Brief content of lectures on discipline “**Agricultural biotechnology”**

Module 1. Plant Biotechnology

**Lecture 1.** Introduction. The aim of Plant biotechnology. Basic direction in Plant biotechnology. Cell technologies for receiving important products derived from plant material

L. 1 **Plants biotechnology**

**History of Plant biotechnology**

**Hybrid breeding**

**Modern Plant breeding**

**Why plant biotechnology?**

The word "biotechnology" was first used in 1917 to describe

processes using living organisms to make a product or run a process, such as industrial fermentations. Biotechnology began when humans started to plant their own crops, domesticate animals, ferment juice into wine, make cheese, and leaven bread.

***Present definition of biotechnology*** “Any technological application that uses biological systems, living organisms, or derivatives theory, to make or modify

products or processes for specific use’’

**Plant biotechnology**

**“Plant biotechnology** describes a precise process in which scientific techniques are used to develop useful and beneficial plants’’

The field of plant biotechnology is concerned with developing ways to improve the production of plants in order to supply the world’s needs for food, fiber and fuel.

In addition, plants provide us with many pharmaceuticals and industrial compounds.

As our population grows, our needs also grow.

To increase the quantity of crop production as well as to produce specific characteristics in plants, biotechnologists are using selective gene techniques.

**History of Plant biotechnology**

Early Plant breeding

Humans domesticate crops.

• Breed plants to further improve desirable characteristics.

Plant breeding 12,000 years ago

**Traditional plant breeding** selects mutants for best yield and quality (e.g., tomatoes).

1. Classical Plant breeding.

2. Cross-breeding to strengthen traits.

Charles Darwin publishes the theory of evolution by natural selection. The founding of the science of genetics.

Gregor Mendel discovers the laws of inheritance by studying flowers in his garden. The science of genetic begins.

**Hybrid breeding**

Two parental lines of normally outbreeding species are inbred through several self-pollinations.

• When crossing such lines the first generation has hybrid vigour.

• The vigour gradually disappears over the next generations so new sowing seeds have to be purchased every year .

• Selection operates on desirable traits, not on survival in the wild.

**Modern Plant breeding**

A basic type of modern plant breeding:

- Mutation breeding

- Green revolution

- Plant tissue culture breeding

**Mutation breeding**

Seeds are treated with

either radiation or

mutagenic chemicals to induce larger or smaller

lesions in the genes.

The mutations are at random over the genome.

Usually mutation results in a loss of function of Genes.

Green revolution (1960-1970)

Green revolution’ leads to greatly increased crop yields based on the incorporation of **dwarfing genes** discovered by Norman Borlaug and the widespread use of agrochemicals.

Plant tissue culture breeding

The process of selectively mating plants in aseptic culture.

- Embryo rescue

- Somaclonal variation selection

- Somatic hybrid (i.e. fusion protoplast).

- Generation of haploid (i.e. anther/microspore culture)

Highlights of Plant tissue culture

1902. Gottlieb Haberlandt proposed that all cells are totipotent. Totipotent.

Totipotency (Lat. *totipotentia,* "ability for all [things]") is the ability of a single [cell](https://en.wikipedia.org/wiki/Cell_%28biology%29) to divide and produce all of the differentiated cells in an **organism.**

**Spores** and **zygotes** are examples of totipotent cells.

 In the spectrum of cell potency, totipotency represents the cell with the greatest **differentiation** potential, being able to differentiate into any **embryonic** cell, as well as extraembryonic cells. In contrast, pluripotent cells can only differentiate into embryonic cells

**History of Plant biotechnology**

**1904**

**Hanning isolated nearly mature zygotic embryos from seeds of Crucifers and successfully grew them to maturity in a defined medium.**

**1925**

**Laibach isolated and grew embryos of interspecific cross *Linum perenne* and *L. austriacum* that aborted in vivo**

**1948**

Folke Skoog discovered that kinetin could induce organogenesis in callus culture of tobacco.

**1957.**

**Skoog and Miller demonstrated the effects and interaction of phytohormones**

**Auxin : cytokin > 1 root formation**

**Auxin : cytokin <1 shoot formation**

**Auxin : cytokin = 1 callus formation**

**History of Plant biotechnology**

**1964**

Haploid plants derived from cultured Datura anthers

**1972**

First interspecific hybridization of Nicotiana sp. by fusing protoplast

**1974**

Haploid plants derived from cultured tobacco microspores

**1977**

Successful integration of T-DNA in plants.

**Two major areas of plant biotechnology:**

**Plant Tissue Culture (plants cloning)**

**Recombinant DNA technology (gene cloning)**

Why plant biotechnology?

Human population is rapidly outgrowing.

Worlds’ current status

For higher yield

Conventional Breeding

Ideotype Breeding

Hybrid Breeding

Wide Hybridization

Mutation Breeding

Germplasm Breeding

*The scope of plant biotechnology*

**Plant genetic engineering**

**Plants micropropagation**

**Plant mutation cloning**

**Plant cells technology**

**Impact of plant biotechnology**

More crop

More money

**Environmental impact**

In vitro conservation

Pesticide reduction

Health impact Society impact

More food

Better food

Society impact

**Plant Tissue Culture (PTC):**

**Plant tissue culture is the sterile, in vitro cultivation of plant parts. Plants have the ability for differentiated cells revert to an undifferentiated state called callus.**

**These cells will then divide and then differentiate back to somatic embryo cells that will regenerate the entire plant.**

Plant Tissue Culture (PTC):

Plants cultured in vitro yield thousands of genetically identical plants (clones) from a single plant.

This process is called micropropagation and is used to commercially propagate plants asexually.

The rapid multiplication allows breeders and growers to introduce new cultivars much earlier than they could by using conventional propagation techniques, such as cuttings.

Plant Tissue Culture (PTC):

**Through the use of biotechnology, desirable genetic traits can be transferred from one organism to another by transfer of DNA.**

**Many more plants with the desirable DNA can be regenerated from small pieces of the transformed plant tissue.**

**Examples of plants produced using tissue culture include the large variety of ornamental plants; agricultural crops such as strawberry, banana, potato, and tomato; and a variety of medicinal plants.**

Plant Tissue Culture (PTC):

**Commercial tissue culture involves exposing plant tissue to a specific regimen of nutrients, hormones, and light under sterile conditions to produce many new plants over a very short period of time.**

**Lecture 2.** Micropropagation technologies of plants. Technology for production of virus-free plants.

**L. 2 Micropropagation technologies of plants.**

**What is Tissue Culture? Types,**

**Techniques and**  **Major Steps of Tissue Culture (Plants)**

**Process**

**Plant tissue culture**

**is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition.**

**Plant tissue culture is widely used to produce clones of a plant in a method known as** [**micropropagation**](https://en.wikipedia.org/wiki/Micropropagation)**.**

**Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including**

**Plant tissue culture**

1. **The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.**
2. **To quickly produce mature plants.**
3. **The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.**

**The regeneration of whole plants from plant cells that have been genetically modified.**

**The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.**

**The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e.:** [**orchids**](https://en.wikipedia.org/wiki/Orchids) **and** [***Nepenthes***](https://en.wikipedia.org/wiki/Nepenthes)**.**

**To clear particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.**

**Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant ([totipotency](https://en.wikipedia.org/wiki/Totipotency)).**

**What is Tissue Culture? Types, Techniques and Process**

**In biological research, tissue culture refers to a method in which fragments of a tissue (plant or animal tissue) are introduced into a new, artificial environment.**

 **In artificial environment fragments of a tissue continue to function or grow.**

**While fragments of a tissue are often used, it is important to note that entire organs are also used for tissue culture purposes.**

**The application of plant tissue cultures**

in fundamental and applied studies on various biological species is rapidly growing.

