

VU LEARNING HUB

vulearninghub.xyz

HIGHLIGHTED HANDOUT

BT402

Microbiology

BT402 by VU Learning HUB

Prepared for VU students • Free study resources

<https://vulearninghub.xyz>

BT 402 Fund Microbiology

Microorganisms

A **microorganism** or **microbe** is an organism which is microscopic, making it too small to be seen by the unaided human eye. The study of **microorganisms** is called microbiology. **Microorganisms** include bacteria, fungi, archaea, protists and viruses.

Evolution of Microorganisms

Microorganisms came into being on earth over a period of about 1.2 to 1.5 billion years. Fossil microbes have been found in rocks 3.3 to 3.5 billion years old. Since then, microorganisms have had the principal task of recycling organic matter in the environment. As such they are absolutely essential to the health of the earth. Without them, the earth would be a gigantic, permanent waste dump.

- **Endosymbiosis:** A condition of living within the body or cells of another organism.
- **Panspermia:** The hypothesis that microorganisms may transmit life from outer space to habitable bodies, or the process of such transmission.

Scientific evidence suggests that life began on Earth some 3.5 billion years ago. Since then, life has evolved into a wide variety of forms, which biologists have classified into a hierarchy of taxa. Some of the oldest cells on Earth are single-cell organisms called archaea and bacteria. Fossil records indicate that mounds of bacteria once covered young Earth. Some began making their own food using carbon dioxide in the atmosphere and energy they harvested from the sun. This process (called photosynthesis) produced enough oxygen to change Earth's atmosphere.

Soon afterward, new oxygen-breathing life forms came onto the scene. With a population of increasingly diverse bacterial life, the stage was set for more life to form. There is compelling evidence that mitochondria and chloroplasts were once primitive bacterial cells. This evidence is described in the endosymbiotic theory. Symbiosis occurs when two different species benefit from living and working together. When one organism actually lives inside the other it's called

endosymbiosis. The endosymbiotic theory describes how a large host cell and ingested bacteria could easily become dependent on one another for survival, resulting in a permanent relationship.

Over millions of years of evolution, mitochondria and chloroplasts have become more specialized and today they cannot live outside the cell. Mitochondria and chloroplasts have striking similarities to bacteria cells. They have their own DNA, which is separate from the DNA found in the nucleus of the cell. And both organelles use their DNA to produce many proteins and enzymes required for their function. A double membrane surrounding both mitochondria and chloroplasts is further evidence that each was ingested by a primitive host. The two organelles also reproduce like bacteria, replicating their own DNA and directing their own division.

Mitochondrial DNA (mtDNA) has a unique pattern of inheritance. It is passed down directly from mother to child, and it accumulates changes much more slowly than other types of DNA. Because of its unique characteristics, mtDNA has provided important clues about evolutionary history. For example, differences in mtDNA are examined to estimate how closely related one species is to another.

Conditions on Earth 4 billion years ago were very different than they are today. The atmosphere lacked oxygen, and an ozone layer did not yet protect Earth from harmful radiation. Heavy rain, lightning, and volcanic activity were common. Yet the earliest cells originated in this extreme environment. Extremophiles archaea still thrive in extreme habitats. Astrobiologists are now using archaea to study the origin of life on Earth and other planets. Because archaea inhabit places previously considered incompatible with life, they may provide clues that will improve our ability to detect extraterrestrial life. Interestingly, current research suggests archaea may be capable of space travel by meteorite. Such an event termed panspermia could have seeded life on Earth or elsewhere.

The presence of archaea and bacteria changed Earth dramatically. They helped establish a stable atmosphere and produced oxygen in such quantities that eventually life forms could evolve that needed oxygen. The new atmospheric conditions calmed the weather so that the extremes were less severe. Life had created the conditions for new life to be formed. This process is one of the great wonders of nature.

Types of microorganisms



Life on Earth is known for its diversity. Throughout the world we can find many millions of different forms of life. Biologic classification helps identify each form according to common properties (similarities) using a set of rules and an estimate as to how closely related it is to a common ancestor (evolutionary relationship) in a way to create an order. By learning to recognize certain patterns and classify them into specific groups, biologists are better able to understand the relationships that exist among a variety of living forms that inhabit the planet.

The first, largest, and most inclusive group under which organisms are classified is called a domain and has three subgroups: bacteria, archae, and eukarya. This first group defines whether an organism is a prokaryote or a eukaryote. The domain was proposed by the microbiologist and physicist Carl Woese in 1978 and is based on identifying similarities in ribosomal RNA sequences of microorganisms.

The second largest group is called a kingdom. Five major kingdoms have been described and include prokaryota (e.g. archaea and bacteria), protocista (e.g. protozoa and algae), fungi, plantae, and animalia. A kingdom is further split into phylum or division, class, order, family, genus, and species, which is the smallest group.

The science of classifying organisms is called taxonomy and the groups making up the classification hierarchy are called taxa. Taxonomy consists of classifying new organisms or reclassifying existing ones. Microorganisms are scientifically recognized using a standard nomenclature using two words that refer to the genus and the species. The names assigned to microorganisms are in Latin. The first letter of the genus name is always capitalised. Classification of microorganisms has been largely aided by studies on fossils and recently by DNA sequencing. Methods of classification are constantly evolving. The most widely employed methods for classifying microbes are morphological characteristics, differential staining, biochemical testing, DNA fingerprinting or DNA base composition, polymerase chain reaction, and DNA chips.

Bacteria

Bacteria are single celled microbes. The cell structure is simpler than that of other organisms as there is no nucleus or membrane bound organelles. Instead their control centre containing the genetic information is contained in a single loop of DNA. Some bacteria have an extra circle of genetic material called a plasmid. The plasmid often contains genes that give the bacterium some advantage over other bacteria. For example it may contain a gene that makes the bacterium resistant to a certain antibiotic.

