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Insect cell culture in research: Indian scenario

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Insect cell cultures are widely used in viral diagnosis and biotechnology, for the production of recombinant proteins, viral pesticides and vaccines as well as in basic research in genetics, molecular biology, biochemistry, endocrinology and virology. Following KRP Singh's pioneering research in 1967, a large number of cell lines from diptera, hemiptera, and lepidopteran insects were established and characterized in India. With the availability of the modern tools in molecular biology and the advancements made in biotechnology, the indigenous cell lines may prove useful in creating a future without biohazardous chemical pesticides as well as producing life saving pharmaceuticals and vaccines for many diseases. This review summarizes information gathered regarding the insect cell lines established so far in India. It also covers the familiarization of the well characterized continuous cell lines and their potential applications. Special attention is given to virus susceptibility of the cell lines, the yield of virus with a comparative analysis with other conventional systems. The potential applications of dipteran and lepidopteran cell lines in agriculture and biotechnology are also briefly discussed for prospective studies.

Key words Baculoviruses - dipteran and lepidopteran cell lines - insect cell culture - recombinant proteins

The successful demonstration of growth of fibrillae from embryonic frog neuroblasts in clotted lymph by Harrison[1] and subsequent workers has opened up a new scientific field of animal tissue culture. The importance achieved in a short span in vertebrate cell culture research has warranted an urgent need to have a parallel system from arthropods especially from insects. Arthropods have become the centre of attraction of scientists due to their importance in medicine and agriculture. They played an important role as intermediate hosts or vectors of pathogens causing diseases to man and pet animals. The growing interest in arbovirus infections of man and domestic animals has stimulated interest in developing cell cultures from arthropods of medical importance especially from mosquitoes and ticks in order to study the vector virus relationship at cellular level. Similarly, in the field of agriculture, the damage caused by caterpillars and the failure of insecticides in their control stimulated urgency in developing cell cultures from lepidopteran insects for large-scale production of insect viruses. The first breakthrough in the development of insect cell culture was the report of maturation of testicular follicle cells to spermatocytes in Cercopia moth in an artificial medium[2]. This result gave encouragement to many workers to initiate work on the development of insect cell culture[3-5]; however, most of these earlier attempts were disappointing[6].

The basic reason for the failure was the lack of a suitable cell culture medium for culturing insect cells. Efforts were made in the late 1940s and 1950s for a medium whose composition resembled that of insect hemolymph. The first such medium was formulated by Wyatt which proved useful for cultivating silk moth ovarian tissues[7]. Subsequently many formulations, which gave promising results, were reported[8-11]. However, the establishment of a viable self-sustainable insect cell line was not possible. Nonavailability of a nutritionally optimum growth medium to stimulate multiplication of cells *in vitro*, difficulty in obtaining adequate quantity of sterile tissues as well as the lack of adequate knowledge of tissue culture techniques *etc*., could have been the possible reasons for this. However, a few promising results appeared in the late 1950s[12-13] though these studies did not culminate in the establishment of continuous cell lines. Vago and Chastang[12] cultivated the larval ovary cells of *Bombyx mori* up to the 12th passage level while Gaw and colleagues[13] subcultured the epithelial cells of *B. mori* gonads up to the 22nd passage level. These studies indicated the probability of growing insect cells *in vitro* for the first time that have stimulated further research.

The first true cell line from an arthropod was established from the pupal tissues of the moth *Antherea eucalypti* by TDC Grace of Australia in 1962[14]. He modified Wyatt's medium[7] with the addition of 10 vitamins and formulated a new medium known as Grace's insect tissue culture medium, which supported the growth of the cell line. This medium became popular due to its usefulness in the establishment and maintenance of many insect cell lines. Today, this formulation is available commercially and several workers have modified this medium to suit their demands[15,16]. The study of insect cells made rapid progress in the following years and more than 500 cell lines from different insect species have been described till today[17]. The system has found wide application in the studies on morphogenesis, virology, pathology, biochemistry, genetics and other fields of biology and medicine.

In recent years, there is renewed interest in developing new lepidopteran cell lines due to their potential application in biotechnology. The advances made in the genetic engineering technology in the early eighties have helped in the production of new recombinant proteins and genes useful in medicine and agriculture[18,19]. Lepidopteran cell lines were primarily being established to propagate insect viruses as a biopesticide for the control of insect pests. Lately, the baculovirus expression vector system combined with insect cell cultures has become more attractive for the expression of many heterologous proteins than other systems *viz*., bacterial, yeast, vertebrate viruses, *etc*., due to its unique characteristics. This technology is also being used in the construction of recombinant baculoviruses to use as biopesticides that offered comparatively faster killing of insect pests than by wild type baculoviruses[20-23].