The use of in vitro technology for commercial propagation of plant species and for the production of bioactive components from them has become profitable industry worldwide.

A whole plant can be regenerated from a small tissue or plant cells in a suitable culture medium under controlled environment.

The plantlets so produced are called tissue-culture raised plants.

These plantlets are a true copy of the mother plant and show characteristics identical to the mother plant.

For example, if the mother plant is a high yielding plant the plantlets will also be high yielding.

Many plant species are presently being propagated through tissue culture successfully.

The capacity of a single cell to grow into a complete plant is termed as Totipotency,

Plant tissue culture can be initiated from almost any part of a plant however, for micropropagation or direct shoot regeneration, meristemetic tissue such as shoot tip is ideal.

The physiological state of the plant has an influence on its response to tissue culture.

The mother plant must be healthy and free from obvious signs of disease or pest.

 The shoot tip explants being juvenile contain a higher proportion of actively dividing cells. It is important to use quality mother plant stock to initiate cultures.

Tissue culture is the *in vitro* aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions often to produce the clones of plants.

The resultant clones are true-to type of the selected genotype.

The controlled conditions provide the culture an environment conducive for their growth and multiplication.

**These conditions include:**

 ***proper supply of nutrients,***

***pH medium,***

***adequate temperature and***

***proper gaseous and liquid environment.***

**Types of Tissue Culture.**

**Seed Culture**

Seed culture is the type of tissue culture that is primarily used for plants such as orchids.

For this method, explants (tissue from the plant) are obtained from an in-vitro derived plant and introduced in to an artificial environment, where they get to proliferate.

In the event that a plant material is used directly for this process, then it has to be sterilized to prevent tissue damage and ensure optimum regeneration.

**Embryo Culture**

Embryo culture is the type of tissue culture that involves the isolation of an embryo from a given organism for in vitro growth.

\*Note, the term embryo culture is used to refer to sexually produced zygotic embryo culture.

Embryo culture may involve the use of a mature of immature embryo. Whereas mature embryos for culture are essentially obtained from ripe seeds, immature embryo (embryo rescue) involves the use of immature embryos from unripe/hybrid seeds that failed to germinate. In doing so, the embryo is ultimately able to produce a viable plant

**Embryo Culture**

For embryo culture, the ovule, seed or fruit from which the embryo is to be obtained is sterilized, and therefore the embryo does not have to be sterilized again.

 Salt sucrose may be used to provide the embryo with nutrients.

The culture is enriched with organic or inorganic compounds, inorganic salts as well as growth regulators.

Embryo culture

is a type of plant tissue culture that is used to grow embryos from seeds and ovules in a nutrient medium.

In embryo culture, the plant develops directly from the embryo or indirectly through the formation of callus and then subsequent formation of shoots and roots.

 The technique is developed to break seed dormancy, test the vitality of seeds, production of rare species and haploid plants.

 It is an effective technique that is employed to shorten the breeding cycle of plants by growing excised embryos and results in the reduction of long dormancy period of seeds.

**Embryo Culture**

 Intra-varietal hybrids of an economically important energy plant “Jatropha” have been produced successfully with the specific objective of mass multiplication.

Somatic embryogenesis and plant regeneration has been carried out in embryo cultures of Jucara Palm for rapid cloning and improvement of selected individuals.

In addition, conservation of endangered species can also be attained by practicing embryo culture technique. Recently a successful protocol has been developed for the *in vitro* propagation of *Khayagrandifoliola* by excising embryos from mature seeds.

The plant has a high economic value for timber wood and for medicinal purposes as well. This technique has an important application in forestry by offering a mean of propagation of elite individuals where the selection and improvement of natural population is difficult.

**Callus Culture**

Callus - This is the term used to refer to unspecialized, unorganized and a dividing mass of cells. A callus is produced when explants (cells) are cultured in an appropriate medium - A good example of this is the tumor tissue that grows out of the wounds of differentiated tissues/organs.

In practice, callus culture involves the growth of a callus (composed of differentiated and non- differentiated cells), which is the followed by a procedure that induces organ differentiation.

**Callus Culture**

For this type of tissue culture, the culture is often sustained on a gel medium, which is composed **of agar and a mixture of given macro and micronutrients** depending on the type of cells.

 Different types of basal salt mixtures such as murashige and skoog medium are also used in addition to vitamins to enhance growth.

**Organ Culture**

Organ culture is a type of tissue culture that involves isolating an organ for in vitro growth.

Any organ plant can be used as an explant for the culture process (Shoot, root, leaf, and flower).

**Organ Culture**

With organ culture, or as is with their various tissue components, the method is used for preserve their structure or functions, which allows the organ to still resemble and retain the characteristics they would have in vivo.

New growth (differentiated structures) continues given that the organ retains its physiological features.

As such, an organ helps provide information on patterns of growth, differentiation as well as development.

There are number of methods that can be used for organ culture

These include;

**Plasma clot method - The method involves the use of a clot that is composed of plasma and chick embryo extract (or any other extract) in a watch glass.**

**This method is particularly used for the purposes of studying morphogenesis in embryonic organ rudiments and more recently for studying the actions of various hormones, vitamins and carcinogens of adult mammalian tissues.**

There are number of methods that can be used for organ culture. These include;

**Raft method –**

**For this method, the explant is placed on a raft of lens paper/rayon acetate and floated on a serum in a watch glass.**

**Method of organ culture**

**Agar gel method –**

The medium used for this method is composed of a salt solution, serum as well as the embryo extract or a mixture of various amino acids and vitamin with 1 percent agar.

The explant has to be subcultured every 5 to 7 days.

The method is largely used for the study of developmental aspects of normal organs and tumors.

**Method of organ culture**

**Grid method** –

Grid method, as the name suggests involves the use of perforated stainless steel sheet, on which the tissue of interest is placed before being placed in a culture chamber containing fluid medium.

**Protoplast Culture**

Protoplast -cells without cell walls.

A protoplast is the term used to refer to cell (fungi, bacteria, plant cells etc) in which the cell wall has been removed, which is why they are also referred to as naked cells.

Protoplasts may be cultured in the following ways;

Hanging-drop cultures

Micro culture chambers

Soft agars matrix

Once a protoplast has regenerated a cell wall, then it goes through the process of cell division to form a callus, which may then be subcultured for continued growth.

**Protoplast culture** is an important method that provides numerous cells (single cells) that can be used for various studies.

**These include;**

***Protoplast culture regenerated into a whole plant***

***Development of hybrids***

***Cell cloning***

***Genetic transformations***

***Membrane studies***

**In protoplast culture**, a number of phases can be observed.

These include;

***Development of a cell wall***

***Cell division***

***Continuous growth or regeneration to a whole plant***

**For plants, some of the special requirements include in protoplast culture**

1. **Less amounts of iron and zinc and no ammonium**
2. **Higher concentration of calcium**
3. **High auxin/kinetic ratio for cell division and high kinetin/auxin ration for regeneration**
4. **Glucose and vitamins**

**Protoplast fusion**

Somatic hybridization is an important tool of plant breeding and crop improvement by the production of interspecific and intergeneric hybrids.

 The technique involves the fusion of protoplasts of two different genomes followed by the selection of desired somatic hybrid cells and regeneration of hybrid plants.

Protoplast fusion provides an efficient mean of gene transfer with desired trait from one species to another and has an increasing impact on crop improvement.

Somatic hybrids were produced by fusion of protoplasts from rice and ditch reed using electrofusion treatment for salt tolerance

*In vitro* fusion

of protoplast opens a way of developing unique hybrid plants by overcoming the barriers of sexual incompatibility. The technique has been applicable in horticultural industry to create new hybrids with increased fruit yield and better resistance to diseases. Successful viable hybrid plants were obtained when protoplasts from citrus were fused with other related citrinae species.