Viruses

Viruses are the smallest of all the microbes. They are said to be so small that 500 million rhinoviruses (which cause the common cold) could fit on to the head of a pin. They are unique because they are only alive and able to multiply inside the cells of other living things. The cell they multiply in is called the host cell. A virus is made up of a core of genetic material, either DNA or RNA, surrounded by a protective coat called a capsid which is made up of protein. Sometimes the capsid is surrounded by an additional spiky coat called the envelope. Viruses are capable of latching onto host cells and getting inside them.

Fungi

Fungi can be single celled or very complex multicellular organisms. They are found in just about any habitat but most live on the land, mainly in soil or on plant material rather than in sea or fresh water. A group called the decomposers grow in the soil or on dead plant matter where they play an important role in the cycling of carbon and other elements. Some are parasites of plants causing diseases such as mildews, rots, scabs or canker. In crops fungal diseases can lead to significant monetary loss for the farmer. A very small number of fungi cause diseases in animals. In humans these include skin diseases such as athlete's foot, ringworm and thrush.

Protozoa

Protozoa are single celled organisms. They come in many different shapes and sizes ranging from an *Amoeba* which can change its shape to *Paramecium* with its fixed shape and complex structure. They live in a wide variety of moist habitats including fresh water, marine environments and the soil.

Algae

Algae can exist as single cells, an example of which is *Chlamydomonas*, or joined together in chains like *Spirogyra* or made up of many cells, for instance *Rhodospira* (red seaweeds).

Archaea

Archaea can be spherical, rod, spiral, lobed, rectangular or irregular in shape. An unusual flat, square-shaped species that lives in salty ponds has also been discovered. Some exist as single cells, others form filaments or clusters. Until the 1970s this group of microbes was classified as bacteria.

Usefulness of microbes

Most of the microbes are either

Necessary for life

Good for us

Can be used for our benefit in the industry

In nature: microbe-plant interaction

- Many microbes are found in nature and help plants to grow
- Rhizobacteria found in the soil fixate nitrogen which is required to grow many crops

Recycling of wastes

Many microbes are found in nature and help plants to grow

Rhizobacteria found in the soil fixate nitrogen which is required to grow many crops. Furthermore, microorganisms—the cyanobacteria or their DNA in the chloroplasts in plant cells—were the source of most of the free oxygen in the early atmosphere. They also oxidize ammonia (the universal end product of protein metabolism) to nitrate, which is the only nitrogen

source used by plants and a therefore essential for production of our plant foods

Microorganisms also are responsible for cellular hydrolysis in the rumens (first stomach compartments) of cattle, facilitating the production of animal protein for human consumption.

Oxygen production

Cyanobacteria or blue green algae produce oxygen in the ocean and this oxygen production is very necessary for aquatic life survival. Blue-green algae (cyanobacteria) are prokaryotes (that is, their cells have no distinct nucleus). They are very independent nutritionally since they can perform photosynthesis using chlorophyll a . Thus they can synthesize sugars for energy from carbon dioxide using the sun's radiation. They also release oxygen. They can respire aerobically and can fix nitrogen, generating amino acids and protein. They require only water, nitrogen gas, oxygen, carbon dioxide, some minerals, and sunlight. The evidence is that they were on earth 3.2

billion years ago. The cyanobacteria are among the earliest microorganisms and very important even today.

Decomposition

Decomposers — Defined as the breakdown of raw organic materials to a finished compost — The fungi invade the organic matter in soils first and are then followed by bacteria — Without this recycling of inorganic nutrients, primary productivity on the globe would stop.

- In food industry
- Cheese and yogurt

- Bread and dough products
- Alcohol production
- Medicine industry
- For the production of medicine

Vaccine production uses bacterial or viral antigens which may be either killed or live but attenuated. A vaccine is a mixture of dead or weakened pathogens which induces the production of antibodies against this pathogen.

- Recombinant insulin is produced either in yeast or *E. coli*. In yeast insulin may be engineered as a single-chain protein. A chemically synthesized C-terminal tail is then grafted onto insulin by inverse proteolysis using the sensitive protease trypsin, typically the lysine on the C-terminal tail is protected with a chemical protecting group to prevent proteolysis.

Uses of Microorganisms in Food

Food may be contaminated from outside sources on the way from the field to the processing plant, or during transport, storage and distribution. There are thousands of different types of micro-organisms everywhere in air, water, soil and foods, and in the digestive tract of animals and human. Fortunately, the majority of microbes usefully function in the environment and in some branches of food industry, such as production of wine, beer, dairy products, bakery products, etc. On the other hand unwanted food spoilage is generally caused by microbes and contamination of food with pathogens causes food safety problems. The micro-organisms occurring on and in foods are divided into three groups: bacteria, yeast and molds. Molds are generally concerned in the food spoilage, their use in the food industry is limited. Yeasts are the most widely used microbes in the food industry due to their ability to ferment sugars to ethanol and carbon-dioxide. Some types of yeast, like baker's yeast are grown industrially, and some may be used as protein sources, mainly as animal feed. Bacteria are important in food microbiology may be divided into groups according to the fermentation product, e.g. lactic acid bacteria, propionic acid bacteria, acetic acid bacteria. Bearing in mind the food constituent attacked, proteolytic, saccharolytic and lipolytic bacteria may be distinguished.

Microorganisms are essential for the production of foods such as cheese, yogurt, bread, beer,

5

wine and other fermented foods. Fermentation is one of the methods to preserve food and alter its quality. Yeast, especially *Saccharomyces cerevisiae*, is used to leaven bread, brew beer and make wine. Certain bacteria, including lactic acid bacteria, are used to make yogurt, cheese, hot sauce, pickles, fermented sausages and dishes. A common effect of these fermentations is that the food product is less hospitable to other microorganisms, including pathogens and spoilage-causing microorganisms, thus extending the food's shelf-life. Some cheese varieties also require molds to ripen and develop their characteristic flavors.

