Cytotechnology **20**: 3--11, 1996.
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Development And Characterization Of Insect Cell Lines

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Keywords: cell line establishment; Cryopreservation; insect cells, development of; insect cells, characterization of; isoenzymes; lepidopteran cell cultures.

Abbreviations: ICD - Isocitrate dehydrogenase; ME - malic enzyme; PG - phosphoglucose isomerase; PGM - phosphoglucose mutase.

Introduction

Continuous insect cell lines were first established in culture over three decades ago when Grace (1962) succeeded in growing cells from the *Antherea eucalypti* female moth ovaries. This breakthrough was the result of patience, the availability of antibiotics, and an improved medium. Since Grace's first report on four cell lines, over 400 lines have been established from more that 100 insect species representing every economically important insect order (see Hink, 1972; 1976; 1980; Hink and Bezanson, 1985; and Hink and Hall, 1989 for information on most of these cell lines.) These cell lines have been used in diverse fields of research as described in the other chapters of this book.

In this chapter, I will provide a brief overview of how new cell lines can be established and, once obtained, how they should be handled and characterized. The use of insect cells in baculovirus expression vectors (described elsewhere in this book) has proven to be a blessing to the whole field of insect cell culture by creating a reliable market for insect cell culture media. This means that, where twenty years ago only a couple of companies were selling insect culture media, today every major media company and many smaller companies supply these important components of successful cell culturing.

Since the baculovirus expression vector system has driven the field in recent years, I will be focusing on lepidopteran cells in this chapter. The reader should realize, however, that the techniques that I will describe here are generally relevant to the culture of cells from any insect order.

Development of Cell Lines

Two factors make primary tissue culture of insects particularly arduous. The first is their generally small size. Grace (1962) overcame this problem by selecting a relatively large moth, but we all cannot be as lucky since our interest may lie with small insect species. The other problem is that insects often live in a dirty environment. Having an insect colony may alleviate both of these problems to some extent. With a colony, a larger number of insects can make up for the relatively small size of the individual. Also, a colony can be cared for in a way to minimize microbial contaminants. I also overcome these problems by setting up primary cultures in small volumes and through the use of antibiotics. While it is generally **not** a good idea to use antibiotics in continuous cell lines (for reasons I'll describe later), they are beneficial in initiating new cell lines. In any case, cell lines have been successfully established from *Trichogramma* wasps (Lynn and Hung, 1991), a genus in which the adult's body is much smaller than the period at the end of this sentence, and from house flies (Eide, 1975) which breed in all kinds of filth.

Selection of medium

The single most important point to consider in attempting to develop a new cell line is the medium. While perhaps the easiest way to do this is with a shotgun approach in which every commercially available medium is tried, a certain amount of thought can go into selecting the order in which these are tried. Many commercial media are sold specifically for Lepidoptera. These range from the "old standby" of Grace's medium (sold by most major media manufacturers) to highly defined, serum-free media such as ExCell 401 (JRH Biosciences, Lenexa, KS[1]), SF-900 (GIBCO, Grand Island, NY) and Insect-Xpress (Whittaker, Walkersville, MD). I personally prefer a modified formulation of BML/TC-10 (Gardiner and Stockdale, 1975) sold commercially as TC-100 (GIBCO, JRH, Sigma Chemical Co., St. Louis, MO and others) to which I add additional peptides (such as 1.25% phytone peptone (BBL Microbiological Associates, Gaithersburg, MD) and 0.075% liver digest (Oxoid USA, Columbia, MD) or 1.25% peptone #P0521 and 0.075% peptone P7750 (Sigma)) and 5-10% fetal bovine serum (Sigma and many other commercial media companies). The other commonly available media are for dipteran cell lines, such as Schneider's *Drosophila* medium (GIBCO, Sigma, and others) and Shields and Sang's M3 medium for mosquito cell cultures (Sigma).

The main points you should consider in selecting a medium for insects other than these two orders are the pH, osmolarity, and the amount and ratio of the inorganic salts. Although it's somewhat outdated, a useful reference for this purpose is Altman (1961). This paper gives information such as concentrations of inorganic salts, freezing point depression (i.e. osmolality), amino acid concentrations and pH of hemolymph from many insects. Based on the information in Altman's paper, you can compare the values of these factors with published formulations of media to select the most appropriate medium for your insect (or a related species) and make modifications as necessary.