Mosquito cell lines

Mosquitoes transmit a number of diseases to human beings and domestic animals. The importance of mosquitoes as vectors of many protozoan, viral and filarial pathogens is well documented. Efforts to control the mosquitoes have not been very successful due to their ability to adapt to different environmental conditions as well as to develop resistance to synthetic insecticides[24]. Susceptibility of mosquitoes to these pathogens is a very complex phenomenon, which is governed by several genes and environmental conditions. The availability of established mosquito cell lines came handy for understanding the interaction of pathogens at the cellular level. The available information indicated that attempts to develop mosquito cell lines have started in the early 1930s[3].

Grace in 1966 reported the establishment of the first mosquito cell line in the world from *Aedes aegypti* mosquitoes[25]. Subsequent studies using isoenzyme analysis indicated this cell line as a contaminant of *Antherea eucalypti* cell line[26]. Singh[27] established two cell lines at the National Institute of Virology (NIV), Pune from the larval tissues of *Ae. aegypti* and *Ae. albopictus* in Mitsuhashi Maramorosch (MM) medium, which was originally designed for culturing leaf hopper cells by Mitsuhashi and Maramorosch[28]. Singh's cell lines are therefore considered as the first, true mosquito cell lines established in the world. He solved the problems associated with obtaining sterile tissues for initiation of cell cultures using a simple technique for surface sterilization of mosquito eggs.

In addition, he used commercially available foetal bovine serum (FBS) as a supplement in place of insect haemolymph, which was considered essential for establishment and maintenance of mosquito cell cultures. Subsequently, a number of mosquito cell lines from different species were established in India and abroad. Table I depicts the number of mosquito cell lines established at NIV, Pune, India.

Attempts were made in the early 1980s to develop cell lines from *Culex* mosquitoes due to their importance in the transmission of Japanese encephalitis (JE) virus in India. The virus has established itself in several parts of India and became a major cause of concern[35]. The first *Culex* cell line in the country was established from the embryonic tissue of *C. bitaeniorhynchus* mosquitoes[32]. This species is considered as one of the important vectors of JE in India[35,36] and Banerjee *et al*[37] have shown the transmission of JE virus by this mosquito in the laboratory. Cell lines from other two species of *Culicine* mosquitoes *viz*., *C. infula*, *C. ambiguous*, which are members of *C. bitaeniorhynchus* complex were also established subsequently[38]. Among these cell lines, the *C. bitaeniorhynchus* cell line was found highly susceptible to JE and West Nile (WN) viruses. The cell line also supported the multiplication of other arboviruses of public health importance in India *viz*., Chikungunya (CHIK), dengue (DEN) and Sindbis viruses[39]. This cell line was also used in the epitope analysis of different strains of JE virus using monoclonal antibodies[40].

A new cell line from the forest mosquito *Ae. krombeini* was established in the early 1990s, which proved highly sensitive to several arboviruses[33]. The new cell line supported the replication of 13 mosquito borne arboviruses[41,42]. It was extremely sensitive to JE and DEN viruses and very low titre of these viruses could be detected on the 1st post infection day (PID) using indirect immunofluorescent technique (IFA) while *C. bitaeniorhynchus* and C6/36 cell lines could not detect these viruses when infected with same dilution[43]. Unfortunately, due to the presence of certain cytoplasmic inclusions in the cells[44], the cell line was not used for further research especially for isolation of virus from field collected mosquitoes.

Recently, a new cell line from *C. tritaeniorhynchus* mosquitoes was established from the embryonic tissue[34]. This mosquito, an important member of the *C. vishnui* complex, is the main vector of JE virus in India[35,45]. The cell line supported the multiplication of JE and WN viruses but did not support the multiplication of any of the dengue serotypes[34].

Applications of mosquito cell lines in virus research

Most of these cell lines are interesting from an academic point of view and much of the early research work was concerned with characterizing the basic physiology of cells in culture. The development of continuous cell lines has proved useful in diverse areas of research especially in virology. The arbovirus-arthropod cell relationships have been the prime consideration due to the subsequent application of mosquito cells in various aspects of arboviral research. Singh's *Ae. albopictus* cell line, which is popularly known as ATC-15 cell line was the most widely studied cell line due to its susceptibility to nearly 50 viruses transmitted by mosquitoes, ticks *etc*[46]. Singh's other cell line derived from *Ae. aegypti* (ATC-10) mosquitoes was less susceptible to arboviruses compared to ATC-15. Singh also observed that with the exception of Colarado tick fever and Ganjam viruses, those arboviruses that are naturally transmitted by mosquitoes or infect mosquitoes experimentally, multiplied in one or both of his cell lines. Extending this argument, Buckley also reported that partially deoxycholate resistant viruses can multiply in one of these cell lines where as lipid solvent sensitive arboviruses isolated from ticks and sand flies cannot multiply in either cell lines[47].