*In vitro* fusion

The potential of somatic hybridization in important crop plants is best illustrated by the production of intergeneric hybrid plants among the members of *Brassicaceae*.

To resolve the problem of loss of chromosomes and decreased regeneration capacity, successful protocol has been established for the production of somatic hybrid plants by using two types of wheat protoplast as recipient and protoplast of *Haynaldiavillosa* as a fusion donor.

It is also employed as an important gene source for wheat improvement.

Some of the other types of tissue culture include

**Single cell culture**

**Suspension culture**

**Anther culture**

**Pollen culture**

**Somatic Embryogenesis**

 **Major Steps of Tissue Culture (Plants)**

**Initiation Phase (Stage 1)**

The initiation phase is the first phase of tissue culture. Here, the tissue of interest is obtained and introduced and sterilized in order to prevent any microorganism from negatively affecting the process. It is during this stage that the tissue is initiated in to culture

**Multiplication Phase (Stage 2)**

**The multiplication phase is the second step of tissue culture where the in vitro plant material is re- divided and then introduced in to the medium.**

**The medium is composed of appropriate components for growth including regulators and nutrients.**

 **These are responsible for the proliferation of the tissue and the production of multiple shoots.**

**\*This step is often repeated several times in order to obtain the desired number of plants**

**Root formation (Stage 3)**

**It is at this phase that roots are formed.**

**Root formation hormones are required in order to induce rooting, and consequently complete plantlets**

**Plant Tissue Culture**

Tissue culture is applied in plant research for such purposes as the growing of new plants, which in some cases undergo genetic alterations.

The plant of interest is taken through the tissue culture process and grown in a controlled environment.

**The Process of Plant Tissue Culture**

This process involves the use of small pieces of a given plant tissue (plant of interest). Once the tissue is obtained, it is then cultured in the appropriate medium under sterile conditions so as to prevent various types of microorganisms from affecting the process

The following is a general procedure for plant tissue culture

Medium preparation

The appropriate mixture (such as the MS mixture) is mixed with distilled water and stirred while adding the appropriate amount of sugar and sugar mixture. Here, sodium hydroxide or hydrochloric acid is used to adjust the pH - Contents used here will depend on the plant to be cultured and the number of tissues to be cultured.

Agar is added to the mixture, heat and stirred to dissolve

After cooling, the warm medium is poured into polycarbonate tubes (to a depth of about 4 cm)

With lids sitting on the tubes, the tubes are placed in a pressure cooker and sterilized for 20 minutes

1. [**Gene cloning using viruses**](https://image1.slideserve.com/3090721/gene-cloning-using-viruses1-l.jpg)
2. [**Transgenesis**](https://image1.slideserve.com/3090721/transgenesis-l.jpg) • Trangenesis, using genetic engineering techniques, is concerned with the movement of genes from one species to another • An organism that develops from a cell into which foreign DNA has been introduced is called a transgenic organism • Because of their immense economic importance, plants have been the subject of traditional breeding programmes aimed at developing improved varieties • Recombinant DNA technology now allows direct modification of a plant’s genome allowing traits to be introduced that are not even present in the species naturally • DNA can now be introduced from other plant species, animals or even bacteria • Micropropagation techniques allow introduced genes to become par of the germ line for plants (the trait is inherited) • Animal cells may become transformed (receive foreign DNA) to provide new enhanced characteristics in livestock as well as providing a means of curing genetic defects in humans through gene therapy
3. [**Transformation using a plasmid**](https://image1.slideserve.com/3090721/transformation-using-a-plasmid-l.jpg) • Ti plasmid isolated from bacteria Agrobacterium tumefaciens. Agrobacterium tumefaciens causes tumours (galls) in plants. • The Ti plasmid can be succesfully transferred to plant cells where a segment of its DNA can be integrated into the plant’s chromosome. • Restriction enzyme and DNA ligase splice the gene of interest into the plasmid as discussed previously for cloning into plasmids • Introduce plasmid into plant cells • Part of the plasmid containing the gene of interest integrates into the plant’s chromosomal DNA • Transformed plant cells are grown by tissue culture
4. [**Transformation using a plasmid**](https://image1.slideserve.com/3090721/transformation-using-a-plasmid1-l.jpg)
5. [**Transformation by protoplast fusion**](https://image1.slideserve.com/3090721/transformation-by-protoplast-fusion-l.jpg) • This process requires the cell walls of plant to be removed by digesting enzymes • The resulting protoplasts (cells that have lost their cell walls) are then treated with polyethylene glycol (PEG) which causes them to fuse • In the new hybrid cell, the DNA derived from the 2 “parent” cells may undergo natural recombination (they may merge)
6. [**Transformation by protoplast fusion**](https://image1.slideserve.com/3090721/transformation-by-protoplast-fusion1-l.jpg)
7. [**Transformation using a gene gun**](https://image1.slideserve.com/3090721/transformation-using-a-gene-gun-l.jpg) • This method of introducing foreign DNA into plant cells, literally shoots it directly through cell walls using a “gene gun” • Microscopic particles of gold or tungsten are coated with DNA and propelled by a burst of helium through the cell wall and membrane • Some of the cells express the introduced DNA as if it were their own
8. [**Transformation using a gene gun**](https://image1.slideserve.com/3090721/transformation-using-a-gene-gun1-l.jpg)
9. [**Transformation using liposomes**](https://image1.slideserve.com/3090721/transformation-using-liposomes-l.jpg) • Liposomes are small spherical vesicles made of a single membrane. They can be made commercially to precise specifications • When coated with appropriate surface molecules, they are attracted to specific cell types in the body • DNA carried by the liposome can enter the cell by endocytosis or fusion • They can be used to deliver genes to these cells to correct defective or missing genes
10. [**Transformation using liposomes**](https://image1.slideserve.com/3090721/transformation-using-liposomes1-l.jpg)
11. [**Transformation using viral vectors**](https://image1.slideserve.com/3090721/transformation-using-viral-vectors-l.jpg) • Some viruses are well suited for gene therapy – they can accommodate up to 7.5kbp of inserted DNA in their protein capsule • When viruses infect and reproduce inside the target cells, they are also spreading the recombinant DNA gene • A problem with this method involves the host’s immune system reacting to and killing the virus • Common viruses used for viral transformation of target cells are retroviruses, lentiviruses and adenoviruses
12. [**Transformation using viral vectors**](https://image1.slideserve.com/3090721/transformation-using-viral-vectors1-l.jpg)
13. [**Transformation using microinjection**](https://image1.slideserve.com/3090721/transformation-using-microinjection-l.jpg) • DNA can be introduced directly into an animal cell (usually an egg cell) by microinjection • This technique requires the use a glass micropipette with a diameter that is much smaller than the cell itself – the sharp tip can then be used to puncture the cell membrane • The DNA is then injected through it and into the nucleus
14. [**Transformation using microinjection**](https://image1.slideserve.com/3090721/transformation-using-microinjection1-l.jpg)
15. [**Making an artificial gene**](https://image1.slideserve.com/3090721/making-an-artificial-gene-l.jpg) • Biologists get genes for cloning from two main sources • DNA isolated directly from an organism • complementary DNA (cDNA) made in the laboratory from mRNA templates • One problem with cloning DNA directly from an organism’s cell is that it often contains long non-coding regions called introns • These introns can be enormous in length and cause problems when the gene as a whole is inserted into plasmids or viral DNA vectors for cloning: • Plasmids tend to lose large inserts of foreign DNA • Viruses cannot fit the extra long DNA into their protein coats • To avoid this problem, it is possible to make an artificial gene that lacks introns • This is possible by using the enzyme reverse transcriptase which is able to reverse the process of transcription • The important feature of this process is that mRNA has already had the introns removed, so by using them as the template to recreate the gene, the cDNA will also lack the intron region
16. [**Gene Therapy**](https://image1.slideserve.com/3090721/gene-therapy-l.jpg) • By using the techniques of recombinant DNA technology, medical researchers attempt to insert a functional gene into a patient’s somatic cells • This should make the patient capable of producing the protein encoded by that allele • Genetic material delivered to a patient’s cells could be used to treat a number of conditions: • Restore the function of a gene that has been lost as a result of a mutation (i.e. possesses a harmful allele) • Kill abnormal cells such as those in cancerous tumours • Introduce genes that inhibit the reproduction of infectious agents such as viruses, bacteria and endoparasites • Render cells resistant to toxic drugs used in the medical treatment of diseases • By replacing missing genes or modifying faulty genes, it may be possible to treat genetic diseases • There have been suggestions that the techniques of gene therapy may also be put to use to create “designer babies” that have traits that are selected by the parents
17. [**Gene Therapy**](https://image1.slideserve.com/3090721/gene-therapy1-l.jpg) • Genetic disorders that are currently undergoing clinical trials include: • SCIDS • Cancers (including melanoma, breast and colon) • Cystic fibrosis • Haemophilia • Rheumatoid arthritis • Peripheral vascular disease • Inherited high blood cholesterol • First attempt at gene therapy was when Ashanti DeSilva was treated for adenosine deaminase (ADA) deficiency on 14 September 1990 • She received new infusions of ADA restored cells every 1-2 months for the first year, then every 3-6 months thereafter. • Ashanti is not completely cured - she still takes a low dose of PEG-ADA. Normally the dose size would increase with the patient's age, but her doses have remained fixed at her four-year-old level. It's possible that she could be taken off the PEG-ADA therapy entirely, but her doctors don't think it's yet worth the risk. • The fact that she's alive today-let alone healthy and active-is due to her gene therapy, and also helps prove a crucial point: genes can be inserted into humans to cure genetic diseases.
18. [**Gene Therapy**](https://image1.slideserve.com/3090721/gene-therapy2-l.jpg) • In contrast, eighteen-year-old Jesse Gelsinger died on September 17th, 1999 while enrolled in gene therapy trial. • Jesse Gelsinger was not sick before died. He suffered from ornithine transcarbamylase (OTC) deficiency, a rare metabolic disorder, but it was controlled with a low-protein diet and drugs, 32 pills a day. • He was not expecting that he would benefit from the study, its purpose was to test the safety of a treatment for babies with a fatal form of his disorder. • Still, it offered hope, the promise that someday Jesse might be rid of the cumbersome medications and diet so restrictive that half a hot dog was a treat. "What's the worst that can happen to me?" he told a friend shortly before he left for the Penn hospital, in Philadelphia. "I die, and it's for the babies." • The researchers had tested their vector, at the same dose Jesse got, in mice, monkeys, baboons and one human patient, and had seen expected, flulike side effects, along with some mild liver inflammation, which disappeared on its own. • When Jesse got the vector, he suffered a chain reaction that the testing had not predicted – jaundice, a blood-clotting disorder, kidney failure, lung failure and brain death. It is thought that the adenovirus triggered an overwhelming inflammatory reaction -- in essence, an immune-system revolt.

