell line or cell strain (Goosen 1993, Weiss et al. 1993). Cells thus removed from tissues of a variety of living organisms can be maintained in culture vessels under appropriate conditions (Annathur et al. 2003, Rieffel et al. 2014, Schlaeger 1996) including suitable culture media, incubation temperature, atmospheric CO₂ concentration, and humidity (Landauer 2014, Spasojevic 2016). In general, the culture medium is composed of various amino acids, salts, vitamins, serum or other proteins, and antibiotics (Abitorabi and Wilcox 2011). Primary cell cultures are those derived from cells removed from tissue without any cell multiplication. Any subsequent cultures from cell multiplication are termed secondary cultures (Fischer et al. 2010, Sullivan 2010). Most cell lines are anchor-dependent and are cultured while attached to a substrate and are known as monolayer cultures or adherent cell cultures (Danielsson et al. 2010), whereas others that are able to be grown suspended in the culture medium are called suspension cultures (Rowghani et al. 2004, Willems and Jorissen 2004).

History of Insect Cell Culture

Harrison (1907) is the first reporter of cell culture use in research in his paper on the nerve cells of frogs in a hanging drop culture. Earle (1944), who had previously created a continuous cell line from a rodent, developed the first human cancer cell line in culture. Gey et al. (1952) produced the first human immortalized cell line (HeLa) from cervical cancer cells from cancer patient Henrietta Lacks. The continued existence and use of the HeLa cell line has produced noteworthy results, including the development of the polio virus vaccine by Jonas Salk, while posing ethical issues involved in its initial collection and continued use. In this regard Skloot (2010) strongly believes that views of individuals should be respected and not taken for granted. Eagle's (1955a, b,c) development of growth media led to the extensive use of cell cultures *in vitro* for biological research. Most of cell culture media currently used in cell biology research are based on the Eagle medium.

Prior to those developments, several entomologists had already reported use of insect cell cultures developed *in vitro* as a research tool. Goldschmidt (1915) placed spermatocysts from the cecropia moth (*Hyalophota cecropia* (L.)) into culture to monitor the development of the spermatozoa, while Glaser and Chapman (1912) successfully followed the development of wilt disease in cultured insect hemocytes. These early experiments utilized a basic salt solution or hemolymph as the culture growth medium, but those cultures usually could not be maintained for more than few days. Almost 50 yr later, Grace (1962a,b) successfully developed insect cell cultures that he maintained for long periods of time in the laboratory. With this development, by 1999 over 500 continuous insect cell lines from more than 100 species of insects had reportedly been developed and maintained (Lynn 1999a, b).

Continuous cell lines of insects are important for research on the molecular mechanisms involved in viral infections of insect cells. From the time when the insect cell lines were first developed (Grace 1962b) and cell lines were successfully infected with plant virus *in vitro* (Chiu and Black 1967), numerous insect cell lines have been developed for studies of the kinetics by which these cell lines are infected and the characterization of the viral genomes. These investigations prompted a series of studies in which leafhopper continuous cell lines were utilized to study many plant-infecting rhabdoviruses and reoviruses (Chiu et al. 1970, Hsu and Black 1973). Grace's (1962a,b) successes were also followed by the development of various insect cell lines from coleopteran, dipteran, lepidopteran, and orthopteran insects (Li and Bonning 2007, Lynn 2007, Vlak 2007). More notable insect cell lines are listed in Table 1 with the source of each and its applications in entomological research.

Establishment and Maintenance of Insect Cell Lines

Cell lines can be successfully established from tissues or cells of insect larvae or pupae. The Sf21, Sf9, and the BTI-TN-5B1-4 (or High Five) cells are the most commonly used insect cell lines in research (Table 1). These three cell lines are derived from lepidopterans. The Sf9 and Sf21 are from pupal ovarian tissue of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), and the High Five cells are from ovarian tissue of the cabbage looper, *Trichoplusia ni* (Hübner). Transformed stable insect cell lines are often from dipteran insects including the fruit flies (Tephritidae, Drosophilidae) and mosquitoes (Culicidae), among which the cell line *Drosophila melanogaster* Schneider 2 (S2), derived from *D. melanogaster* Meigen, is most commonly used in insect cell line research.

Insect cell lines are isolated from different tissues and organs (i.e., fat body, embryonic tissues, ovaries, imaginal discs, testis, dorsal vessel, etc.) at different stages of development. Overall, the greatest number of insect cell lines have been developed from the embryonic tissues, followed by ovarian tissue; however, in India, the majority of lepidopteran cell lines have been developed from the ovarian tissue, followed by embryonic tissue (Pant et al. 2002).