(i) In primary isolation of arboviruses: The availability of continuous cell lines has been very useful in the study of arboviruses. The absence of cytopathic effect (CPE) in majority of mosquito cell lines infected with arboviruses has limited the use of this cell system for primary isolation work. However, several successful attempts have been made using those cell lines, which showed CPE for virus isolation from original field materials. Using ATC-15 cell line, Singh and Paul[48] successfully demonstrated the isolation of four serotypes of DEN viruses from human sera. In their later studies, Paul and Singh[49] compared the sensitivity of ATC-15 cell line, VERO cells and intracerebral inoculation of mice to some arboviruses and showed the superiority of the mosquito cell culture system. The results indicated that *Ae. albopictus* cell

line was equally or slightly more sensitive to infection with CHIK, WN and JE viruses comparing the other two systems. However, with DEN-2 virus, the mosquito cell culture system was 100 and 40 times more sensitive than VERO and infant mice system, respectively. Supporting results were also published by Chappel and coworkers in the isolation of dengue viruses over infant mice and LLC-MK2 cells[50].

IFA has become an important tool in the detection of virus antigen with its simplicity and specificity[51]. This technique is now routinely being used in the detection of virus antigen in infected cell lines and mosquitoes[42,52]. This new technique has helped to re-discover the potential of mosquito cell lines in the study of arboviruses, and several virus isolations were made in mosquito cell lines from field collected mosquitoes at NIV, Pune[53-56]. The *C. bitaeniorhynchus* cell line for JE virus and *Ae. albopictus* cell line or C6/36 cells[57] for DEN viruses are mainly being used in the isolation of these viruses from mosquitoes collected during epidemic periods.

(ii) In serology: The use of mosquito cell lines in the serological typing of arboviruses has been restricted due to the lack of CPE in these cell lines. However, several successful attempts have been made to use those cell lines, which showed CPE[58-63]. A few studies were also reported from India. Paul and coworkers blocked the production of CPE in JE infected cultures with JE virus antiserum in *Ae. albopictus* cell line[64]. In another study, Pavri and Ghosh[65] successfully used tissue culture fluid from DEN infected *Ae. albopictus* cells directly in complement fixation (CF) test. Subsequently, Singh and Paul followed this method for serotyping of dengue viruses[48]. Ghosh and associates[66] observed CF antigens of Chandipura (CHP) and JE viruses in infected *Ae. albopictus* and *Ae. aegypti* cells. In JE virus infected cells, the CF antigen was found in both intra- and extra-cellular fluid whereas in CHP infected cells CF antigen was retained intracellularly. Ghosh and Bhat[67] also reported haemagglutinin activity in normal culture fluid of *Ae. albopictus* cells.

(iii) In rickettsial studies: The replication of *Coxiella burnetti* was detected on the 7th PID in *C. bitaeniorhynchus* cell line using IFA[68]. The rupturing of cell walls and subsequent release of the organism into the tissue culture fluid (TCF) was also reported by the authors. This TCF was effectively used to infect embryonated chicken eggs. The IFA technique has proved quicker in detecting the presence of rickettsiae as in other conventional methods like staining with Gimanez staining, *etc*., the detection was possible only on the 11th PID.

(iv) In biochemical and molecular studies: In India, though many mosquito cell lines are available, these were used mainly in the isolation of arboviruses except for a few other studies[69]. Mosquitoes gained considerable importance in biochemical and genetic research due to their increasing resistance to insecticides. Following the pioneering work of Stollar and coworkers[70], mosquito cell lines are routinely being used to carry out genetic and biochemical aspects[71-73]. This system has been employed effectively in various fields of molecular biology and genetics in recent years especially to explore the suitability of established mosquito cell lines for investigating cellular and humoral immunity[73]. The cell cultures are also being used in the exploration of immune responses in mosquitoes, receptor studies, host parasite interaction, *etc*., in addition to their various other applications in molecular biology. Better results can be achieved by generating highly susceptible cell lines or cell clones, which show little or very low cellular responses against a particular pathogen.