**Module 2. “**Microbial biotechnology**”**

L. 6. Microbial biotechnology: fundamentals of applied microbiology (metabolism. control and monitoring of aseptic processing in biotechnology).

Microorganisms

* Minute living things unseen by the naked eyes
* Diverse and unique life form
* Ubiquitous in nature live in soil, water, food,
animal intestines as well as in extreme settings
such as glaciers, hot springs and deep-sea
thermal vents

Microorganisms
Protozoan
Algae
Yeast
Mold
Archaeon
Bacterium
Virus
***Microorganisms.***Application of scientific and engineering principles to the processing of materials by
microorganisms to create useful products or processes.

* Microorganisms utilized may be natural isolates,
laboratory selected mutants or microbes that have been genetically engineered using recombinant DNA methods.

**7.**  Microbial Biotechnology
Deals with the prevention of deterioration of
processed or manufactured goods, environmental
protection and with waste disposal system.

* Production of antibiotics, organic acids and enzymes by fermentation of natural microbes,
laboratory selected mutants or microbes genetically engineered using recombinant DNA
methods.

**8.** Microbial Biotechnology in Foods and Agriculture

* Benefits
* development of genetically engineered plants with
internal resistance to drought, frost, insect pests and infestation
* reduction in dependency of plants on chemical fertilizers and identification of alternatives to
expensive fertilizers
* replacement of dangerous chemical pesticides with microbial pesticides to manage and control the problem of pests

**9.** Microbial Biotechnology in Foods and Agriculture

* reduction in the reliance on chemical treatments to control weeds by engineering herbicide
tolerance into crops
* production of products that have high yield and
enhanced nutritional value
* development of novel biomass products as foodstuffs, using organisms such as algae, fungi,
bacteria and yeast.

**10.** Microbial Biotechnology in Foods and Agriculture

* A. FOOD
* improved and preserved by fermentation.
* FERMENTATION is
* Any process that produces alcoholic beverages or
acidic dairy products
* Any spoilage of food by microorganisms
* Any large-scale microbial process occurring with
or without air
* All metabolic processes that release energy
from a sugar or other inorganic molecule.

*L.7. Prokaryotic cells in biotechnology production. Fermentation Biotech-*

*nology. scientific, technical and economic aspects of microbial products. Research and development*

**1.** Microbial Biotechnology in Foods and Agriculture
Fermentation End-products
Pyruvate
During the fermentation process, microbial growth
and metabolism result in the production of

* 1. enzymes capable of breaking down
carbohydrates, lipids and proteins.
* 2. vitamins
* 3. antimicrobial compounds
* 4. texture-forming agents
* 5. amino acids
* 6. glutamic acid
* 7. organic acids
* 8. flavor compounds

Genetically Modified (GM) Beer

* Fermentation carried by a genetically modified
brewers yeast, Saccharomyces cerevisiae,
containing glucoamylase gene from a
closely-related yeast, Saccharomyces diastaticus.
* GM S. cerevisiae increases the yield of alcohol
and enable the production of a full-strength,
low-carbohydrate diet beer without the use of
extra enzymes after the beer had been brewed.
* Produced by John Hammond and his colleagues at
the Brewing Research Foundation International (a
UK research organization).

Nutfield Lyte
**14**
Microbial Biotechnology in Foods and Agriculture
Oenococcus oeni (malolactic enzyme gene)
Schizosaccharomyces pombe (malate permease gene)
GM S. cerevisiae ML01

* Genetically Modified (GM) Wine
* Fermentation carried by a genetically modified
yeast, Saccharomyces cerevisiae ML01, containing
gene for malolactic enzyme from the bacterium
Oenococcus oeni and a malate permease gene from
the fission yeast, Schizosaccharomyces pombe.
* The recombinant yeast softened the wines mouth
feel by decreasing its acidity, reduces buttery
flavor due to lactic acid secondary metabolism.
* The GM yeast is distributed by Springer
Oenologie, Lesaffre Group, North America.

*L.9. Process management in Microbial Biotechnology: Genome management and analysis in microbial Biotechnology. Microbial process kinetics*

Microbial Biotechnology in Foods and Agriculture.