- Bacteria
- Yeast
- Molds
- Fungi
- Citric acid production by fungi
- Production of enzymes
- Production of vinegar
- Lactic Acid Bacteria
- Lactic Acid Bacteria
- Lactic Acid Bacteria

Finally, essential uses of microorganisms can be seen in the environment, as they play a vital role

in many of the nutrient cycles. For instance, carbon fixation during the carbon cycle by autotrophic bacteria, such as cyanobacteria, synthesise organic molecule using CO_2 from the atmosphere to be used by other organisms and release oxygen for our consumption. In addition,

microorganisms are vital participants in the food chain since they act as decomposers, breaking

down dead organisms and organic materials and releasing minerals for uptake by living organisms and CO_2 back into the atmosphere to be used by photosynthetic organisms.

Microorganisms, known as methanogens, influence the carbon cycle by converting CO_2 in their cells to methane and releasing it into atmosphere, thus increasing methane concentration whereas methanotrophs consume methane from the atmosphere, leading to a decrease in the greenhouse gas and global warming.

Involvement of microorganisms in the nitrogen cycle demonstrates that they are not just beneficial for humans, but are significant to plants as well, especially diazotrophs. Plants and diazotrophs have developed a symbiotic relationship, for example, *Rhizobium* present in the nodules on legume roots, fixes nitrogen enabling the plant to flourish in nitrogen-deficient

growth. Microorganisms are crucial for all three steps of nitrogen cycle: firstly, *Nitrosomonas*

and *Nitrosococcus* convert ammonium ions to nitrite and *Nitrobacter* convert nitrite to nitrate during nitrification; secondly, during denitrification *Pseudomonas denitrificans* reduces nitrate to nitrogen gas and thirdly nitrogen fixation, with the diazotrophs reducing nitrogen from the air into ammonia which are utilized by the plants to synthesise DNA and amino acid. Another microorganism interacting with the plants are mycorrhizal fungi, which have a symbiotic relationship with plant roots. This association is beneficial for plant as fungal hyphae increases surface area enabling the plant roots to absorb more nutrients and advantageous to fungi since they gain sugars produced by plants during photosynthesis.

Moreover, microorganisms digest harmful chemicals, such as pollutants and chemical wastes produced by the industry through a process known as bioremediation, thus protecting the environment and human health. In this process, microorganisms grow over a solid substrate to form a biofilm, through which the fluid containing the contaminants are poured through, so that the enzymes produced by the microorganisms can degrade the contaminant and the resulting fluid is non-toxic. In a similar process, microorganisms aid sewage water treatment: primary treatment, such as screening, results in sludge which is digested by anaerobic microorganisms; during secondary treatment, microorganisms enable floc formation, biodegradation and neutralization of toxins when passing over the microbial films, e.g., *Geobacter sulfur* reduces/purifies contaminated water by precipitating metals such as uranium.

Applications of microorganisms in the food industry, mainly in the production of dairy products are another example where microorganisms are beneficial to humans. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* converts lactose in milk into lactic acid causing the milk to coagulate, during fermentation (reduction and oxidation of organic molecules), and form yogurt in the process. Furthermore, probiotic yogurt with live bacteria's is also produced nowadays, to maintain the balance of microbial flora in our gut and prevent the growth of pathogens. Fermentation of milk by lactic acid bacteria also causes the milk to coagulate and form curd, then additional organisms is added to form the various cheeses, such as *Penicillium camemberti*.

to produce Camembert. Another example is the addition of *Streptococcus lactis* and *Lactococcus citrovorum* to cream during fermentation to produce lactic acid, which causes thickening of the cream, and gives rise to the flavor that is attributed to sour cream. Furthermore, microbes such as yeasts which help us in the process of bread making, alcohol production and food preservation are also part of our diet. For example, marmite and vegemite are made from "spent brewer's yeast" and are rich in vitamins. Additionally, *Fusarium graminearum*, a type of fungus has been developed into meat substitute for human consumption. The success of the food industry is partially due to the careful selection and addition of beneficial microorganisms, leading to a variety of cultured food for a wide range of people.

Understanding microbe's genetics has enabled us to use microorganisms in genetic-engineering techniques, such as gene cloning, and has given numerous benefits to the biotechnological industry. Microorganisms, such as bacteria, viruses and bacteriophages, act as cloning vectors to transfer specific sequence of gene, into the plasmids of the bacterial cell using restriction enzymes. The purpose of the restriction enzyme is to bind to the inverted palindromic in both chromosomal and vector DNA, thus cleaving the DNA and producing sticky ends. The sticky ends of both DNA are joined together by DNA ligase producing a recombinant DNA, used to transform the bacteria host cell. The bacteria can be induced to produce the protein which these genes encode as the vector is replicated and divides to produce new cells. Since the vector contains a selectable marker, such as coding for antibiotic resistant gene, the bacterial cells which has taken up the recombinant DNA can be identified. Proteins from recombinant technology can be used to produce recombinant vaccines and other vital substances, such as insulin for diabetic individual. Application of microorganisms in the medical industry is beneficial to human health, for also to the economy because huge amount of medicines produced using microorganisms, such as insulin from *E. coli*, lowers the cost of production.

Retrovirus acts as a vector in gene therapy to treat ADA deficiency; patient's ADA-deficient lymphocytes is cultured in the laboratory and infected with the retrovirus that contains the normal ADA gene, creating a recombinant retroviruses. During the replication of the retrovirus, its own DNA containing the normal ADA gene is inserted into the host's DNA causing the patient's lymphocytes to synthesize ADA.