Lepidopteran cell lines

In the last two decades, a lot of work has been done in India to develop cell lines from lepidopteran insects due to their potential application in the fields of agriculture and medicine. The cell lines are used as a tool to grow many entomopathogenic viruses, which have the potential as biopesticides. These viruses are highly virulent to susceptible insect hosts and are ecofriendly. Members of the family baculoviridae, in particular, have been projected as bio-pesticides of the future and are being used in many countries for pest control[74]. Baculoviruses have been isolated from the members of Phylum Arthropoda (insects and crustaceans) and no isolations have been reported from vertebrates so far[75]. Baculoviruses are extremely virulent to their susceptible hosts and are safe for

Table I. New cell lines established from mosquitoes in India

Mosquito species	Tissue of origin	Designation	Reference
Aedes aegypti	Neonate larvae	ATC-10	Singh, 1967[27]
Aedes albopictus	Neonate larvae	ATC-15	Singh, 1967[27]
Aedes vittatus	Neonate larvae	ATC-121	Bhat & Singh, 1970[29]
Aedes W-albus	Neonate larvae	ATC-136	Singh & Bhat, 1971[30]
Aedes novalbopictus	Neonate larvae	ATC-173	Bhat & Guru, 1973[31]
Culex bitaeniorhynchus	Embryonic tissue	ATC-415	Pant & Dhanda, 1982[32]
Aedes krombeini	Neonate larvae	NIV-AK-453	Pant *et al*, 1992a[33]
Culex tritaeniorhynchus	Embryonic tissue	NIV-CT-894	Athawale *et al*, 2002[34]

humans. In nature, baculoviruses play an important role in bringing down the pest population to economic thresholds as and when their population increases[20]. In the field of medicine, the cell lines play an important role in the abundant expression of many life saving molecules, which are otherwise difficult to synthesize. The application of baculovirus expression vector system (BEVS) for the expression of foreign proteins in insect cells has stimulated tremendous interest in the recent years. The following unique characteristics distinguish BEVS from other systems and a wide variety of genes and medically important proteins from viruses, fungi, bacteria, plants and animals have been expressed in insect cells using BEVS[76,77]. These biomolecules are used effectively in pharmaceuticals and in the development of vaccines and diagnostics.

(*i*) Abundant expression of soluble recombinant proteins, which are antigenically, immunologically and functionally similar to their native counter parts. (*ii*) Ability to accommodate large DNA inserts. (*iii*) Two or more proteins can be expressed simultaneously by using dual expression vectors or by coinfection with two or more different recombinant viruses. (*iv*) Baculovirus infected insect cells perform many of the post-translational modifications such as phosphorylation, glycosylation, correct signal peptide cleavage *etc*. (*v*) Very late transcription from the polyhedrin promoter allows expression of cytotoxic recombinant gene products. (*vi*) Baculoviruses and the cell system are easy to handle and safe.

In India, though NIV scientists were the pioneers in developing mosquito cell lines, not much attention was given to develop lepidopteran cell lines until recently. The establishment of the first lepidopteran cell line was reported by Pant *et al* in 1977[78]. They established a cell line from the embryonic tissue of potato tuber moth (*Gnorimoschema/Phthoramea operculella*) and characterized the cell line in detail. The cell line was initiated and maintained in MM medium supplemented with 10 per cent FBS which was later replaced with 0.8 per cent bovine serum albumin supplemented with yeastolate and lactalbumin hydrolysate. This is one of the very few insect cell lines maintained in growth medium without supplementing FBS. The cells could also be grown as suspension culture in conical flasks on a rotary shaker giving higher yield of cells. However, the authors did not study the susceptibility of the newly established cell line to any of the baculoviruses.

It took nearly two decades to establish another lepidopteran cell line in India. In the last decade, though many scientists at different laboratories actively worked on development of new lepidopteran cell lines, only NIV, Pune succeeded in the establishment of cell lines and reported eight new cell lines during 1997-2002 (Table II). In India, lepidopteran cell lines *i.e.*, Sf-9, Sf-21, BMN, *etc*., are being used for various studies and all these cell lines are procured from abroad. The new, indigenously developed lepidopteran cell lines were characterized in detail using the routine parameters including their susceptibility to several baculoviruses. Some of the cell lines have already been proved useful in propagating baculoviruses due to their high susceptibility and virus yield while some expressed