Initiation of primary cultures

I have found the most useful source of cells for developing new cell lines to be embryos, especially if you have a colony of insects available. These can usually be obtained in large quantities and the insect chorion is sufficiently impervious to simple disinfectants (such as 70% ethanol) so these can be used to decontaminate the eggs. The general procedure I use for isolating cells is shown in Figure 1. I normally submerge insect eggs for 5 to 10 min followed by two rinses in sterile distilled H_2O. You can, at this point, simply disrupt the eggs in culture medium in a tissue homogenizer (Bellco Glass, Vineland, NJ), transfer the cell suspension to a tissue culture petri dish or flask (Corning, Costar, Falcon, and Nunc are common brands of tissue cultureware) and wait for cell attachment. Various other methods have been used to obtain embryonic cells, including dechorionating the eggs with chlorox prior to disrupting or using enzymatic treatments (trypsin, collagenase, hyaluronidase, and elastase have all been used) rather than mechanical disruption.

I obtain the best results by using micro dissecting forceps (Roboz Surgical Instruments Co., Inc., Washington, DC) to mechanically break open the chorion in culture medium after disinfection. The embryos are then teased away from the yolk material and transferred to a standing drop (0.1-0.2 ml) of medium (supplemented with 50µg gentamicin sulfate/ml) in a 35 mm tissue culture petri dish (Falcon #3001). A microscalpel (Roboz) is used to cut each embryo into 4-8 pieces. During the cutting, many of the tissue fragments become attached to the scratches formed by the scalpel in the plastic, from which they will migrate during subsequent days. I generally use 10-20 embryos for each culture. After cutting up the embryos, the dish is sealed by stretching a 5 X 75 mm piece of Parafilm® around the edge. The dish is then placed in a tightly sealed plastic container with a small beaker of distilled water, and the entire plastic container is incubated at 27°C[2]. After 1-2 days, an additional 1.0 ml culture medium is added to the dish. It is resealed with Parafilm and replaced in the plastic container in the incubator.

Patience becomes the greatest virtue at this stage. After an initial period in which the cells migrate from the tissue fragments, little growth may be seen for weeks. During this period, additional culture medium should be added to the dish (about 0.5 ml per week). When the petri dish contains about 3 ml medium, all except 0.5 ml should be replaced with 0.5 ml fresh medium. Prior to making this exchange, the culture

[1] Mention of proprietary or brand names is necessary to report factually on the available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

[2] 27°C is near optimum for many insects. Your own specific insect may warrant a higher or lower temperature.