Another application of recombinant techniques in the biotechnology industry, is the production of heat stable enzymes from thermophilic organisms that live in an extremely hot environment. For instance, DNA polymerase produced from *Thermus aquaticus* and *Thermotoccus litoralis* is used in the PCR and DNA fingerprinting, has enabled tracing of the fate of genes in plant and animal populations and improved gathering of evidence at crime scenes. Similarly, another enzyme, which breaks down lignin, obtained from *Phanerochaete chrysosporium* is used in the paper industry to bleach paper without the production of dioxin, thus preventing pollution.

Application of genetic-engineering techniques in agriculture is the production of transgenic plants, using *Agrobacterium tumefaciens* to introduce cloned genes, such as resistant to environmental stress. This is achieved by replacing the genes in T-DNA of Ti plasmid that causes the crown gall tumour with the gene of interest and a selectable marker gene, usually resistance to kanamycin. The T-DNA is then incorporated into the plant genome when exposed to *A. tumefaciens*, and these cells grown in a medium containing kanamycin and carbenicillin. Transgenic plants have led to the development of biodegradable plastics and medical products such as production of vaccines from altering transgenic plants.

Another example of benefits of microorganisms in agriculture is their use as biological pest control, for example, *Bacillus thuringiensis* produce Bt-toxin which is lethal for insects if ingested, but non-pathogenic to humans and other animals. Bt-toxin gene is genetically engineered into crops to increase their yield. Some viruses are also microbial biopesticides, bacteriophages, and they specifically target caterpillars, by releasing nucleocapsids – causes the insect to die – when ingested; therefore they are efficient pest control without huge environmental implications.

Usually microorganisms are associated with causing cancer, but recent developments have highlighted they could be used in treating cancer. William B. Coley showed that the tumours regress when cancer patients were injected with various pathogens, such as Coley's toxin. Recent research findings have revealed that *Mycobacterium bovis* can be used in treating bladder cancer, as the BCG strain in microorganism kills the tumor. Similarly, recent development has suggested that Bacteriophages bacteriophages could be used as antibiotics, since they ingest *E.coli*, *Salmonella* spp. and other disease causing gram-negative bacteria, without affecting the human.

An additional important application of microorganism in medical industry is using bacteriophage to make antibiotics for curing anthrax. Schuch and his colleagues (2002) obtained enzyme, PhG, from the bacteriophage; its function is to lyse the bacterial wall of *B. anthracis*. Their research revealed that PhG was a strong lytic enzyme, as even when applied externally they still lysed the *B. anthracis* strains, and that 80% of *B. anthracis* infected mice was saved when injected with the enzyme.

Another example of importance of microorganism in medical industry is synthesis of steroid hormones, for instance *Rhizopus nigricans*, which convert steroid compound into steroid hormones through biotransformation. Also, antibiotics are produced by the secondary metabolites of microorganisms, for example *Streptomyces griseus* produces streptomycin and penicillin is produced from *Penicillium*, when its growth is prevented due to low nutrients. (Prescott, 1999).

Human's digestive system include different species of friendly bacteria which are vital for metabolism of food, production of enzymes and vitamins for digestion (e.g., β -galactosidase, amylase); denaturation of disease-causing microorganisms and regulation of intestinal acidity. Non-pathogenic bacteria, such as *Lactobacillus* spp., form a symbiotic relationship with most multicellular organisms and is essential to the maintenance of our health, as they ensure that pathogenic bacteria is prevented from growing, aids our immune system in the process. Similarly, microorganisms exist in ruminants' digestive system break down cellulose into monosaccharides, releasing metabolic energy in the process, and the ruminant also use the microorganism as a source of amino acids and other products.

Microorganisms being used in various researches throughout history, led to the advancement of science and technology. For example, using pathogenic and non-pathogenic strains of *Streptococcus pneumoniae* by Frederick Griffith to show that there was genetic material through the process of transformation; and then Avery, MacLeod and McCarty used the same microorganisms to show that DNA was the transforming principle. Another crucial experiment was Hershey & Chase experiment using E.coli and T2 bacteriophage which showed that DNA is the hereditary material (Prescott, 1999).

Microbial mining is where acidophilic bacteria, such as *Thiobacillus ferrooxidans* are used to obtain copper from their ore. Acidic solution is showered over the copper ore, to increase

bacterial growth residing in the soil surrounding the ore. The metabolic waste produced by the bacteria, sulphuric acid, causes the copper to leach out of the rock, preventing toxic chemicals to be released to the environment and is cheaper. Although, microbial mining can only occur where the soil around the ore deposits contains the microorganisms, further research has been conducted to produce genetically engineered bacterial strains, with the ability to excavate minerals from ores containing low-levels of minerals. Microorganisms have been beneficial to humans in the past too - the Widal-Felix test for typhus. A patient infected with typhus will have antibodies to this bacterial species circulating in their blood which can bind to Proteus OX19, harmless soil bacterium. Physicians used to diagnose typhus by mixing patient's blood serum with Proteus OX19, positive test for typhus is observed when Proteus OX19 is clumped.

Overall, microorganisms are vital for life on Earth and are not just disease causing agents. 

Few microorganisms are pathogenic, but many more have an important role in various ecological and industrial processes, maintaining human health and every day new discoveries are made that shows microorganisms are crucial for scientific research to be made.

What is biotechnology?

The applications of biotechnology includes plant tissue culture, production of transgenic animals and plants, applications in medicine as tools and diagnostics, creation of new enzymes and their immobilisation for industrial use, development of monoclonal antibodies and control of pollution, etc.

Definition according to different scientists:

Biotechnology is defined as the 'application of scientific and engineering principles to the processing of material by biological agents to provide goods and services. It ranges from traditional biotechnology to the most advanced modern biotechnology. Biotechnology is not a separate science but rather a mix of disciplines (genetics, molecular biology, biochemistry,

embryology, and cell biology) converted into productive processes by linking them with such practical disciplines as chemical engineering, information technology, and robotics.