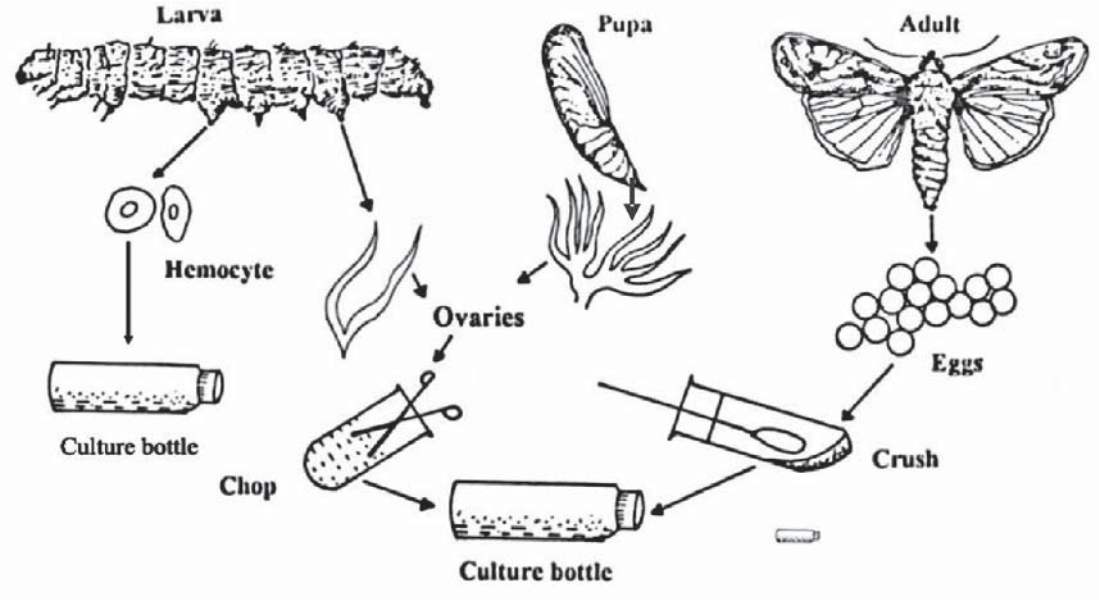

Fig. Diagrammatic representation of establishing primary cultures from different tissues of lepidopteran insects.

recombinant proteins with better efficiency than Sf-9 cells (NIV unpublished data). Further research in these directions may produce the full potential of these cell lines in expressing foreign proteins and genes.

(i) Establishment of lepidopteran cell lines: Cell lines were established from different organs/tissues *i.e.*, embryonic, ovarian, fat body, testis, imaginal discs, dorsal vessel, *etc*., at different stages of development from lepidopteran insects. This is possible due to the comparatively larger size of these insects. The maximum number of cell lines has been established from the embryonic tissues followed by ovarian tissue[17]. In India, majority of lepidopteran cell lines established were from the ovarian tissue followed by embryonic tissue (Table II). The initiation of cell cultures from different tissues of lepidopteran insects is shown diagrammatically in the Fig.

(ii) Characterization of cell lines: The newly established cell lines were characterized using routine parameters *i.e.*, morphology, karyology, growth curve, species specificity, *etc*., as done for all other established cell lines in the world. Morphological studies carried out for all the newly established lepidopteran cell lines indicated that epithelial-like cells dominated in most of the cell lines followed by fibroblast-like cells and a few giant or vacuolated cells. Karyological analysis indicated a typical lepidopteran pattern showing a large number of dot-like, darkly stained chromosomes in all the cell lines and their number ranged from 45 to over 300. For morphological and karyological studies, the method described by Schneider[85] was used. Growth of the cell lines differed individually and ranged from 4- to 10-fold weekly. Species specificity of all the cell lines was determined using isoenzyme profile analysis and heteroduplex analysis. Isoenzyme profile analysis is an important parameter used in the characterization of a new cell line to rule out the chances of cross contamination from the existing cell lines using the method described by Tabachnik and Knudson[86]. Four enzymes *i.e.*, lactate dehydrogenase, malate dehydrogenase, Glucose-6-phosphate dehydrogenase and isocitric dehydrogenase, were used in the present study. The enzyme profiles indicated the cell lines as distinct, new cell lines, and not a contaminant of any of the cell lines maintained in the laboratory. The heteroduplex analysis is a new molecular technique used to authenticate cell lines and used effectively to differentiate cell lines recently[87,88]. The 12S or 16S rRNA gene of the mitochondrial DNA was PCR amplified and run on a poly acrylamide gel along with the corresponding amplified fraction of DNA from parent insect. The appearance of a homoduplex