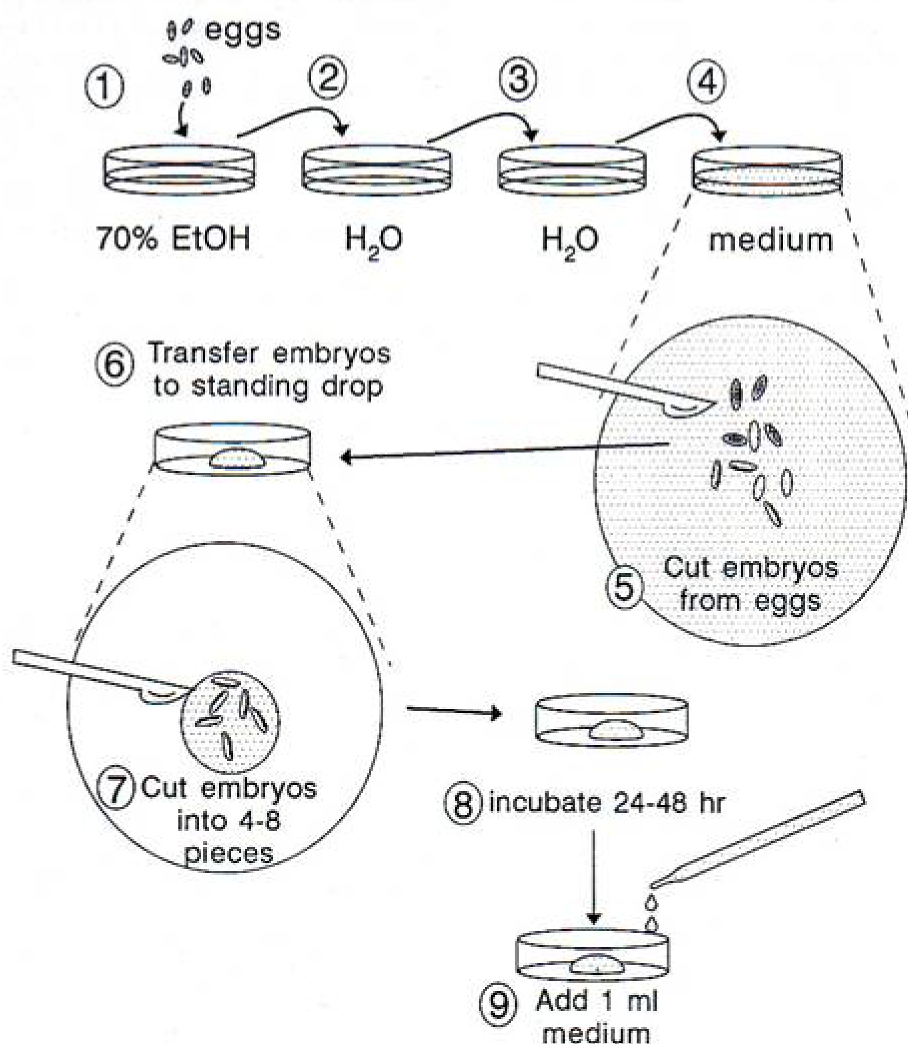

Figure 1. Steps for preparing primary insect cell cultures. Details are described in the text, but the steps include: 1. Disinfect eggs (or whole adult or immature insect if other tissue is desired) in 70% ethanol for 5-10 min. 2. Rinse in sterile distilled water, 5 min. 3. Transfer to fresh sterile distilled water. Hold excess material at this point while proceeding to step 4 with some of the material. 4. Transfer to tissue culture medium containing gentamicin (50 µg/ml). 5. Place a microscalpel and fine-pointed forceps in 70% ethanol. Ignite alcohol in a flame (Use a small bunsen burner or alcohol lamp. Do not hold instrument in flame, simply ignite and hold at an angle to allow alcohol to burn off. Take care not to hold hand over burning alcohol or to allow alcohol to flow onto your hand.) Use the cooled forceps and scalpel to remove embryos from eggs. 6. Transfer embryos to 35 mm tissue culture petri dish containing a standing drop (0.1-0.2 ml) medium with gentamicin. 7. Cut embryos into 4-8 pieces with microscalpel. 8. Seal petri dish lid to bottom by stretching parafilm® around the edge of dish and incubate at 27°C in a humidified chamber made from a tightly sealed container (such as Tupperwear®) holding a small beaker of water. 9. After 24-48 hr, remove parafilm and add 1.0 ml additional medium containing gentamicin. Reseal with a fresh piece of parafilm and return to humidified container in incubator. (Reprinted with permission from Hackett and Lynn, 1995).

should be examined with an inverted phase contrast microscope. If there are many non-attached cells, the old medium should be transferred to a sterile centrifuge tube. The unattached cells can be recovered by low speed (50Xg, 5-10 min) centrifugation, and then resuspended in the fresh medium before adding it to the original culture. Alternatively, if the original culture contains a substantial number of attached cells, the medium and non-attached cells removed from the primary culture can be transferred to a new dish. I

have often found that these secondary cultures will initiate consistent growth earlier than the primary culture.

This process of adding and replacing medium should be continued as long as living cells are observed in the culture(s). As mentioned above, it may take weeks before the culture contains a substantial number of cells. When the culture reaches about 80% confluence, a subculture may be attempted. The method of subcultivation depends largely on how tightly attached the cells are to the culture dish. I usually attempt a gentle flushing procedure for performing the first subculture. For this, the medium is drawn into a transfer pipet and sprayed across the cell surface to dislodge the cells. The cell suspension is transferred to a new dish (or if there are many cells, to a small (12.5 or 25 cm_2) tissue culture flask with fresh medium. Fresh medium is also returned to the original dish since all the cells are seldom removed by this method.