The Spinks Report (1980) defined biotechnology as 'the application of biological organisms, systems or processes to the manufacturing and service industries'.

United States Congress's Office of Technology Assessment defined biotechnology as 'any technique that used living organisms to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses'.

The key components of modern biotechnology are listed below:

(i) Genomics

The molecular characterization of all genes in a species.

(ii) Bioinformatics

The assembly of data from genomic analysis into accessible forms, involving the application of information technology to analyse and manage large data sets resulting from gene sequencing or related techniques.

(iii) Transformation

The introduction of one or more genes conferring potentially useful traits into plants, livestock, fish and tree species. Genetically improved organism, genetically modified organism (GMO), Living modified organism (LMO).

Molecular breeding

Identification and evaluation of useful traits in breeding programs by the use of marker-assisted selection (MAS).

Diagnosis

The use of molecular characterization to provide more accurate and quicker identification of pathogens.

(iv) Vaccine technology

The use of modern immunology to develop recombinant deoxyribonucleic acid (rDNA) vaccines for improved control of livestock and fish diseases.

History of Biotechnology

The history of biotechnology begins with zymotechnology, which commenced with a focus on brewing techniques for beer. By World War I, however, zymotechnology would expand to tackle larger industrial issues, and the potential of industrial fermentation gave rise to biotechnology. The oldest biotechnological processes are found in microbial fermentations, as borne out by a Babylonian tablet circa 6000 B.C., unearthed in 1881 and explaining the preparation of beer. In about 4000 B.C., leavened bread was produced with the aid of yeast. The Sumerians were able to brew as many as twenty types of beer in the third millennium B.C. In the 14th century, first vinegar manufacturing industry was established in France near Orleans. In 1680 Antony Van Leeuwenhoek first observed yeast cells with his newly designed microscope. In 1857, Louis Pasteur highlighted the lactic acid fermentation by microbes. By the end of 19th century large number of industries and group of scientists were involved in the field of biotechnology and developed large scale sewage purification system employing microbes were established in Germany and France. In 1914 to 1916, Delbruck, Heybach and Hensenberg discovered the large-scale use of yeast in food industry. In the same period, acetone, butanol and glycerol were obtained from bacteria. In 1920, Alexander Fleming discovered penicillin and large scale manufacturing of penicillin started in 1944.

Fermentation to Produce Foods

Fermentation is perhaps the most ancient biotechnological discovery. Over 10,000 years ago mankind was producing wine, beer, vinegar and bread using microorganisms, primarily yeast. Yogurt was produced by lactic acid bacteria in milk and milks were used to produce cheese. These processes are still in use today for the production of modern foods. However, the cultures that are used have been purified and often genetically refined to maintain the most desirable traits and highest quality of products.

Industrial Fermentation

In 1897, the discovery that enzymes from yeast can convert sugar to alcohol led to industrial processes for chemicals such as butanol, acetone and glycerol. Fermentation processes are still in

use today in many modern biotech organizations, often for the production of enzymes to be used in pharmaceutical processes, environmental remediation and other industrial processes.

Food Preservation:

Drying, salting and freezing foods to prevent spoilage by microorganisms were practiced long before anyone really understood why they worked or even fully knew what caused the food to spoil in the first place.

Quarantines:

The practice of quarantining to prevent the spread of disease was in place long before the origins of disease were known. However, it demonstrates early acceptance that illness could be passed from an infected individual to another healthy individual, who would then begin to have symptoms of the disease.

Selective Plant Breeding:

Crop improvement, by selecting seeds from the most successful or healthiest plants, to obtain a new crop having the most desirable traits, is a form of early crop technology. Farmers learned that using only the seeds from the best plants would eventually enhance and strengthen the desired traits in subsequent crops. In the mid-1880's, Gregor Mendel's studies on inheritable traits of peas improved our understanding of genetic inheritance and led to practices of cross-breeding (now known as hybridization). In just fifteen years progress have been made by microbiologists and genetic engineers, and we are hopeful to solve many fold problems of the present day, specially energy and food crisis to cater the need of growing population of the world. Mineral ore deposits are also becoming more scarce and expensive to recover from earth's crust. Microorganisms can be used to enhance the recovery of metals from low-grade ores and from effluents containing undesirable quantities of heavy metals or other toxins. When these technologies are applied at industrial level, they constitute bio-industry.

Scope of Biotechnology:

Genetic engineering in biotechnology stimulated hopes for both therapeutic proteins, drugs and biological organisms themselves, such as seeds, pesticides, engineered yeasts, and modified human cells for treating genetic diseases. The field of genetic engineering remains a heated topic.

of discussion in today's society with the advent of gene therapy, stem cell research, cloning, and genetically-modified food. Biotechnology is the applied science and has made advances in two major areas, viz., molecular biology and production of industrially important bio-chemical. The scientists are now diverting themselves toward biotechnological companies; this has caused the development of many biotechnological industries. In USA alone more than 225 companies have been established and successfully working, like Biogen, Cetus, Genentech, Hybritech, etc. In world, USA, Japan, and many countries of Europe are leaders in biotechnological researches encouraged by industrialists. The advances in recombinant DNA technology have occurred in parallel with the development of genetic processes and biological variations. The development of new technologies have resulted into production of large amount of biochemically-derived proteins of medical significance and created an enormous potential for pharmaceutical industries. Biotechnology in itself is a vast subject and its scope is extended to various branches of biology. This includes plant tissue culture, production of transgenic in animal and plants, applications in medicine as tools and therapeutics, creation of new enzymes and their immobilization for industrial use, development of monoclonal antibodies and control of pollution, etc.