Table II. Lepidopteran cell lines established in India

Name of species	Designation	Tissue of origin	References
Gnorimoschema operculella	Not designated	Embryonic (eggs)	Pant *et al*, 1977[78]
Spodoptera litura	NIV-SU-992	Larval ovary	Pant *et al*, 1997[79]
Spodoptera litura	NIV-SU-893	Pupal ovary	Pant *et al*, 1998[80]
Spodoptera litura	NIV-SU-1095	Larval haemocyte	Pant *et al*, 2000[81]
Helicoverpa armigera	NIV-HA-1195	Larval haemocyte	Sudeep *et al*, 2002[82]
Helicoverpa armigera	NIV-HA-197	Embryonic (eggs)	Sudeep *et al*, 2002[83]
Bombyx mori	NIV-HA-1296	Larval ovary	Sudeep *et al*, 2002[84]
Bombyx mori	NIV-HA-197	Pupal ovary	Sudeep *et al*, 2002[84]
Gnorimoschema operculella	NIV-PTM-1095	Embryonic (eggs)	(in press)

Table III. Susceptibility of newly developed lepidopteran cell lines to different baculoviruses

Cell line	Baculoviruses				
	AcMNPV	HaSNPV	SpltMNPV	BmMNPV	PTM-GV
NIV-SU-992	+++	—	++	—	—
NIV-SU-893	++	—	++	—	—
NIV-SU-1095	++	—	+	—	—
NIV-HA-1195	+	++	+	—	—
NIV-HA-197	++	+++	++	—	—
NIV-BM-1296	++	—	—	+++	—
NIV-BM-197	++	—	—	++	—
NIV-PTM-1095	—	—	—	—	?

+Approximately 25per cent cells showing occlusion bodies (OBs) on 7th PID; ++approximately 50 per cent cells showing OBs; +++approx.75 per cent cells showing OBs; —Not susceptible (No OBs); MNPV, multiple nucleopolyhedrovirus; SNPV, single nucleopolyhedrovirus; GV, granulosis virus; Ac, *Autographa californica*, Splt, *Spodoptera litura*; Ha, *Helicoverpa armigera*; PTM, potato tuber moth; BM, *Bombyx mori*

indicated 100 per cent homology while heteroduplex indicated cross contamination. The analysis of all the cell lines gave homoduplex with their parent DNA indicating the cell lines as new, unique cell lines (Shouche *et al*, personal communication).

(iii) Susceptibility of the established cell lines to certain baculoviruses: Spodoptera litura cell lines- *S. litura is* an important agricultural pest in India causing serious damage to tobacco and vegetables. Three new continuous cell lines from larval ovaries (NIV-SU-992), pupal ovaries (NIV-SU-893) and larval haemocyte (NIV-SU-1095) were established and characterized in detail[79-81]. Susceptibility of these cell lines to certain baculoviruses was also studied and found susceptible to the homologous virus *Spodoptera litura* multiple nucleopolyhedrovirus (SpltMNPV) as well as *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). On infection with SpltMNPV, the three cell lines yielded 5.6, 5.3 and 3.0 x10^6 occlusion bodies (OBs)/ml, respectively on the 7th PID[89]. With AcMNPV, the cell lines yielded 5.5, 3.0 and 1.96 x 10^6 OBs/ml, respectively. However, none of these cell lines supported the growth of *Helicoverpa armigera* single nucleopolyhedrovirus (HaSNPV), *Bombyx mori* multinucleopolyhedrovirus (BmMNPV) and potato tuber moth granulosis (PTM-GV).

The susceptibility status of these cell lines to different baculoviruses is given in Table III.

Helicoverpa armigera cell lines: *H. armigera* is a polyphagous insect causing damage to over a hundred species of plants especially cash crops *i.e.*, cotton, vegetables and ornamental plants causing damage worth billions of dollars globally[90]. In India, this is a major pest of cotton, vegetables and oilseeds. Being a borer, the larvae remain inside the stem or fruits for most of the time; hence, the application of chemical insecticides (contact poison) does not make much impact in the control of the larvae. Therefore, efforts are being made to control this insect using baculoviruses *i.e.*, HaSNPV.

Presently, the production of HaSNPV is made in the larval system, which is labour intensive and expensive. Therefore, efforts were made to establish cell lines from this insect for production of HaSNPV in large scale. Two cell lines from the *H. armigera* were developed *i.e.*, from the larval haemocyte and embryonic tissue and designated as NIV-HA-1195 and NIV-HA-197, respectively. Both the cell lines were tested for their susceptibility to different baculoviruses (Table III). The NIV-HA-197 cell line was highly susceptible to HaSNPV, yielding a very high titre (2.88×10^7 NPV/ml) on the 10th PID[83]. The cell line was also susceptible to AcMNPV and SpltMNPV yielding high titres[91]. The HaSNPV grown in the cell culture was bioassayed in different instar larvae of *H. armigera*. The results indicated that the OBs produced *in vitro* were highly virulent to 2nd and 3rd instar *H. armigera* larvae causing cessation of feeding on the 2nd day and mortality in 6 days. This cell line is also growing in goat serum (GS) supplemented medium producing a comparable yield of OBs. These OBs were found more virulent than the former and 100 per cent mortality was observed within 5 days of infection to 2nd instar *H. armigera* larvae (Sudeep, unpublished data). Goat serum, being cheap and locally available will help in the large scale production of HaSNPV for use as a biopesticide in future.