If few cells are removed by flushing, a more vigorous subculture method can be used. My next attempt normally is to cool the culture at 4°C for 20 min before using the flushing technique described above. Cooling causes depolymerization of microtubules which are important in attachment of some cells. If cooling does not work, an enzymatic treatment can be used. I first attempt to use collagenase (Worthington Biochemicals, 0.05-0.1 mg/ml Calcium/Magnesium-free phosphate buffered saline osmotically adjusted to the same concentration as the medium, for lepidopteran cells this is 320-370 mOsm/kg). If collagenase does njot remove the cells, I try VMF trypsin (Worthington, 0.05-0.1 mg/ml saline as described for collagenase). Finally, if all these methods fail to dislodge a substantial proportion of the cells, you can use a cell scraper to remove the cells (a sterile rubber policeman or a specially designed cell scraper available from tissue culture equipment manufacturers). After each of these treatments (flushing, cooling, enzyme) you should wait at least a day before attempting the next harsher treatment since, even if you do not dislodge many cells, you probably cause some cell damage and need to give the culture a chance to recover. You also may find that using these different subculture protocols will result in strains of the original culture with distinct characteristics. (Figure 2).

The secondary cultures are generally treated like the primary culture with fresh medium being added or replaced and subculturing attempted when warranted by cell densities. Eventually with sufficient diligence, you will be able to put the culture(s) on a regular subculture routine. I often find that a cell line will continue to improve in growth rates during the first year or two of regular subculturing. During this period, you are selecting for cells which grow faster, survive the subculture procedure better or, most likely, a combination of these factors . If you have a particular goal in mind of what you want these cells to do for you (virus replication, specific biochemical products, responsivity to hormones, etc.), you should test for the desired properties as soon as you can spare some cells. If you find a culture with the desired characteristics, you should: 1) freeze some cells in liquid nitrogen (see procedure later in this chapter) and 2) attempt to isolate a uniform culture by cloning (Lynn, 1989) or other selection technique. (For example, if you notice cells subcultured by one technique has a greater proportion of desirable cells, use this subculture method to maintain a selection pressure on the cells.)

Maintenance of Cell Lines

A number of important rules should be followed in maintaining a cell culture laboratory. First, you should **always** use a different bottle of culture medium for each cell line you maintain in the lab. A scandal of sorts exists in cell culture history in which many cell lines (many reported to be normal human diploid) used for experiments were later found to be HeLa cells (cervical cancer cells; Nelson-Rees, et al., 1981). The accepted explanation for these mixups was that HeLa cells were maintained in the laboratory where the research was being done and, during subculturing, the bottle of medium shared between the various cultures in the lab was inadvertently contaminated with the HeLa cell line. Since HeLa cells are very vigorous, fast-growing cells, they often outgrew the other cells being kept in the laboratory until they were the only cells present. A similar event occurred in the early history of insect cell culture when Grace (1966) developed an *Aedes aegypti* cell line which subsequently was determined to be *A. eucalypti* cells.

Also, you should only handle **one** cell line at a time. I maintain from 10-20 different cell lines in my lab at any one time. It's obviously useful to handle these cultures at the same time for use in initiating experiments, but, as Einstein reportedly said, time is relative. When handling your primary stock of cells (as opposed to cells being used in "deadend" experiments), you should only have that cell line and it's own bottle of maintenance medium (use a different