 Genetically Modified Vitamin B2 - Riboflavin, a water-soluble vitamin that is synthesized by
plants and many microorganisms but is not
produced by higher animals occurs naturally in
peas, beans, grains, yeast, milk, egg yolk and
liver. - chemically synthesized for use in food
and feed fortification and in small amounts as a
colouring agent in foods e.g. ice cream,
processed meat, fish products, sauces and soups.
- a very pure product could be produced using a
genetically modified strain of Bacillus subtilis.
Vitamin B2 Crystals from GM Bacillus subtilis

Microbial Biotechnology in Foods and Agriculture. Genetically modified red yeast,
(Xanthophyllomyces dendrorhous) - Produce large quantities of astaxanthin, a pigment currently
produced by chemical synthesis and used as a coloring agent by the food, pharmaceutical and
cosmetic industries.
 Microbial Biotechnology in Foods and Agriculture

* Molecular Diagnostics
* Provide outstanding tools for detection, identification and characterization of microbial
strains for bio-processing applications and for
the improvement of fermentation processes as well
as detection of spoilage microflora (microbes
causing food to become unfit for eating).
* Genetic based methods are more specific, sensitive and rapid than the classic
microbiological methods.
* Used for the detection of pathogens, pesticides
and toxins.
* Offer considerable potential for facilitating
process and fermentation control and monitoring
the quality and safety of raw materials and
by-products.

*L.10. Measurement, monitoring, modelling and control in Microbial Biotechnology*

GENETIC-BASED METHODS
Enhanced specificity, sensitivity speed
Microarray or gene chip
Polymerase chain reaction (PCR)
Each dot on this microarray chip represents one gene.
Classic diagnostic methods

* culture-based
* microorganisms grown on agar plates/tubes and
detected thru biochemical identification
* tedious, labor intensive and slow

Enzyme-Linked ImmunoSorbent Assay (ELISA)
DNA-sequencer
 Microbial Biotechnology in Foods and Agriculture

* B. Functional Genomics
* New area of research that aims to determine patterns of gene expression and interaction in
the genome, based on the knowledge of extensive or complete genomic sequence of an organism.

Genomics hold promise for advances in fields ranging from medicine and agriculture, all the
way to energy production.
From Genes to Proteins
Genomics unlock the secrets of what DNA is making which proteins.
C. Agriculture

* Production of proteins from genetically modified (GM) microorganisms improved plant and animal production and their food processing properties.

Microbial Biotechnology in Foods and Agriculture

* Genetically Modified Squash (ZW 20)
* Yellow crookneck squash (Cucurbita pepo L.) that contains the coat protein genes of watermelon
mosaic virus 2 (WMV2) and zucchini yellow mosaic
virus (ZYMV).
* Created by Agrobacterium-mediated transformation in which the transfer-DNA (T-DNA) contained the
coat protein genes from each of the two viruses.
* Demonstrates remarkable field resistance against the two viruses.
Discoloration of Yellow crookneck squash due to
mosaic virus
Yellow crookneck squash with coat protein genes of mosaic viruses
Microbial Biotechnology in Foods and Agriculture GRAPEVINE FANLEAF VIRUS (GFLV) - oldest disease of grapes)
LEAVES SYMPTOMS RANGE FROM SLIGHT CHLOROSIS (YELLOWING) AND FEATHERING OF LEAF VEINLETS TO MOTTLED LEAVES (*Пестрые листья*) WITH WIDENED SINUSES.
* Genetically Modified Grape
* Chardonnay grape variety introduced with GFLV coat protein gene through a bacterium vector.
* GM grape exhibits resistance to infection by the GFLV.
* Herbicide-tolerant plants
* Herbicide-tolerance (tolerance to weeds) of agricultural plants eliminates the environmental
risk of using the traditional chemical herbicides.
* Using rDNA technology, the genes that code for the phytotoxic compound can be identified,
isolated and modified by mutagenesis and
re-introduced into plant cultivars to confer
herbicide-tolerance.

Herbicide-tolerant sugar beet Non-Herbicide-tolerant sugar beet
Chemical industries - involve in the production of specialty chemicals such as amino acids,
enzymes, polysaccharides, vitamins, sweeteners,
food additives, flavors, fragrances etc. -
involve in the conversion of biomass into
specialty chemicals from either plants or
biological wastes generated from agriculture and
food processing.

*L. 11 Microbial Biotechnology in Chemical Industries*
A. Amino acids - building blocks of proteins in animals, plants and microorganisms. - produced
either by isolation from natural materials, from hydrolysis of plant proteins, or by chemical,
microbial or enzymatic synthesis.

Coryne bacterium efficiens
- a bacterium used commercially to produce amino
acids and other materials. Since the discovery in
the 1950s that these bacteria could produce
large amounts of glutamic acid, researchers have
genetically modified strains to increase their
yields.
**2.**  Microbial Biotechnology in Chemical Industries
B. Enzymes - biological catalysts that facilitates and speed up metabolic reaction in living organisms. –

 *industrial applications*
include production of cheese (chymotrypsin), clarification of apple juice and wine (pectinase
and protease), laundry detergents (subtilisin),
pulp and paper production (cellulase and
xylanase), treatment of sewage (lipase and
protease) - enhanced activity, specificity,
stability at unusual optimum conditions are
achieved thru recombinant DNA techniques and protein engineering.

Hard cheese sold in the UK made using an enzyme from GM yeast rather than animal rennet.
Microbial Biotechnology in Chemical Industries
C. Polysaccharide - produced by yeast, fungi and bacteria and is used in food, cosmetics,
chemical, medical and oil industries. - used as lubricants, viscosifiers, flocculating and
gelling agents in food processing and for stabilizing liquid suspensions.

***L.12****. Microbial Biotechnology in Medicine and Pharmaceutical Industries*E. coli with human insulin gene
A. Insulin - production of human insulin for the treatment of diabetes.
Drop of insulin
B. Vaccine - production of vaccines which use
only a part of a specific antigen of the
pathogenic organism, that eliminates the disease-causing capability of vaccines
Cancer-killing vaccinia virus, JX-963 (Stanford
University and Jennerex Biotherapeutics)

*Microbial Biotechnology in Medicine and Pharmaceutical Industries*C. Interferon - proteins of 2 biological
effects (1) inhibition of cellular proliferation
and (2) modulation of the immune system.
Intefen, a recombinant Human Interferon a2a exerts broad-spectrum antitumor, antiviral and immuno-regulatory activities (produced by 3SBIO)
D. DNA probes - used in identifying defects and mutations that cause diseases. - used in the
diagnosis of the bacteria that cause gum disease and variety of genetic diseases such as muscular
dystrophy, cystic fibrosis and Huntingtons disease.
fluorescent DNA probes that can attach to a specific DNA gene sequence, and detect single
nucleotide alterations (RIKEN, Japan)
Microbial Biotechnology in Medicine and
Pharmaceutical Industries
E. Gene therapy - modification of microorganisms
to increase yield or improved action of
antibiotics and other antimicrobial agents. -
useful in the treatment of hypertension, obesity,
coronary heart disease, cancer and inflammation.
Hemophilia gene therapy
*Microbial Biotechnology in the Industries*
A. Energy - production of biofuels from organic matter via biomass conversion which is a
renewable and less environmentally hazardous source of energy.

* various microorganisms also produces hydrogen, methane and bio-diesel by bacteria consuming sewage sludge in anaerobic condition.

AN IDEAL GM ORGANISM?