Bombyx mori cell lines: *B. mori* cell lines are effectively being used in the expression of foreign proteins using BEVS[77,84]. In India, research work has been carried out with *B. mori* cell lines, (BMN, BM5, *etc*.) brought from abroad, for expression studies[92,93]. Efforts were therefore, made to establish cell lines from this important insect and two cell lines were established from the larval and pupal ovaries. The newly developed larval and pupal ovary cell lines were designated as NIV-BM-1296 and NIV-BM-197, respectively and characterized in detail[84]. Both the cell lines were susceptible to BmMNPV and AcMNPV. However, the cell lines did not support the multiplication of HaSNPV and SpltMNPV (Table III).

Potato tuber moth (Gnorimoschema/Phthorimaea operculella Zeller) cell line: PTM is an important pest of potatoes in India causing extensive damage to the crop. This pest attacks the crop in the field as well as in storage, and efforts to control this insect using conventional methods has not been satisfactory[94]. Release of parasitoids and predators has also not helped in the control of this insect and the damage is increasing steeply. Efforts are being made to control with granulovirus (GV) in many laboratories and agricultural universities but with limited success. Large scale production of GV in the insect (*in vivo*) is not feasible due to the small size of the insect. The growth of PTM-GV in a newly established PTM cell line was reported by Lery and coworkers in Egypt[95]. Efforts were made in our laboratory and an embryonic cell line (NIV-PTM-1095) was established from the moth and characterized including the susceptibility to PTM-GV (unpublished data). On infection with PTM-GV, a few virus-like particles were observed in the tissue culture fluid in electron microscopic studies[89]. The cell line did not support the replication of any of the other baculoviruses tested (Table III). However, subsequent studies with HaSNPV showed a few occlusion bodies in a few cells. In AcMNPV infected cultures also, the morphology of the cells was found significantly changed in comparison to the control cells, however, occlusion bodies were not observed (Sudeep, unpublished data).

(iv) Application of the newly established lepidopteran cell lines for research- Nucleopolyhedrovirus production: Two new cell lines, *H. armigera* embryonic cell line (NIV-HA-197) and *S. litura* larval ovary cell line (NIV-SL-992), produced very high yield of OBs on infection with respective homologous viruses. OBs grown *in vitro* were found highly virulent to 2nd and 3rd instar larvae causing mortality within 6 days post infection (PI). Due to their importance in the field of agriculture, efforts are

being made to scale up the production of these baculoviruses. The HaSNPV yield in FBS supplemented medium was 28.8 x 10^6 OBs/ml on the 10th PID in the NIV-HA-197 cell line. Efforts were also made to clone this cell line to get high virus yielding cell populations and seven clones were developed. The clones were studied for their virus yielding properties and two clones were found better than the other clones in virus production. Both the cell clones *i.e.*, cl-1 and cl-7, yielded >32 x 10^6 OBs/ml on the 7th day PI in FBS supplemented medium. The clones were adapted to goat serum supplemented medium to reduce the production cost. One of the clones (cl-1) yielded 32.3 x 10^6 OBs per ml in GS supplemented medium on the 10th day PI (Sudeep, unpublished data). Bioassay of GS supplemented medium grown OBs caused 100 per cent mortality to 2nd instar larvae of *H. armigera* on the 5th PID. The GS adapted sub-line may be useful in the large scale production of HaSNPV for use as a biopesticide. GS is locally available and can be processed for cell culture use at a very negligible cost which will be a plus point for commercial competitiveness.