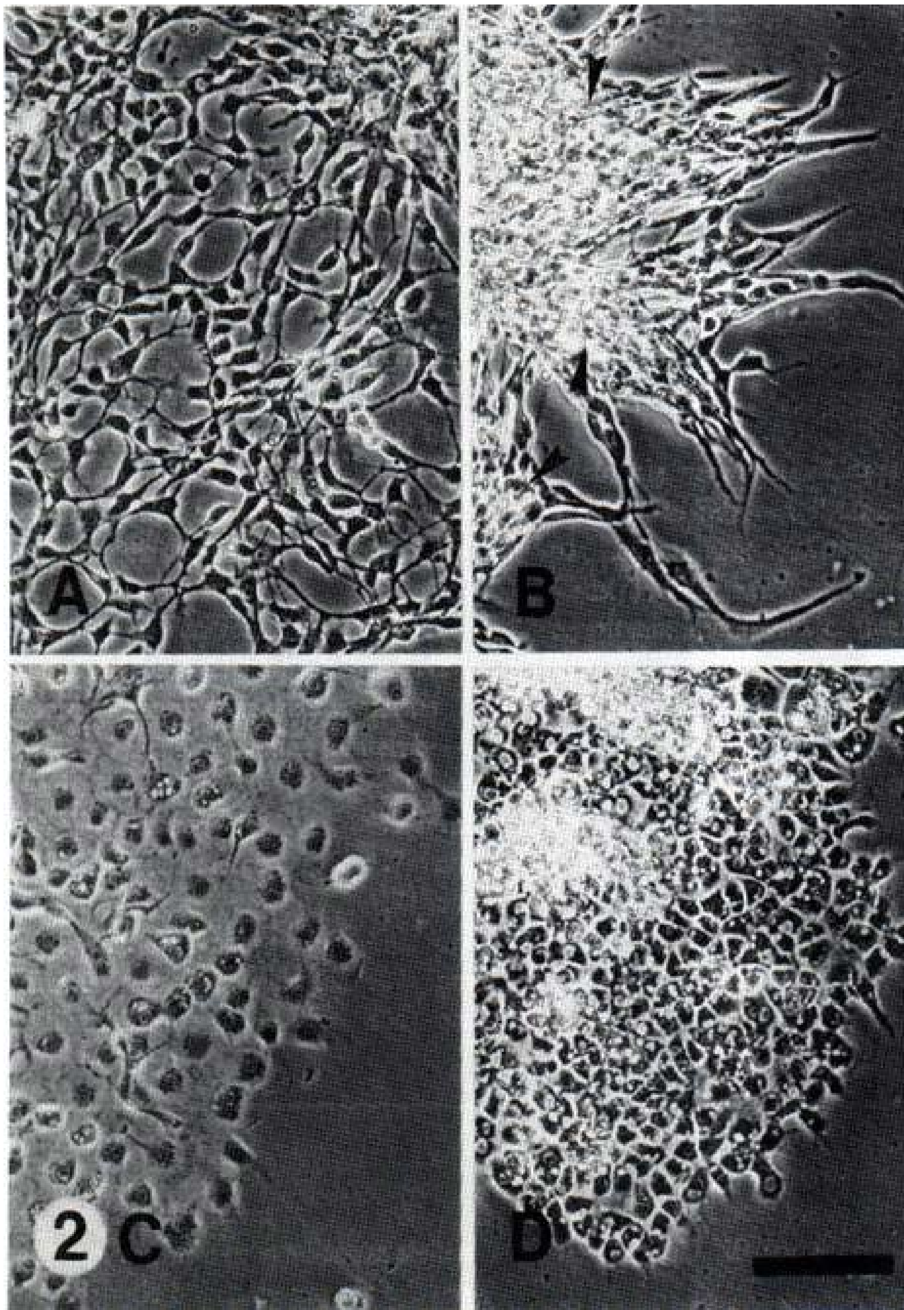

Figure 2. Variability in cell types forming aggregates in an early passage of diamondback moth embryo cells. The presence of such colonies suggest various cell strains can be isolated from a single primary culture of insect embryos. A: Neuroblast-like cells. B: Myoblast cells (cell aggregates indicated by arrow were actively pulsating). C and D: Epithelial-like cell colonies with different cell sizes and morphologies. Scale bar = 100µm. (Reprinted from Lynn, 1989.)

bottle of medium for experiments) in the transfer hood at the time. This means you should only have one parent culture, a new flask(s) (already labeled with the cell's identity), one bottle of medium (and a container with the enzyme if you're using one), and a pipettor and pipet in the hood at one time. Any additional objects could affect the air movement in a laminar flow hood and are unnecessary.

In the process of subculturing, as I mentioned above, you should prelabel the new culture flask prior

to putting cells into it. The best method is to keep a log of the subculture procedure in a notebook. When you write in it what you plan to do with the parent culture, for example, in setting up two new cultures of TND1 cells, you would write:

20 Sept 94
Split culture A of passage 29 of TND1 1:10 with TNM-FH (7 Sept. 94)
new cultures = TND1-30A and -30B

you would also write on the two new culture flasks:

20 Sept. 94 **TND1-30A**   **20 Sept. 94** **TND1-30B**

before you put them under the hood. This procedure should avoid improper labeling of a culture after it has cells in it. You can (and should) compare how a new flask is labeled with the parent flask as you add the cells.

The next rule concerns pipets. It is best to use single-use, disposable pipets, but whichever type of pipet you use, you should *never* use a pipet to go into a bottle of medium twice. This rule will avoid the possibility of accidentally contaminating the medium with the cells. If you use reusable pipets, they should be washed with detergent, thoroughly rinsed with demineralized water and sterilized by autoclaving (at least 121°C, 15 lb pressure for 15 min) or dry heat (180°C for 2 hr). Of course, since we've already determined we are never going to use one bottle of medium for two cell lines, this rule of only using a pipet once might seem extraneous, but it's a very good backup rule to follow. And, of course, we **never** mouth pipet. Use a rubber bulb or one of the mechanical pipettors. The major source of microbial contamination in cell cultures is not the medium or the serum, it's the laboratory worker!