Culture media for maintaining cell culture lines are complex combinations of amino acids, salts, vitamins, growth factors, carbohydrates, metabolic precursors, trace elements, and hormones. The requirements for these constituents differ among various cell lines. Carbohydrates are generally provided as glucose, but in few instances, glucose is substituted with galactose in order to reduce lactic acid accumulation in the cultures. Other carbon sources include some amino acids (especially L-glutamine) and pyruvate. pH is maintained by one or more buffering systems with CO₂/sodium bicarbonate, phosphate, and HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) as the most commonly used. Serum also is found in a complete medium. Commonly used culture media are the Eagle Minimal Medium, Dulbecco's Modified Eagle's Medium, and Roswell Park Memorial Institute Medium (Arora 2013).

Insect cell lines are usually maintained at 26–28°C. Maintenance below the optimal temperature range will yield decreased cell growth rate. Above 30°C, cells

Table 1. Available list of significant insect cell lines and their applications with source details.

No.	Name of Insect Cell Line	Source	Applications	References
1	FTRS-AoL	<i>Adoxophyes orana</i> neonate larvae	Research on viruses; susceptible to insect viruses	Mitsuhashi 1989
2	AC20	<i>Agallia constricta</i>	Virus infectivity tests	Chiu and Black 1967
3	AS-H 1	<i>Agrotis segetum</i>	Research on granulosis virus	Kozlov et al. 1990
4	LPC-Aa98-19	<i>Anacridium aegyptium</i>	Suitable for virus multiplication and manipulation and also used in biopesticides	Hernandez-Crespo et al. 2000
5	BCIRL/AMCY-AfO(TV)-CLG	<i>Anagrapha falcifera</i> adult ovaries (testes) fat body	Production of viral pesticides, mainly baculoviruses	Goodman et al. 2001a, b
6	BCIRL/AMCY-AgE-CLG	<i>Anticarsia gemmatilis</i> Embryos	Production of recombinant proteins and viral pesticides	Goodman et al. 2001b
7	APE1	<i>Antitrogus parvulus</i>	Development of engineered entomopoxviruses as microbial control agents	Femon et al. 1996
8	WIV-BS-02	<i>Biston suppressaria</i>	Infection studies of homoceros tubulosa nuclear polyhedrosis virus	Mitsuhashi 1989
9	RIRI-BR1	<i>Blaps rhynchoptera</i>	Used as folk medicine in Yunnan Province, China	Zhang et al. 2015
10	WIV-BS-481	<i>Buzura suppressaria</i> larval hemocytes	Research on baculovirus studies	Grasela et al. 2012

Table 1. Continued.

No.	Name of Insect Cell Line	Source	Applications	References
11	BCI RL-Cc-AM	<i>Cactoblastis cactorum</i>	Support baculovirus infection and used in alternative biocontrol method	Grasela et al. 2012
12	KLBIQ-Chsu-I	<i>Chilo suppressalis</i>	Production of insect virus expression vector	Liu et al. 2015
13	FPMI-CF-1	<i>Choristoneura fumiferana</i> midguts	Expression of recombinant proteins using baculovirus vectors	Hink et al. 1991
14	Schneider 2 (S2)	<i>Drosophila melanogaster</i>	Useful in transfection research	Benting et al. 2000, Suske 2000
15	IPLB-Ekx4T	<i>Ephesia kuehniella</i> embryos	Useful in biocontrol research and susceptible to nucleopolyhedro viruses	Lynn and Ferkovich 2004
16	BTI-EAA	<i>Estigmene acrea</i> larval hemocytes	Recombinant protein production insect cell lines	Hink et al. 1991
17	NIV-HA-197	<i>Helicoverpa armigera</i> embryonic tissue	Application in the mass production of this baculovirus as a bioinsecticide	Sudeep et al. 2002
18	CSIRO-BCI RL-HP1	<i>Helicoverpa punctigera</i> embryos	Useful in virological studies	McIntosh et al. 1999
19	NIAS-LeSe-11	<i>Leucania separata</i> larval fat body	Research on nucleopolyhedrovirus	Yanase et al. 1998
20	Lulo	<i>Lutzomyia longipalpis</i>	Can be used in vaccines and diagnostic tests	Rey et al. 2000

Table 1. Continued.

No.	Name of Insect Cell Line	Source	Applications	References
21	IPLB-LD-64	<i>Lymantria dispar</i> pupal ovaries	Used to quantitate infectivity	Goodwin et al. 1978
22	IPRI 108	<i>Malacosoma disstria</i> larval hemocytes	Nuclear polyhedrosis virus infectivity <i>in vitro</i> assay	Volkman and Goldsmith 1982
23	NN-1	<i>Nephotetix nigropictus</i>	Used in phytoreovirus research	Duan and Zhang 2014
24	AFKM-On-H	<i>Ostrinia nubilalis</i>	Useful in virus transfection research	Belloncik et al. 2007
25	RAE25	<i>Rhipicephalus appendiculatus</i>	Useful tool in defining the complex nature of the host vector and pathogen relationship	Bell-Sakyi et al. 2007
26	NIH-SaPe-4	<i>Sarcophaga peregrina</i>	Used in study of host parasite relationship in insect borne pathogenic microbes	Komano et al. 1987
27	Se6FHA	<i>Spodoptera exigua</i>	Useful in nuclear polyhedrosis virus research and in production of recombinant proteins	Hara et al. 1993
28	Sf9 cells	<i>Spodoptera frugiperda</i> pupal ovarian tissue	Recombinant protein production using baculovirus and in evaluation of host-virus interaction	Davis et al. 1993, Ma et al. 2014, Wilde et al. 2014
29	Sf21 cells	<i>Spodoptera frugiperda</i> pupal ovarian tissue	Research on baculoviruses and their use for producing recombinant proteins	Chen et al. 2005, Lynn 2003
30	IBL-SLO-1A	<i>Spodoptera litura</i>	Useful in studies like replication of <i>S. litura</i> nuclear polyhedrosis virus <i>in vitro</i>	Shih et al. 1997

