Inspections, Compliance, Enforcement, and Criminal Investigations

Industrial Applications of a New Biochemical Technology

[Previous Chapter[1]] [Table of Contents[2]] [Next Chapter[3]]

DEPT. OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION *ORA/ORO/DEIO/IB*

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The first in vitro diagnostic reagent containing monoclonal antibodies was approved by FDA in 1981 and the first drug manufactured by recombinant DNA technology was approved a year later. These exciting industrial applications were the results of fundamental research which was successfully demonstrated in the laboratory only six and nine years ago respectively (5 and 1). These new technologies will have significant impact on all of the industries regulated by FDA (7). This ITG describes the basics of two new biochemical techniques, the industrial applications for large scale production, and the related containment and safety practices.

HYBRIDOMA TECHNOLOGY

Monoclonal Antibodies

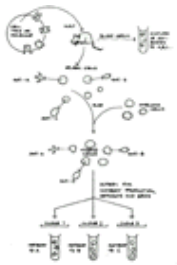
Antibodies are protein components of the immume system in the blood and other body fluids, and they are composed of two heavy and two light polypeptide chains with a total molecular weight of 150,000 or greater. They are produced in reaction to a specific structural site (determinant) of a foreign substance called antigen (immunogen). Because an antigen, such as a bacterium, virus or a relatively large molecule, usually has several antigenic determinants, the antiserum prepared by injecting animals with a selected antigen will contain a mixture of antibodies. Monoclonal antibodies, on the other hand, are pure antibodies derived from cells which recognize a single determinant (and other structurally similar determinants).

A simplified production procedure of monoclonal antibodies is illustrated in Fig. 1 (Figure) (6). The procedure involves injecting the mouse with an antigen, which elicits the production of antibodies by plasma cells which are concentrated in the spleen. The cells are taken from the spleen and mixed in a special selective growth media with myeloma (tumor) cells. The fused cells (hybridoma) are then screened for antibody production and can be individually separated by using a fluorescence-activated cell sorter (FACS).

The hybridoma have two outstanding features: (1) secretion of the antibody specified by the spleen cell and (2) vigorous growth and longevity that is inherent of the myeloma cell. They can be grown in tissue culture and produce the desired antibody for a long time. They can also be injected into the abdomen of a mouse, where tumors will be induced. The tumor-bearing mouse develops ascites and the ascitic fluid is a rich source of monoclonal antibodies with the predefined specificity.

New perfusion reactors up to 100 liters recently have been designed to grow large quantities of tumor cells (3). The reactors can be adapted to grow anchorage-dependent normal cells by adding tiny beads made of dextran or synthetic polymers. Because the large scale systems are more efficient, they will replace the conventional roller bottles and spinner bottles for culturing a wide range of mammalian cells.

FIG. 1 (6)**[4]



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RECOMBINANT DNA TECHNOLOGY

The Construction of a R-DNA Molecule

Genetic information in almost all living cells is stored in the double helix of deoxyribonucleic acids (DNA). The expression of DNA into protein involves two major steps: transcription of DNA into messenger ribonucleic acid (mRNA) and translation of mRNA on ribosomes into protein. There are two forms of DNA in a bacterial cell. Most of the DNAs (genes) are found in the chromosome. The second is a much smaller circular DNA called a plasmid which can replicate itself in the cell. Plasmids can be extracted from cells, by softening the cell wall with chemical reagents, and used as the acceptors for DNA coding for the desired product. Two methods commonly used to obtain the DNA are: (1) direc chemical synthesis and (2) isolation of the mRNA and preparation of the cDNA (complementary) by reverse transcription.

A Plasmid can be cleaved by an endonuclease at a particular site leaving two single-stranded, chemically reactive (sticky) ends (Fig. 2)[7]. Treatment of a foreign DNA with the same endonuclease will result in pieces of DNA with the same two sticky ends. The cleaved plasmid is mixed with the foreign DNA and recombined at the site of cleavage in a process called annealing. With the help of a DNA ligase, the recombinant plasmid is closed and reformed into a circle. The recombinant plasmid is then transferred into a cell where it can be replicated. The original plasmid usually contains antibiotic resistant gene(s) called markers, which will facilitate the selection of the desired cells with recombinant plasmid during the initial screening, and later will help prevent contamination during fermentation. The selected recombinant microorganisms which contain the DNA coding for the desired product are the seed pool, and are kept in appropriate media stored in liquid nitrogen or under refrigeration.