The above covers some of the common mistakes made by new cell culturists. For more extensive information on general procedures for cell culture, see Freshney (1987) or Griffiths et al. (1992). These books were written primarily about vertebrate cell culture, but most of the procedures are similar to those used in insect cell culture. Also, for specific techniques on insect cells and tissues which may not be covered in the rest of this volume, see Hink (1989).

Characterization of Cell Lines

Historically, cell lines have been characterized by morphology and karyology as being a specific cell type or from a particular species. However, cell morphology alone has never been sufficient for characterizing cells. This is because changes in general morphology can occur under different conditions and with time in culture. Karyology is more reliable except for certain cells. Unfortunately, lepidopteran insects are one of the exceptions. Most cell lines from Lepidoptera are highly polyploid and made up of small chromosomes which are impossible to properly karyotype. Better chromosome spreads can be obtained by not using colchicine or colcemid (Disney and McCarthy, 1982), but I recommend using a molecular technique for identifying cells. While DNA fingerprinting may ultimately be a useful technique for this purpose, little effort has been made thus far to determine minimum numbers of probes required for this procedure to be reliable. The isoenzyme technique has been analyzed for use with insect cells (Greene et al. 1972, Tabachnick and Knudson, 1980; Brown and Knudson, 1980; 1982).

The use of isoenzymes relies on the fact that, while organisms have many shared enzyme systems, the particular enzyme protein from a specific organism may differ from other, even closely related, organisms. Thus the protein which acts as the catalyst for converting glucose 6-phosphate to glucose 1-phosphate (phosphoglucomutase, PGM) may be made up of different amino acids in insect A as compared to insect B. These differences can be discerned through electrophoretic techniques.

The electrophoretic method used is not particularly important. Greene and coworkers (1972) used polyacrylamide gels while Knudson's group initially used starch gels (Tabachnick and Knudson, 1980), but later reported that cellulose acetate was a more reliable method (Brown and Knudson, 1980; 1982). Since those reports, a system has been developed commercially (the Authentikit™, Innovative Chemistry, Inc., Marshfield, MA) which uses preformed agarose gels, thus eliminating a major problem with this technique of obtaining consistent results between different gels. Although the reaction buffers needed for staining for the enzymes can be prepared from scratch (see Brown and Knudson, 1980), Innovative Chemistry, Inc. also supplies the reaction buffers as lyophilized powders. While the Authentikit™ is sold with reaction buffers for eight particular enzymes, Tabachnick and Knudson (1980) determined four enzyme

systems were sufficient for discriminating 16 different lines to the species. These enzymes, PGM, phosphoglucose isomerase (PGI), malic enzyme (ME) and isocitrate dehydrogenase (ICD) were subsequently used by Brown and Knudson (1980; 1982) to discern, to the species level, 14 lepidopteran, 20 dipteran and a tick cell line. I have adopted these same four enzymes to characterize cell lines used in my laboratory, an example of which is shown in Figure 3.

Since I use the procedures as outlined in the manufacturer's instructions, I will only provide a brief summary here (all solutions mentioned are obtained from Innovative Chemistry, Inc.). A nearly confluent 25 cm^2 culture flask of cells is suspended by the normal subculture method, transferred to a centrifuge tube and placed on ice. The cells are centrifuged (100Xg, 5 min[3]), washed once in cold PBS and then recentrifuged. The resulting cell pellet is suspended in extraction buffer, the cells lysed, and centrifuged (800Xg, 10 min). The resulting supernatant is mixed with an equal volume of stabilization buffer and stored at -20°C until electrophoresis. One µl of this mixture (or a dilution of the mixture if enzyme activity is too high) is applied to an agarose gel, electrophoresed 25 min at 160V at 4-10°C and then stained with the individual reaction buffer at 27°C[4] for 20-40 min. The gels are washed with distilled water to remove excess reaction buffer, dried and kept as a permanent record of the cell's isoenzyme pattern.