Break down cellulose like a bacterium, ferment sugar like a yeast, tolerate high concentrations of ethanol, and devote most of its metabolic resources to producing just ethanol. (Lind, 2006)
Colonies of recombinant *Streptomyces* bacteria are designed to produce enzymes called cellulases.
With these enzymes, the bacteria can break down cellulose on the way to producing ethanol.
*Microbial Biotechnology in the Environment*
*A. Bioremediation* - uses microorganisms to degrade waste materials into less toxic or non-toxic material in the environment - GM
bioremediation provide a safer, healthier and cleaner environment.
*S. cerevisiae*, Baker's yeast
-comes in handy for cleaning up radioactive waste because it can bind uranium to its cell walls.
*Rhodococcus sp*.
-used in bioremediation of PCBs (polychlorinated biphenyls)
 Microbial Biotechnology in the Environment
B. Waste and Wastewater management – microbial communities detoxify contaminants in water, soils, sediments and sludge.
*Azoarcus tolulyticus*
- degrades toluene, one of the most toxic components of gasoline, and is useful in cleaning up chemical spills.
A strain of *Pseudomonas stutzeri* that degrades the solvent carbon tetrachloride
*Clostridium bifermentans*
an anaerobic bacterium that degrades the explosive TNT

**Module 3. “** Animal biotechnology

**Lec. 13.** *The main directions and tasks of modern animal biotechnology. Bioethics issues in animal biotechnology.Totipotency, multipotency, pluripotency of animal cells.**Hormonal regulation of mammalian**reproduction. Sexual cycles.*

*Definition.* The application of scientific and engineering principles to the processing or production of materials by animals or aquatic species to provide goods and services (NRC 2003)

  Animals are playing a growing role in the advancement of biotechnology, as well as increasingly benefiting from biotechnology. Combining animals and biotechnology results in advances in four primary areas: 1. Advances in human health 2. Improved animal health and welfare 3. Enhancements to animal products 4. Environmental and conservation benefits

1- AnimalAnimals provide a number of products we use in every day life:Milk, Leather, Meat, Wool, Egg, Enzymes And many more e-g medicine

  Animal biotechnology includes all animals: livestock, poultry, fish, insects, companion animals and laboratory animals.

  Applications developed through research have led to the emergence of three scientific agricultural animal biotechnology sectors: 1. Animal genomics 2. Animal cloning 3. Genetic engineering of animals

  Animal genomics Genomics defines and characterizes the complete genetic makeup of an animal. By understanding the genomes of animals, we can better understand the basis for disease resistance, disease susceptibility, weight gain, and determinants of nutritional value.

*Animal cloning*. Using somatic cell nuclear transfer, livestock breeders can create an exact genetic copy of an existing animal – essentially an identical twin. Cloning does not manipulate the animal’s genetic makeup nor change an animal’s DNA: it is simply another form of sophisticated assisted reproduction.

*Transgenic animals* A transgenic animal is one which has had genetic material from another species added to its DNA. This breakthrough technology allows scientists to precisely transfer beneficial genes from one species to another.

 **Animal Biotechnology to Advance Human Health**
Animals have been used for years to produce medicines for humans. Animal-made pharmaceuticals (AMPs) transform biotech animals into “factories” to produce therapeutic proteins in their milk, eggs, and blood, which can be used in the development of biopharmaceuticals. In addition, biotechnology can be used to produce human-compatible transplant organs, tissues and cells in pigs that can be vital to enhancing human health.

 **Biotechnology to Improve Animal Health**
For decades, farmers have been improving livestock herds through enhanced animal husbandry practices and more modern technologies, such as artificial insemination, embryo transfer, in vitro fertilization, genetic mapping and cloning. Through biotechnology, farmers can enhance breeding, resulting in healthier herds. Additionally, the animal health industry has developed treatments that can prevent and treat disease. New vaccines, diagnostic tests and practices can help farmers treat animal diseases, while reducing food borne pathogens at the farm level.

 **Biotechnology to Develop More Nutritious Food**
Improved animal health conditions from vaccines, medicines and diagnostic tests result in safer foods for consumers. In addition, food quality may be improved by introducing desirable traits through new genes into farm livestock and poultry. In the future, meat, milk and egg products from animals can be nutritionally enriched with the use of biotechnology.

**Conservation of Environment and Animals**
Biotechnology can help produce environmentally friendly animals, as well as conserve endangered species. Farm animals and their feeds have been improved through biotechnology to reduce animal wastes, minimizing the impact on the environment. Today’s reproductive and cloning techniques offer the possibility of preserving the genetics of endangered species. Genetic studies of endangered animals can also result in increased genetic diversity which can result in healthier populations of species.

 **1- Animal Biotech a) Improve animals or the products they produce**
Animals may be used to produce products that promote human healthIncrease milk productivity , Example Transgenic organisms are organisms that are injected with foreign DNA from another organismCows engineered to produce human hemoglobin

 **Biotechnology can lead to new and improved animal products**
Biotechnology can lead to new and improved animal products. For example, it can modify the composition of milk, or the fat content of meat. Genetically transformed cows can produce designer milks with superior properties for use in various milk products. Added caseins in milk, for instance, can enhance cheese making. Increasing the phosphate group in casein can enhance the level of calcium. Removal of the source of lactose intolerance in milk can have a significant impact on the market for dairy products, especially among the 90 % of people with an Asian or African background who are lactose-intolerant.

 1- Animal Biotechb) Animal CloningCloning is the copying animal gene into many copies,Start with Embryo Twinning (splitting embryos in half)Advantage of cloning: preservation of endangered animals, studying the effect of drugs etc on duplicates, improve agricultural productionDolly and her surrogate mother.

**1- Animal Biotech c) Improvement animal Health.**
Animal health and well being have become increasingly important issues for animal producers and consumers.Biotechnology can improve animal health by producing genetically engineered animal that resist disease.The development of genome resources and technologies allow for identification of several host resistance genes.Aim: to prepare and present about genetic bases of disease resistance in the livestock sector

**1- Animal d) Artificial Insemination (AI) What AI?**
Artificial insemination- the transfer of collected semen to a recipient femaleSemen is collected from males of desired qualitySemen is graded and stored

**Lec. 14.** Artificial insemination, In vitro fertilization, and embryo transfer in animals.Cryopreservation of gametes and embryos.Embryoengineering.

**Animal Biotech: Creating test tube baby**
e) What is test tube baby?In vitro fertilization- fertilization of collected ova outside the reproductive tract; Usually in a test tubeSemen is collected from males of desired qualityOva are removed from femalesSperm and ova are placed in a petri dish or test tube

Animal BiotechnologyIn Vitro Fertilization: procedure in which eggs are fertilized with sperm in a dish. Resulting embryos can be used for embryo transfer or frozen (cryopreserved) for future use

 1- Animal BiotechF) Embryo transferWhat is Embryo Transfer? Embryo transfer- removing fertilized ova (embryos) from donor and implanting in a recipient. Surgical and nonsurgical methods are used to remove and implant. A quality donor female can produce more offspring

 1- Animal biotechG) What is Multiple Ovulation. Multiple ovulation- promoting increased release of ova during estrus. Hormone injections administered prior to estrus. Used with embryo transfer. AI may be used to fertilize ova After fertilization, embryos are removed and placed in recipients

 Animal Biotechnology. Cloning: creation of an organism that is genetically identical of another. Two Ways: Artificial Embryo Twinning & Somatic Cell Nuclear Transfer (SCNT)

Animal BiotechnologyStem Cell Research: process of changing undifferentiated cells into specialized cellsTwo Types of Cells Used: Embryonic and Adult

  Dolly (5 July 1996 – 14 February 2003) was a female domestic sheep, and the first mammal cloned from an adult somatic cell, using the process of nuclear transfer. She was cloned by Sir Ian Wilmut, Keith Campbell and colleagues at the Roslin Institute, part of the University of Edinburgh Scotland, and the biotechnology company PPL Therapeutics, based near Edinburgh. The funding for Dolly's cloning was provided by PPL Therapeutics and the Ministry of Agriculture.

 1996, University of Edinburgh scientists celebrated the birth of Dolly the Sheep, the first mammal to be cloned using adult somatic cells. The Edinburgh team’s success followed its improvements to the single cell nuclear transfer (SCNT) technique used in the cloning process. Dolly became a global scientific icon and SCNT technology has spread around the world and has been used to clone multiple farm animals. The cloning of livestock enables growing large quantities of the most productive, disease-resistant animals, thus providing more food and other animal products.