Applications:

Industrial Applications of Biotechnology:

The industrial application of molecular biotechnology is often subdivided, so that we speak of red, green, gray or white biotechnology. This distinction relates to the use of the technology in the medical field (in human and animal medicine), agriculture, the environment and industry. Some companies also apply knowledge deriving from molecular biotechnology in areas that cut across these distinctions (e.g., in red and green biotechnology, sequencing services). According to an investigation by Ernst and Young relating to the German biotech industry, 92% of companies are currently (2004) working in the field of red biotechnology, 13% in green, and 13% in gray or white biotechnology.

Biotechnology in Medicine:

Biotechnology products for therapeutic use include a very diverse range of products, as outlined in Tables 22.4, 22.5. Some products are intended to mimic the human organism, whereas

others are intended to differ from the human counterpart and may be analogues, chemically modified (e.g., pegylated) or novel products (e.g., single chain or fragment antibody products, gene transfer vectors, tissue-engineered products). Most of these products are regulated as medicinal products; however, the regulatory status of others such as some cell therapies and tissue organ-based products differs globally and falls within the borderline between the practice of medicine, medical devices and medicinal products. Biotechnology-derived pharmaceuticals may be derived from a variety of expression systems such as *Escherichia coli*, yeast, mammalian, insect or plant cells, transgenic animals or other organisms. The expressed protein or gene may have the identical amino acid or nucleotide sequence as the human endogenous form, or may be intentionally different in sequence to confer some technical advantage such as an optimized pharmacokinetic or pharmacodynamic profile.

Red Biotechnology:

Within the field of red biotechnology, which deals with applications in human and animal medicine, there are various further distinctions that can be made: biopharmaceutical drug development, drug delivery cell and gene therapies, tissue engineering/regenerative medicine, pharmacogenomics (personalized medicine), system biology, and diagnosis using molecular medicine.

Biopharmaceutical Drug Development:

In the field of biopharmaceutical drug development, it is the development of therapeutic human proteins by recombinant methods. As mentioned above, recombinant human insulin was the first recombinant medicine in the world, produced by Genentech and brought to market in 1982. Today, recombinant human insulin has almost completely driven the other preparation of insulin (isolated from human or animal sources) from the market. The first therapeutic antibodies, especially monoclonal antibodies, have been on the market since the late 1990s.

Drug Delivery:

Closely linked to the development of therapeutic agents are the means of achieving their targeted delivery to their site of action. These drug delivery systems are mainly used for drugs whose physical and chemical characteristics make them insufficiently stable in reaching their site of

action intact. They can also be used to transport drugs in a targeted way to particular sites of action (tissue specific targeting), or to overcome biological barriers such as the intestinal wall or the blood-brain barrier.

Green Biotechnology:

Green biotechnology is the application of biotechnology processes in agriculture and food production. The main dominant forces in green biotechnology today are agro plants with a worldwide area of operation such as BASF, Bayer Crop-Science, Monsanto and Syngenta. They are concentrating considerable attention on molecular plant biotechnology, which is seen as a future growth factor in agro-industry. The traditional pesticide market, on the other hand has been stagnating for years.

Transgenic Plants:

The main emphasis in modern plant biotechnology is the production of transgenic plants. The first use of gene technology to bring about changes in plants became possible at the beginning of the 1980s, around ten years after the first experiment with bacteria. The market value of transgenic plants is estimated to be in excess of 2 billion euros, according to the calculation of the German Federal Office for the Environment. These figures relate to transgenic crop plants, which were being grown on an area totaling about 40 million hectares worldwide in 1999 and 2000.

Novel and Functional Food:

New types of foodstuffs with novel properties are often called functional food. Another category that is often mentioned in this context is nutraceuticals. These are foods that have a medicinal effect.

Livestock Breeding:

Modern biotechnology is being employed commercially to introduce novel performance features in productive livestock. The transgenic specimens then display for example different wool characteristics for sheep, or improved milk characteristics in cattle.

Grey/White Biotechnology:

The terms Grey and White Biotechnology have been coined for the application of biotechnological processes in environmental and industrial production contexts. The latter is primarily focused on the production of fine chemicals, in particular technical enzymes.

Technical Enzymes

Modern biotechnology already dominates the technical enzymes market. They can be found as proteases, lipases, cellulases and amylases for example in modern detergents, where the soot, amongst other purposes as protein and fat solubilizers.

Safety Concerns

There are a number of safety issues relating to biotechnology products that differ from those raised by low molecular weight products and need to be taken into account when designing the safety evaluation programme for a biotechnology derived pharmaceutical product. The quality and consistency of the product requires careful control in terms of product identity, potency and purity because of concerns about microbiological safety, impurities arising from the manufacturing process (e.g., host-cell contaminants, endotoxin, residual DNA levels and process chemicals), and the fidelity of the protein sequence and post-translational modifications during process improvements and scale-up.

Microbial Biotechnology

Microbial biotechnology is sometimes also referred to as industrial microbiology which is an old field that has been given new dimensions because of the discoveries made in the field of genetic engineering in vitro manipulation of DNA molecules to generate new combinations of genes or sequences, to place the gene under the control of different regulatory systems, to introduce a specific mutation in a molecule, etc.

Industrial microbiology came into existence initially by establishment of alcoholic fermentation processes to produce wine and beer. Thereafter, came the microbial production of antibiotics, and food additives such as amino acids, enzymes, butanol and citric acid. Genetic engineering has enabled us to use microorganisms for production of new substances which the microorganisms could not have produced naturally, such as the production of human insulin a pancreatic hormone which stimulates the transportation of glucose into cells. The production of

made by bacteria has been possible due to genetic engineering techniques of inserting human insulin gene into bacterium.

Microbial biotechnology can be divided under two subheadings:

- (1) Traditional microbial technology which is the large scale manufacture of products which are normally produced by microorganisms.
- (2) Microbial technology with genetically engineered microorganisms in which new genes have been inserted.