In addition to identification, cell lines need to be periodically screened for contaminants. The primary way to avoid bacterial contamination is by **not** using antibiotics in maintaining cell lines. While this may seem contradictory, the reasoning is simple. If you do not have antibiotics in the medium, any bacterial (or fungal) contamination will become apparent in the highly nutritious cell culture medium within a few days. This will allow you to return to a backup culture to recover the cells. Alternatively, with antibiotics, you may passage the cells for weeks or months with a low level contamination which will eventually become apparent when antibiotic resistance develops in the contaminant. By that time, all your cultures will be contaminated and there will be little hope of recovery. For this reason, I reserve antibiotics for use in "deadend" experiments (experiments in which the cells will no longer be used for maintaining a culture) and for primary cultures. In the case of primary cultures, once regular growth is obtained, I replace the medium being used on the cultures with antibiotic-free medium (usually by the 5th passage).

So, since this avoids most bacterial contamination, our main concern is with viruses and mycoplasma. Here again, avoiding the problem is the best solution. Never use mouth pipetting and obtain your culture supplies (medium, serum, cultureware) from a reputable dealer. One practical advantage of working with insect cells is that many of the contaminants vertebrate cell culturists have to contend with are not an issue with insect cells. For example, since the major source of mycoplasma is the lab worker, these organisms are adapted to grow at 37°C. The temperatures at which insect cells are grown is not conducive to very effective growth of these organisms (in fact, the insect cells will usually outgrow the bacteria). In the case of viruses, the major source of contamination is serum. Since this is usually of bovine source, these often will not replicate in the insect cell. However, it is still a good idea to periodically screen your cultures for these contaminants.

In the case of mycoplasma, a number of tests are available. These include growth assays using mycoplasma culture medium (such as Mycotrim™, Hana Media, Inc., Berkeley, CA), screening with fluorescent nuclear dyes (such as Hoechst 33258, see Chen, 1976) or coculture with 6-methylpurine (Mycotect, BRL, Bethesda, MD) which is metabolized by mycoplasma to form toxic components. Of these, the Hoechst 33258 method seems the most reliable, but does require a fluorescent microscope. In addition, there are commercial testing facilities which will screen your cultures for mycoplasma (e.g. Flow Laboratories, McLean, VA, and Microbiological Associates, Rockville, MD). Screening for viruses can only be effectively accomplished with an electron microscope, since these are internal contaminants. This is a complicated technique which obviously cannot be covered in detail here, but what you are looking for is any sign of regular arrays of particles.

As mentioned previously, the best solution to contamination is prevention. In the event you do find your cultures are contaminated, it is best to simply discard them and revert to your frozen stock. For this reason, it is very important that you prepare a frozen stock of any new cell lines as soon as possible. The

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[3] The centrifugation speed listed here are somewhat lower than that recommended by the manufacturer, but are used because of limitations of my equipment. These have been adequate for obtaining good results with the Authentikit™ system.

[4] The manufacturer recommends 37°C. The lower temperature cited here is used to be compatible with the insect cell enzymes.