FIG. 2[8]

9

(image size 12KB)[10]

Fermentation

A vial is removed from liquid nitrogen storage and the preserved microorganisms are streaked on an agar plate. The

isolated colonies are individually transferred to selective agar media for verification of genotype and phenotype. Colonies which are positive on the microbiological tests and are strongly resistant to the antibiotic (s) are selected as the starting inoculum. Depending on the process, several stages with varying medium composition and increasing volume are used before the inoculum reaches sufficient quantity. The final inoculum, from several hundred liters and up, is usually 5 to 15% by volume of the production fermenter.

Chemically defined media are normally used in the inoculum preparation, second stage (s) and production fermentation, because the recombinant microorganisms usually have stringent nutritional requirements. Traces of antibiotics are also added to the medium to help prevent the mutant from taking over the slower growing recombinant cells. Contamination of a fermentation batch by microorganisms and/or phages (in the case of E. coli fermentation) is not uncommon. So it is important that a firm establishes an acceptable level for each type of contamination. Automated equipment and microprocessor controlled instruments will be extensively used to control and monitor the process variables during fermentation. Other novel systems such as immobilization techniques and continuous reactor will be applied to increase the yield and improve the stability of the recombinant culture. (Computer Controlled Manufacturing processes will be covered in another ITG.) Containment

Three levels of physical containment---P1, P2 and P3 for large scale operations (greater than 10 liters) with P3 having the highest containment, are described in the NIH Guidelines (4b and c). In addition to physical containment, NIH guidelines also define laboratory biological containment in terms of three classes of host-vector systems, designated HV-1, 2, and 3, which have progressively diminishing probability of survival outside the specific growth conditions in the laboratory (4a). For a given HV system, a more toxic product will require a higher physical containment level. The appropriate containment of recombinant microorganisms in industrial fermentation is usually decided by the local Institutional Biosafety Committee. However, it is generally recognized that in manufacturing a non-toxic product with HV-1 system (E. coli K-12 and the vector in (Fig. 2)[11] or other similar systems), the NIH's P1 LS recommendations should be followed as a minimum.

The key points of P1-LS conditions are as follow: (1) use a closed vessel, (2) culture fluids should be inactivated by validated procedures before removing from the closed vessel, (3) a closed system should be used to collect samples and to add material, (4) exhaust gasses should be filtered through a HEPA filter before being removed from the close system, (5) the closed vessel should be sterilized by validated procedures before opening, and (6) should have emergency procedures for large losses of culture. Several conditions of P2-LS are also recommended for industrial fermentation: rotating seals and other mechanical devices should be designed to prevent leakage of viable organisms the closed vessel should include sensing devices to monitor the integrity of containment during operation and the integrity of containment should be tested with the host organisms before introducing the recombinant molecules.

Safety

The host organisms most commonly used are derived from E. coli K-12. It has been shown the E. coli K-12 cannot be converted into an epidemic pathogen by laboratory manipulation with R-DNA molecules and it will not colonize the human intestinal tract. All the available information indicates that the hazard of exposure to recombinant E. coli K-12 will not be any greater than to any of the conventional industrial microorganisms and will probably be less than some of the chemicals used in the purification (8).

Lengthy and complex procedures are needed to purify the products made by recombinant microorganisms, because a the products reported thus far are intracellular. Some very potent chemicals (e.g. cyanogen bromide) and a large amount of solvents are used. We recommend that investigators should follow a firm's safety practices and pay special attention to personal safety procedures during an inspection.

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2. Cohen, S. N., "The Manipulation of Genes," Scientific American, July 25, 1975.
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Additional Reading and Viewing Suggestions:

- Cultivation of Mammalian Cells: Paul F. Kruse, Jr., and M. K. Patterson, Jr., Editors, Tissue Culture, Academic Press, New York, 1973
- Introduction to Molecular Biology and Recombinant DNA Technology: Kadar, A. J., "Recombinant DNA Research Technical Bulletin, "A draft, Div. of Field Science, HFO-600, June 1980.
- Watson, J. D. Molecular Biology of the Gene, 3rd ed., W.A. Benjamin, New York, 1976.
- Advanced text and a landmark Symposium sponsored by FDA: Gueriguian, J. L. Editor, Insulin, Growth Hormon, and Recombinant DNA Technology, Raven Press, New York, 1981.
- Introductory Engineering Text on Fermentation: Wang. D.I.C. et al., Fermentation and Ezyme Technology, Wiley, New York, 1979.
- Two excellent videotapes from FDA: "Monoconal Antibodies" A Workshop cosponsored by NCDB and sigma X1, April 20, 1980. The tape may be obtained from HFN-26.
- "Recombinant DNA Technology," a 50-minute tape by H. I. Miller, HFN-130, Oct. 1981. It is available from HFO-600.

[Previous Chapter[12]] [Table of Contents[13]] [Next Chapter[14]]

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