Industrial Microorganisms:

Industrial microorganisms are those microorganisms which have been selected carefully to make one or more specific products. Industrial microorganisms are selected for their metabolic activities which are capable of specific products and give high yield of particular metabolites. To achieve the desired objectives of high metabolic specialization the industrial strains are genetically modified by mutation or recombination using techniques of genetic engineering. The major metabolic pathways are either brought down or eliminated. Though the industrial strains may grow well under highly specialized artificial conditions of the fermenter they may show poor growth characteristics in natural conditions of competitive environments.

Source of Industrial Strains:

The initial and ultimate source of industrial strains has no doubt been nature but through the experience of years of large scale microbial processes perfection has been achieved for greater yield. The strains so developed have been deposited in culture collections. To get a new industrial process patented, the applicant is required to deposit a strain capable of carrying on the process in a recognized culture collection.

 Microbial biotechnology is also defined as any technological application that uses microbiological systems, microbial organisms, or derivatives thereof, to make or modify products or processes for specific use. Current agricultural and industrial practices have led to the generation of large amounts of various low-value or negative cost crude wastes, which are difficult to treat and valorize. Production of agro-industrial waste pollutants has become a major problem for many industries. The olive oil industry generates large amounts of olive mill wastes (OMWs) as by-products that are harmful to the environment. However, OMWs have simple and

complex carbohydrates that represent a possible carbon resource for fermentation processes. In addition, OMWs generally contain variable quantities of residual oil, the amount of which mainly depends on the extraction process. Therefore, OMWs could be used as substrate for the synthesis of biotechnological high-value metabolites but their utilization in this manner may help solve pollution problems. The fermentation of fatty low-value renewable carbon sources like OMWs to production of various added-value metabolites such as lipases, organic acids, microbial biopolymers and lipids, single cell oil, single cell proteins and bio surfactants is very interesting in the sector of industrial microbiology and microbial biotechnologies. But more research is needed on the development of new bioremediation technologies and strategies of OMWs, as well as the valorization by microbial biotechnology. Few investigations dealing with the development of value-added products from these low cost materials, especially OMWs have been conducted. This chapter discusses olive oil microbiology, the most significant recent advances in the various types of biological treatment of OMWs and derived added-value microbial products.

Introduction to microbial biotechnology

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 strains, or microbes that have been genetically modified using recombinant DNA methods. Deals with the prevention of deterioration of processed or manufactured goods, environmental protection and waste disposal system. Production of antibiotics, organic acids and enzymes by fermentation of microbes. Microbial biotechnology, enabled by genetic studies, will lead to breakthroughs such as improved vaccines and better disease diagnosis tools, improved microbial agents for biological control of plant and animal pests, eradication of plant and animal pathogens for reduced violence, development of new industrial catalysts and fermentation sequences, and development of new microbial agents for bioremediation of soil and water contaminated by agricultural runoff. Microbial genomics and microbial biotechnology research is critical for advances in food safety, food security, biotechnology, value-added products, human nutrition and functional foods, plant and animal protection, and furthering fundamental research in the agricultural sciences.

Microorganisms in food

Microorganisms are used in the production of fermented food and beverages. Eg. Yeast, *Penicillium*, *Lactobacillus*.

PREPARATION OF FERMENTED FOODS:

The use of microorganisms to produce fermented foods has a very long history. Microbial fermentation is essential to production of wine, beer, soybeans, mushrooms, cheeses, milk, cheese, salami, sauerkraut, and many more. The metabolic end products produced by the microorganisms flavor fermented foods. For example, mild ripened cheeses owe their distinctive flavors to the mixture of aldehydes, ketones, and short-chain fatty acids produced by the fungi. Lactic acid bacteria are widely used to produce fermented foods. These organisms are also of particular importance in the food fermentation industry because they produce peptides and proteins (bacteriocins) that inhibit the growth of undesirable organisms that cause food spoilage and the multiplication of food borne pathogens. The latter include *Clostridium botulinum* (the cause of botulism) and *Listeria monocytogenes* which produces meningococcal, meningitis, perinatal septicemia, and other disorders in humans.

Microorganisms in Medicine

They also have immense potential in the field of medicine. They are used industrially for the production of antibiotics, vaccines, insulin, growth hormones and diagnostic kits. Eg. *E. coli*, Polio virus. Microbial development in the field of medical microbiology has helped in the protection and improvement of the health of the entire population through the development of various antibiotics. It has also helped aware the peoples regarding their health condition through the public education process. High-quality health services that lead to good physical and mental health conditions are only possible through the education of public health or medical microbiology.