In expression of foreign proteins (recombinant proteins): In India, imported cell lines *i.e.*, *Spodoptera frugiperda* cell line (Sf-9 and Sf-21), *Trichoplusia ni* and *B. mori* cell lines have been used to study the expression of different foreign proteins[92,93,97]. We have made an effort to express recombinant (AcMNPV) proteins in the newly developed cell lines. Preliminary experiments with hepatitis C virus (HCV) core protein indicated significant level of expression in *H. armigera* and *S. litura* cell lines. Further studies with two other recombinant proteins *viz.*, P24 and gp120 of HIV-1 indicated high level of expression of these proteins in NIV-HA-197 and NIV-SU-1095 cell lines. The expression of P24 was also not affected significantly by the absence of FBS in the cell culture. NIV-HA-197 and NIV-SU-1095 cell lines showed higher level of expression of gp120 protein of HIV-1 than that observed in Sf-9 cells. The effect of tunicamycin, which inhibits glycosylation of glycoproteins, was also studied in these cell lines. Tunicamycin affected the gp120 synthesis in all the five cell lines tested. However, the effect was significant in *H. armigera* (embryonic) cells and *S. litura* (haemocyte) cells.

(v) Miscellaneous studies: In addition to diptera and lepidoptera, attempts were made to develop cell lines from other Orders of Class Insecta. Bhat and Singh[98] attempted to develop a cell line from the triatomine bug, *Triatoma rubrofasciatus*. Though three different cell types along with multicellular vesicles were observed in the culture, they degenerated after 30 days. Pant and coworkers[99] successfully established three cell lines from the bed-bug, *Cimex hemipterus* (Hemiptera: Cimicidae) and characterized one cell line (NIVI-CH-440) in detail. The susceptibility of the cell line was studied to important arboviruses *viz*. JE, CHIK, DEN-2, *etc.*, and the rickettsiae, *C. burnettii*. It was interesting to note that neither the viruses nor the rickettsiae multiplied in this cell line.

Prospective study

A number of well characterized, dipteran and lepidopteran cell lines are available with NIV but the potentials of these cell lines are not yet fully exploited. Mosquito cell lines have been used mainly in the isolation of arboviruses. These cell lines with the help of modern tools available in molecular biology may find application in solving many of the complex problems associated with mosquito borne diseases.

Lepidopteran cell cultures are increasingly being used in diverse areas of research *i.e.*, in biotechnology, agriculture and medicine. Many lepidopteran cell lines are being used to propagate insect viruses in large scale and are being used as biopesticides. The successful application of HaSNPV has been reported from China, Australia and other parts of the world[74,90]. Similarly, *Anticarcia gemmatalis* nucleopolyhedrovirus is being used for the control of velvet bean caterpillar of Soya bean in Brazil for the last two decades[74]. The US environment protection agency has approved the use of six baculoviruses for application against pests of agriculture and forestry[100]. In this context, it is high time to think in this direction and recognize the potential baculoviruses for crop protection in India. As mentioned earlier, *H. armigera* and *S. litura* are important pests of agriculture in India and may be controlled by the respective baculoviruses. The production of these viruses are presently made in the *in vivo* system and the new cell lines can replace the production of virulent OBs in large scale at a competitive cost. Already goat serum adapted cell lines

are available for baculovirus propagation, which produce comparative yield with better virulence (Sudeep, unpublished data). Integrating the existing facilities and proper application of potentials may make this true and India can have a future without hazardous chemical pesticides.

Preliminary results obtained in the expression studies indicate that *H. armigera* (NIV-HA-197) and *S. litura* (NIV-SU-1095) cell lines yield better expression of certain proteins than Sf-9 cells, which are used universally for expression studies[101]. The new cell lines therefore, have the potential for expressing heterologous proteins using AcMNPV as a vector. It is important to note that in the cell lines the yield of AcMNPV was much lower compared to the yield of homologous viruses. However, both the cell lines yielded better expression of foreign proteins in comparison to Sf-9 cells. It will be worth to construct recombinants in HaSNPV or SpltMNPV and try to express foreign proteins in these cell lines. Due to their high susceptibility to homologous baculoviruses, these may produce significantly high amount of recombinant proteins. The higher level of expression also contributes in reducing the production cost. Another cell line, which can be used for expression studies, is *B. mori* (larval ovary) cell line, which produced very high yield of BmMNPV. *B. mori* cell lines are extensively used in expression studies with comparable efficiency, and a number of foreign proteins were expressed using BmMNPV as vector[77, 84]. The new cell lines developed in India have not been studied for expression of foreign proteins. However, due to their high susceptibility to BmMNPV, these cell lines may also express heterologous proteins with better efficiency.

In India, though we have developed a few lepidopteran cell lines there is an urgent need to develop more new indigenous cell lines, as these may find better application than the existing cell lines in their virus yielding properties. The unique features of the BEVS and the economic benefits in the production of proteins as reagents, diagnostics or therapeutics are important in the fields of medicine and agriculture suggest that this system has great potential for the future. The new cell lines along with the existing ones will definitely help in future research to develop biological pesticides as well as the large scale production of important life saving pharmaceutical products.

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