Table 1. Continued.

No.	Name of Insect Cell Line	Source	Applications	References
31	TI-1 [Thysanoplusia]	<i>Thysanoplusia intermixta</i>	Studies of insect pathogenic viruses and baculovirus expression vector system	Hashiyama et al. 2011
32	High Five cells	<i>Trichoplusia ni</i> cabbage looper ovarian cells	Recombinant protein expression using baculovirus or transfection	Hink 1970, Zhang et al. 2008
33	XP-1	<i>Xylotrechus pyrrhoderus</i>	Production of insect pathogenic viruses and recombinant proteins	Iwabuchi 1999

may become increasingly less viable and may not recover viability even when the temperature is reduced to the optimal range (Drugmand et al. 2012).

Subculturing of the cell line cultures must be performed periodically to retain log phase growth and maximum viability. Anchor-dependent monolayer, or adherent, cell cultures should be subcultured at 100% confluence, typically at a 1:5 dilution (volume of cells to final volume of medium) in order to maintain log phase growth. Suspension cell cultures should be subcultured to a concentration of 0.7×10^6 to 1.0×10^6 cells/ml before reaching a density of 2.0×10^6 to 2.5×10^6 cells/ml. If surplus cells are available from subculturing, those should be preserved in a suitable protective agent (e.g., glycerol, dimethyl sulfoxide) and stored at temperatures below -130°C (cryopreservation) until they are needed for experimentation (ATCC 2012).

Newly established insect cell lines are characterized using the usual parameters of morphology, growth curve, species specificity, karyology, etc. Morphology and viability must be microscopically recorded at regular intervals. The growth medium also must be monitored for microbial contamination, which may be presented as unusual shifts in pH, increased turbidity, or appearance of suspended particles. In addition to daily inspections, it is important to test a sample of the culture regularly for the presence of bacteria, fungi, and mycoplasma. There are several methods that can be used to check for these contaminants (Sudeep et al. 2005).

Cells grown in suspension cultures develop either as single cells or as groups of cells. Viable cells appear round and refractive while dead cells are smaller and darker. Some cells may attach and grow on the inner surface of the culture vessel and appear round or flattened, resulting in some cell lines developing as mixed adherent and suspension cultures. The percentage of these adherent cells varies with conditions and cell density. Cell debris may also be detected in healthy cell culture populations (ATCC [®]Animal Cell Culture Guidetips and techniques for continuous cell lines, www.atcc.org). Both trypan blue and erythrosine B stains are used to conduct periodic viability assays to quantify the number of viable cells in a cell culture population. Both stains are excluded by the cell membranes of living cells but are taken up and retained by dead cells, which lack intact cell membranes (Krause et al. 1984).

Concluding Comments

Establishing insect cell lines for a specific research objective is often a slow and challenging undertaking. Primary cell cultures may not develop into cell lines in all cases. Many of the isolated cells may take several months to be successfully cultured, and the resulting cells may not be appropriate for the preferred application. Of the approximately 950,000 insect species that reportedly exist, we have only 1,000+ insect cell lines from only approximately 100 insect species.

Additional challenges to insect cell culture reside with development of growth media for specific needs. Media required for all the various sources of cells for culture are presently not available and have not been developed. Furthermore, large-scale production of insect viruses in insect cell culture lines requires media ideal for cell line growth as well as for the replication of the virus agents. Each cell line and virus culture combination may likely require its own unique medium composition.

Development of continuous cell lines from aphids (Homoptera: Aphididae), the most significant insect group of plant virus vectors, have been attempted with no success thus far, perhaps due to the lack of a suitable growth medium or suboptimal use of other factors involved in the establishment of the cell cultures (Peters 1971, Peters and Black 1970). Efforts have also been made to initiate thrips (Thysanoptera) cell lines (Kritzman et al. 2002, Nagata et al. 1997). Although limited success has been reported, secondary cultures have not been successfully maintained. Yet, such cultures are required for research regarding virus infection of host cells, determination of the existence of viral receptors on cell surfaces, virus replication and development within the cell, and virus escape from the cells. Establishing continuous cell culture lines from a natural insect vector of a plant virus is essential for examining insect–virus interactions and, thus, developing innovative management methods.

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