 **Cloning History First animal cloned was a tadpole in 1957**
First animal cloned from diploid cells was Dolly the sheep in 1996In 2002, a private company claimed to have successfully cloned the first human child

 The future of cloning preservation of endangered animals, studying the effect of drugs etc on duplicates, improve agricultural production.Limits to Cloning: The donor cell must come from a living organism. An organism is also shaped by its environment. The success rate for cloning is very low Clones may be old before their time

 **Biotechnology Techniques in Animal Breeding**
Knockout AnimalsUsed to determine the function of specific genes, by creating animals without these genes.

Knockouts are primarily used to understand the role of a specific gene or DNA region by comparing the knockout organism to a wild type with a similar genetic  background. laboratory mouse in which a gene affecting hair growth has been knocked out (left), is shown next to a normal lab mouse.

Of pharmacological proteins

**Creating A New Variety of Fish**

**Transgenic Growth-Enhanced Tilapia**

 **Transgenic Growth-Enhanced Loach**

**Objections to transgenic fish**
Unnatural and undesirable Pleiotropic effects. Novel proteins could be allergens. The fish, although not interbreeding, could be viewed as equivalent to an introduced alien species. Transgenic + wild native fish = cause ecological harm

 **3. Fuels From Algae. Renewable and no damage to the environment.**
Alternatives to fossil fuels may be photosynthetically generated biomass. Renewable and no damage to the environment.

Biomass can be converted by bacteria to fuels such as methane. Dunaliella is an alga that can produce glycerol, which can be converted by bacteria to chemicals such as ethanol and butanol, which can be used as fuels.

.Algae may also be genetically modified to make gasoline-type fuels since brown algae and cyanobacteria already make small amounts from fatty acids.

**Seaweed as Fuel Methane via anaerobic digestion Fermentation**
Highly enriched in sugars-fermented to produce bioethanol or butanol E.g Eucheuma and Kappaphycus Red-seaweed polysaccharide consists of carrageenan can be used for production of bio-ethanol

**Algal Products. a) Macroalgae (seaweed): Uses Food**
*Herbalism* – wound dressing, dental moulds, As biofertilizer Bioethanol production, Can be cultured by producing protoplast and callus tissue from which algae can be regenerated. Cell and tissue culture can be used to select for new genes or traits.Protoplast fusion allows for traits from two organisms to be mixed.

 Algal food products Onigiri and wakame misovsoup, Japan Laver and toast

 **b) *Microalgae (green algae and cyanobacteria*):**

Mostly as food, but also used as pigment sources such as β-carotene.Algae such as Spirulina and Chlorella are of much nutritional value. Spirulina is marketed today as dried flakes that are used in fish food and Japanese food. Spirulina – SCP, capsules for space researchers Phycobiliproteins are pigments involved in algal photosynthesis, and can be used as phycofluors, which can label biological molecules.

4. Anticancer Compounds.Didemnin B cyclic depsipeptide compounds isolated from a tunicate (sea-squirt) of the genus Trididemnum is effective against leukemia and melanoma in mice, and is also an effective immunosuppressive agent that could be used in organ transplants to prevent organ rejection.

Dolastatins are compounds from sea hare Dolabella auricularia are effective against leukemia and melanoma because they inhibit cell division, and may be similar to the anticancer drug vinblastine.

**Bioremediation- oil splills**
Ananda Mohan Chakrabarty, an Indian-born scientist working at GE in the 1960’s and 1970’s, developed the multi-plasmid hydrocarbon-degrading Pseudomonas and patented it in 1971.This was the first time anyone had patented a living organism.Pseudomonas putida- degrades the hydrocarbon present in oil spliis.

 The possible harm to the environment by uncontrolled growth of engineered strains is weighed against the environment. Uncontrolled growth is unlikely because the bacteria need injection of other elemental fertilizers besides the carbon in the oil to grow, it is something that has been brought up.Suicide switch- once bacterial cells they’ve eaten a certain amount of oil they kill themselves.

 **Research on the use of marine collagen in cartilage repairing techniques**

**Research on the use of collagen from marine invertebrates in wound healing and product development** Integrin

 **Transfer of an Arctic Fish Antifreeze Protein (AFP) into Strawberries to Increase Frost Resistance**
Ocean Pout

 **Why Strawberries Need Frost Resistance**
Strawberries grow in temperate climate regions which are capable of having low temperatures and frost. Spring frosts cause damage to the flowers of the plant leading to poor yields and erratic fruiting. Frost on average causes millions of dollars in damages and drives up the price of the fruit for the consumer.

**Why Strawberries Need Frost Resistance Cont.**
Frost or cold resistant plants would also help eliminate after harvest losses. .Strawberries are a soft and fragile fruit therefore they have a very short shelf life and must be stored and shipped at low temperatures.It is not uncommon for the storage and shipping temperature to get to close to freezing and harm the berries.

**Frost Prevention Practices**
Floating row covers – easy and effective but really only for small acreages, so not applicable to large scale producers.Wind machines – only provide a few degrees of protection and work the best with plants that bloom late, so not efficient enough to save a large scale berry farm from a heavy frost.

 **Frost Prevention Practices Cont.**
Sprinkler irrigation – works well for protecting strawberries but it is risky and an expensive irrigation system is required. Growers must be careful not to overwater their crops or to water during a period where evaporative cooling can take place and harm the plants. Genetically engineered plants could provide an opportunity to eliminate expensive and less efficient preventative practices.

**Arthur DeVries and Nototheniid fish**
Discovery of AFP’sAntifreeze proteins or AFP’s were discovered by Arthur DeVries and colleagues in 1969.First discovered in a Antarctic Nototheniid fish; AFP’s opened a whole field of study into cold resistance.Since the discovery of AFP’s in fish several other AFP’s have been discovered in other organisms such as bacteria, fungi, plants, insects, and vertebrates.Arthur DeVries and Nototheniid fish

 What are AFP’s?AFP’s are proteins that reduce and prevent the damage caused to an organism by freezing.These proteins are able to utilize their unique structures that hinder freezing by binding to and preventing young ice crystals from growing. (Fletcher 2001)The AFP we will focus on for this research is type III which comes from the Ocean Pout.

 Type III AFPThere are four types of AFP’s that come from fish and all have different structures, so all have different ways they bind to ice crystals.The type III AFP is made from short β-strands and one helix turn, resulting in a flat faced globular fold. (Fletcher 2001)AFP type III

 **5. Tourism GloFish/Zebra fish**
fluorescent red zebra fish sold as a novel fish, has become the first transgenic animal sold to U.S.Yorktown Technologies, Austin, Texas.expressing a red fluorescent protein from a sea anemone under the transcriptional control of the promoter from the myosin light peptide 2 gene of zebrafish

 **Important challenges does the area of Marine Biotechnology face**
BiosafetyAccess to Marine Organisms/Resources Intellectual Property Rights

**future of Marine Biotechnology as it relates to Agriculture and Industry**
Scientists in this field of Marine Biotechnology are studying the various enzymes and proteins of marine life in hopes of solving many problems that plague the area of Agriculture and Industry today. These problems include trying to find anti-corrosive coatings and "self-cleaning" surfaces for industrial use.

**Lec. 15.** Animal genes cloning.Stem cells and the perspectives of practicalapplication.

Why are cloning and stem cells shown as one topic? Because they are closely related in some ways. Both involve dealing with the progress of cells as an organism develops. A fertilized egg cell develops into a complete organism; that egg cell has the capability to replicate -- and to "differentiate" (change) into different kinds of specialized cells (e.g., heart and kidney). These specialized cells are typically unable to replicate much, if at all.