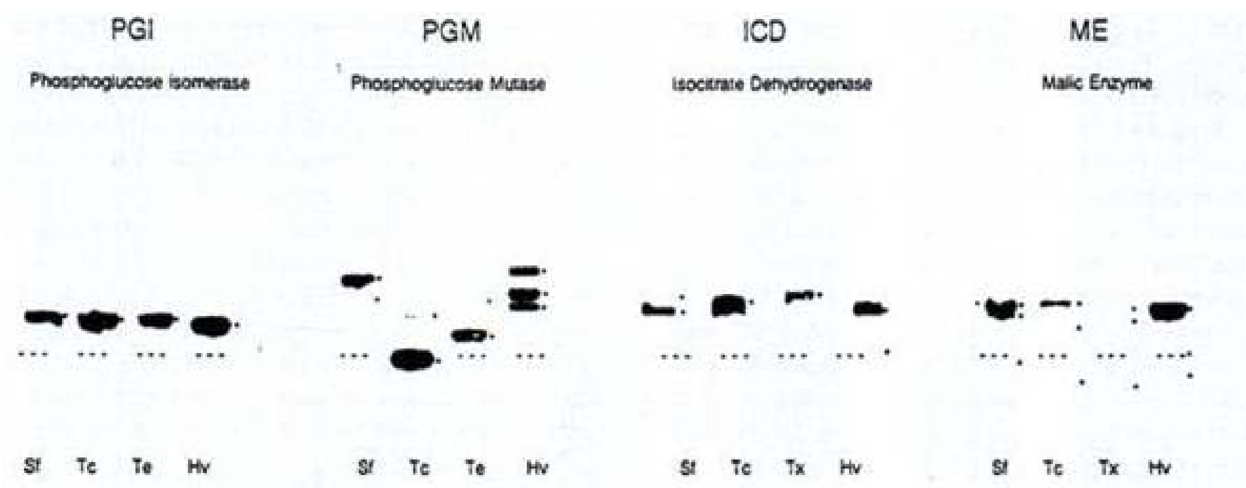

Figure 3. Isoenzyme patterns obtained with various cell lines and staining for four enzymes; PGM, PGI, ICD, and ME. Cell lines shown are IPLB-Sf21AE (Sf, Vaughn et al, 1977), IPLB-Tcon1 (Tc, Lynn and Hung, 1991), IPLB-Tex2 (Te, Lynn and Hung, 1991) and IPLB-HvT1 (Hv, Lynn, et al. 1988). The dashed lines mark the location of the origin (where cell extracts were applied prior to electrophoresis) and the small dots to the right of each band were applied with a pen to mark the migration distance. In some cases, weaker bands seen on the gel may not be apparent on the photoreproduction.

procedure described in Freshney (1987) is similar to the method I use. Briefly, cells are placed in suspension by the normal subculture procedure and centrifuged (50Xg, 10 min). Resuspend the cells in medium containing a cryopreservant. Researchers have used 5-10% dimethyl sulfoxide, but for most insect cells, I prefer 5-10% glycerol. It is best to freeze a few ampules to test the suitability of the cryopreservant prior to making a major freeze for stock purposes. Dispense the cell suspension into 1- or 2-ml glass ampules (Bellco Glass, Vineland, NJ) and seal with a gas/air or gas/O_2 torch. Sealing ampules requires care because improperly seal vials may inspire liquid nitrogen during storage and will explode during thawing. (Plastic cryovials are also available from several manufacturers, but these also require careful use since they should **never** be used in the liquid phase of LN_2.) Sealing ampules should be practiced prior to making a critical freezing of cells. A useful safety technique to test for a good seal is to submerge the sealed ampules in a container of 1% methylene blue in 70% ethanol at 4°C for 10 min. Any improperly sealed ampules will contain the dye. After sealing, ampules are cooled to freezing. While there are specially designed devices for this, a useful alternative is to place the sealed ampules in a styrofoam box (such as used in shipping chemical supplies) and place it in a -70°C mechanical freezer. After at least 2 hr at -70°C, the cells are transferred to a liquid nitrogen freezer (such as Linde freezers, Union Carbide Corp., Indianapolis, IN). An accurate freezer log must be maintained as to the cell line designation and passage number, date of freeze, location in freezer, type of medium, and type/amount of cryoprotectant.

Recovering cells should be done rapidly. A face shield or protective goggles must be worn. This is a precaution for the possibility that the ampule has taken up liquid nitrogen which would cause a dangerous explosion. The ampule is removed from the freezer and placed in warm water (37°C is usually recommended for vertebrate cells, but I use 30-32°C to avoid causing a heat shock response which can occur with some insects at 37°C). As soon as the medium is thawed, wipe the ampule with 70% ethanol, break it open at the neck (scoring the glass with a file if necessary). Transfer the contents to a flask and slowly add 10 ml fresh medium. The cells may be centrifuged at this point and resuspended in fresh medium or left to attach to the flask prior to removing the medium containing the cryopreservant. While initial subcultures following thawing may need to be made at a higher split ratio than before freezing for a few passages, it should be possible to maintain the cells in essentially the same manner as before freezing.

Conclusions

With the wide availability of insect cell culture media, it can generally be considered a routine process

to develop new cell lines. Exceptions to this statement do exist, of course. Difficulties may arise when attempting to culture a specific cell type. For example, while there are a few cell lines from insect fat body and at least one from the midgut, it may not be possible to obtain cell lines from these tissues from all insect species due to terminal differentiation and other factors. Also, researchers have desired cell lines from certain species, such as the honey bee, for which no success has been obtained. As in the early days of tissue culture, it is difficult to discern why negative results occur. However, as more is learned about the physiology and nutrition of various insects and tissues, we may get clues which will help solve these questions.

The remaining chapters in this book will provide the reader with exciting uses for insect cell culture. As I mentioned earlier, the baculovirus expression vector system has provided a stimulus to the field of insect cell culture not seen previously.

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