Stem cells are cells that can replicate and can turn into any of some variety of cells. Potentially, stem cells may be useful in replenishing missing or defective cell populations in an organism.

Cloning (in this context) involves growing a new organism from a single cell of an old organism. In part, this requires that the cell used for cloning be able to revert to the "primitive" state typical of an egg cell -- able to replicate and differentiate. This is particularly a challenge if the cell used for cloning is already differentiated. The common form of cloning that is discussed involves "nuclear transfer"; only the nucleus of the cell to be cloned is used, and it is transferred to an egg cell that has been deprived of its own nucleus. That same nuclear transfer procedure has been used in some procedures for making stem cells -- specifically for making embryonic stem cells.

*The word "cloning" has various meanings in biology. The general meaning is to make an identical copy of something. Some organisms, such as bacteria, normally reproduce by cloning; they get bigger, then divide in two, producing two identical daughter cells. Some plants can reproduce from pieces of an old plant -- a type of cloning. Those working with DNA refer to cloning a gene -- making many copies of it outside its normal environment. Note that the "nuclear transfer" type of cloning actually does not clone the donor cell, but only its nucleus.*

Stem cells are commonly classified two ways: by their origin, and by their potency (capability).

***Stem cell origin***. The most common terms, perhaps, have long been embryonic stem cells and adult stem cells. These terms clearly point to the origin of the cells. The term embryonic stem cells usually refers to a specific procedure for getting stem cells from a particular stage of embryonic development -- one that has been shown to work well. In contrast, the term adult stem cells is general, and encompasses a variety of types of cells. For example, hematopoietic (blood-forming) stem cells and nerve stem cells are both examples of adult stem cells. As these examples illustrate, the "origin" terms are fairly straightforward descriptors. The caution is that the term per se does not imply the characteristics, and we must always be careful to remember that our common views of them may or may not be completely correct. In particular, we should not expect various kinds of adult stem cells to behave similarly.

*Stem cell potency*. This type of term describes what the cells can do. Common terms include pluripotent, multipotent, and unipotent. These terms represent a hierarchy, from having a wide range of capabilities to having only one possible fate. Pluripotent stem cells can become most anything. Unipotent stem cells are restricted to becoming only one special type of cell. Multipotent cells are somewhere in between. As an example, hematopoietic stem cells may become any of various kinds of blood cells, but not other types of cells.

*Relationship between origin and potency*. The common view is that embryonic stem cells, from early in development, are undifferentiated, and therefore pluripotent. As development continues, cells differentiate to one or another fate, and become of lower potency. Thus adult stem cells are generally thought to have restricted potency, being either multipotent or unipotent, depending on the specific case.

*Differentiation.* The broad view in biology is that an organism starts as an undifferentiated (unspecialized) cell (the fertilized egg). As development proceeds, individual cells become progressively more differentiated (specialized). Differentiation is usually thought to be primarily unidirectional, especially in higher organisms. Dedifferentiation refers to the process of becoming less specialized; this is probably uncommon in real life, but we will see that it is an important process in stem cell work. Trans-differentiation refers to the hypothetical process in which a cell that is specialized to be one type changes to become specialized of another type. Whether trans-differentiation actually occurs, either in the animal or in the lab, is a controversial issue.

*Caution.* Stem cells terms are descriptive. Do not take them as definitive. For example, we have said above that adult stem cells have restricted potency. This fits with our general understanding of how differentiation occurs, and agrees with most of our experiences. But it would be improper to conclude that it must always be so. In fact, people are still exploring and debating the properties of adult stem cells -- in part because there are many types. As always in biology, we must take care to not get trapped in our terminology. Biological phenomena often do not classify as cleanly as we would like, or as early work might suggest.

## *Induced pluripotent stem cells (iPSC)*

The hot new kid on the stem cell block is the induced pluripotent stem cell (iPSC). To understand why this development is so exciting, we need to look at the pro and con of embryonic stem cells (ESC). The big plus of ESC is their versatility -- their pluripotency. They can become any kind of cell -- naturally in ordinary development of the embryo into an adult animal, or in the lab. The big minus is that they are hard to get. Getting ESC requires getting a young embryo or newly fertilized egg. In humans, this is technically demanding, and ethically controversial.

So what are iPSC? Briefly, they are cells with ESC capabilities (pluripotency -- the plus side of ESC), but produced without an egg or embryo (thus avoiding the minus side of ESC).

How are iPSC made? The basic idea is to take cells from an adult -- fully differentiated cells such as skin cells, grow then in the lab and treat them, to induce them to dedifferentiate to an ESC-like state.

Why did people think to try that? Because we know it works. Procedures such as the cloning that created Dolly the sheep do something like this. The nucleus of an adult cell is transferred into an unfertilized egg. The new hybrid cell develops into a new organism, a clone of the animal that donated the nucleus. This process is called somatic cell nuclear transfer (SCNT). We understand that the adult nucleus must first have dedifferentiated into an embryonic-like state. If it can happen in an egg, then maybe we can make it happen outside of an egg -- in the lab.

How is it done? And how did people figure it out? Well, the first thing they did was to examine gene expression in ESC. This gave some hints about which genes were likely to be important. Those genes were then checked more carefully. Turns out, adding about four gene products to the adult cells induces them to become ESC-like -- what we now call induced pluripotent stem cells, or iPSC. It's all fairly new, and there are various procedures that work. People are now trying to refine the procedures.

The original procedures used to make iPSC were not particularly efficient, and some aspects of the procedures were undesirable. For example, one of the genes used to induce iPSC was an oncogene -- a gene known to cause cancer. Interestingly, the initial reports from different labs used somewhat different procedures. So, despite the weaknesses, the procedure seems better than isolating ESC from embryos. Even in the few months since the initial reports of iPSC, there have been reports of work on understanding why it works, why it is inefficient, and developing improved procedures.

Are iPSC really just like ESC? That is still an open question. They seem to be quite similar. In particular, they can be made to produce many cell types, as with ESC. On the other hand, they do not seem exactly like ESC when their gene expression patterns are examined. Remember, not all ESCs are the same. It is probably best at this point to be very cautious. The development of iPSC is an exciting new development, but its potential remains to be seen.

Bottom line, are induced pluripotent stem cells the magic answer we have all been waiting for? Whoa. Patience. It is too early to know. We know only a little about them so far. As noted above, they do seem to have some key characteristics of ESC, but are not identical to ESC. The significance of the differences remains to be understood. Further, one of the early procedures for making iPSC used one gene product that may well cause cancer. Better ways to make them are needed -- and are being worked out. So, let's take this as an exciting development, a good story to follow.

Here are a few papers from the iPSC field. They are in reverse chronological order; if you want to read this group of references in historical order, start at the end of this section.

The difference between iPSC and ESC. Although iPSC show many of the key characteristics of "true" ESC, they usually show some differences, and are variable. This paper does a detailed comparison of iPSC and ESC, and shows that transcription of a particular chromosome region is key to the difference, and that this difference is due to imprinting. This would seem to open the door to understanding the iPSC process better, and also to recognizing "better" iPSC lines. A news story: Gene Silencing May Be Responsible for Induced Pluripotent Stem Cells' Limitations

Making human iPSC that cure a disease. They take skin cells from patients with a genetic defect, cure the genetic deficit, and make iPSC. They then show that these stem cells can form hematopoietic (blood forming) cells. They do not yet carry out the final step, showing that these can be used to treat the patient. Press release from the Salk Institute: Genetic Re-disposition:

Making iPSC using only one factor. A German group has shown that a single factor seems to be both necessary and sufficient for making induced pluripotent stem cells -- in one particular case. This is a good step forward both in its practical implications (simplicity, and in avoiding the oncogene factors), and in understanding. Its generality remains to be seen. A news story: Single Factor Converts Adult Stem Cells Into Embryonic-Like