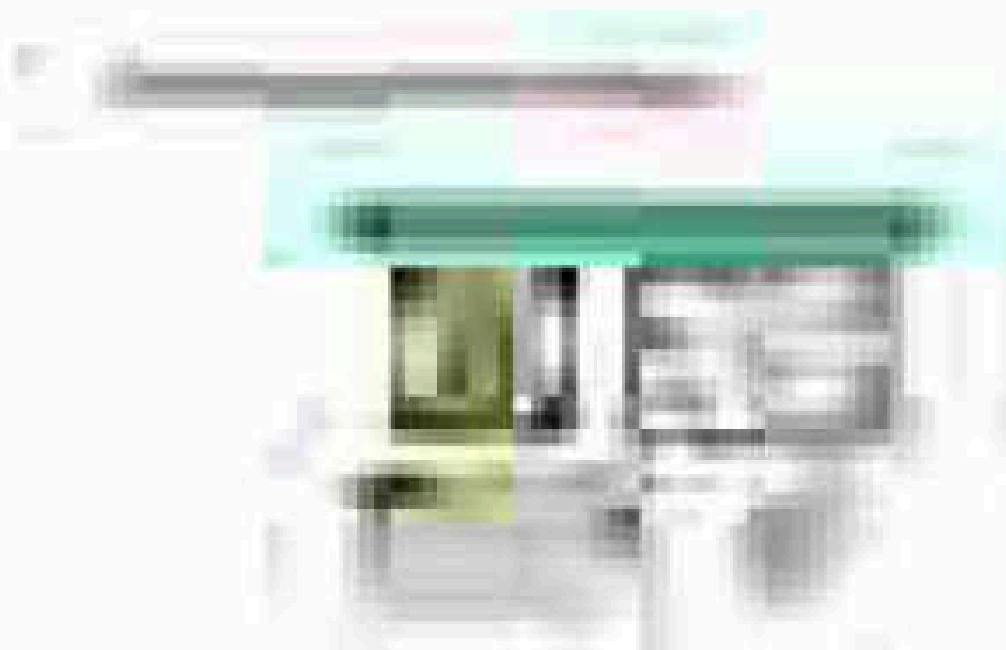
Microorganisms in Industry

Industrially important acids, enzymes, pigments are produced with the help of microorganisms. Eg. *Aspergillus niger*, *Bacillus subtilis*. Microorganisms are also known as the chemical factory to convert raw material into useful products. It may be possible to perform such reactions into large industrial scale if its criteria are followed. Organisms, medium and the products are the necessary criteria to perform such experiments. The microorganisms used in the industry are increasingly improved through genetic manipulation or through recombinant DNA in recent

years it has been found practicable to utilize nutrient containing wastes from a number of commercial operations, whey from the industry, sulfate liquor from the paper industry, and sludge

Microorganisms in Industry

Industrially important acids, enzymes, pigments are produced with the help of microorganisms. Eg. *Aspergillus niger*, *Bacillus subtilis*.



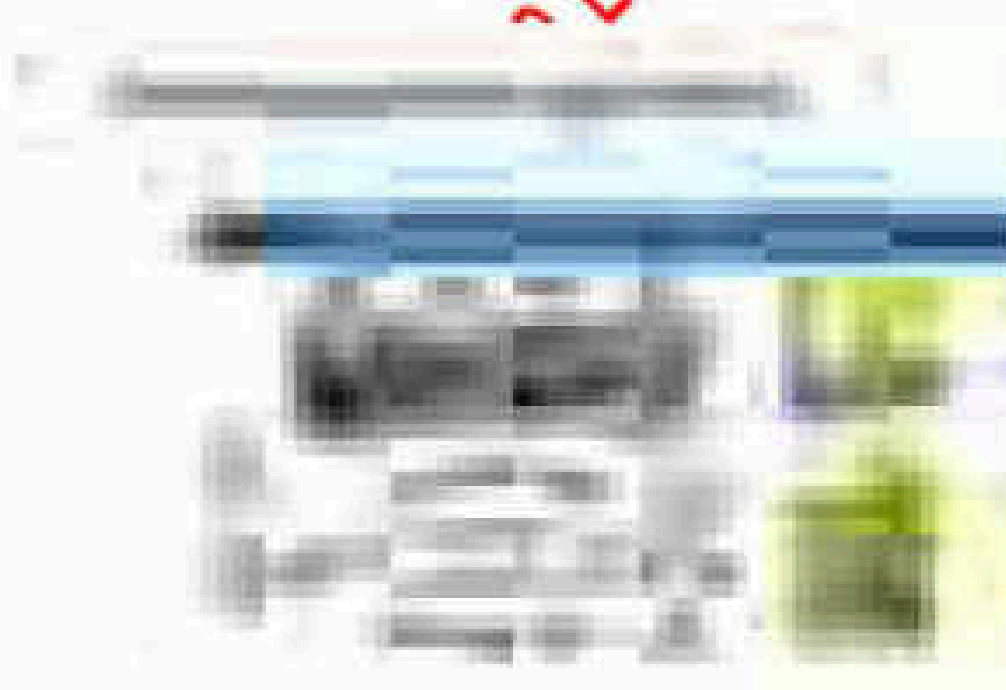
Microorganisms in Agriculture

Microorganisms have been ruling in agriculture from the past century. They are used as Biofertilizers and Biopesticides. Eg. *Rhizobium*, *Bacillus*, *Acetobacter*.

AGRICULTURE

Methods dependent on microbial biotechnology greatly increases the diversity of genes that can be incorporated into crops plants dramatically shorten the time required for the production of new varieties of plants. It is now possible to transfer foreign genes in the plant cells. Transgenic plants that are viable and fertile can be regenerated from these transformed cells, and the genes

that have been introduced into these transgenic plants are as stable as other genes in the plant nuclei and show a normal pattern of inheritance. Transgenic plants are most commonly generated by exploiting a plasmid vector carried by *Agrobacterium tumefaciens*, a bacterium. Foreign DNA carrying from one to 50 genes can be introduced into plants in this manner, with the donor DNA originating from different plant species, animal cells, or microorganisms. Higher plants have genes whose expression shows precise temporal and spatial regulation in various parts of plants – for example, leaves, floral organs, and seeds that appear at specific times in the plant development and/or at specific locations, or whose expression is regulated by environmental stimuli. Other plant genes respond to different stimuli, such as plant hormones, nutrients, loss of oxygen (anoxia/hypoxia), heat shock, and wounding. It is therefore possible to insert the control sequence(s) from such genes into transgenic plants to confine the expression of foreign genes to specific organelles or tissues and to determine the initiation and duration of such expression. Microorganisms that live on or within plants can be manipulated to control insect pests and fungal disease or to establish new symbioses, such as the one between nitrogen-fixing bacteria and plants. In bacteria and yeast, trehalose-6-phosphate is synthesized from UDP-glucose and glucose-6-phosphate in a reaction catalyzed by trehalose-6-phosphate synthase (OtsA). Trehalose-6-phosphate phosphatase (OtsB) then converts trehalose-6-phosphate to trehalose.



Microorganisms in Environment

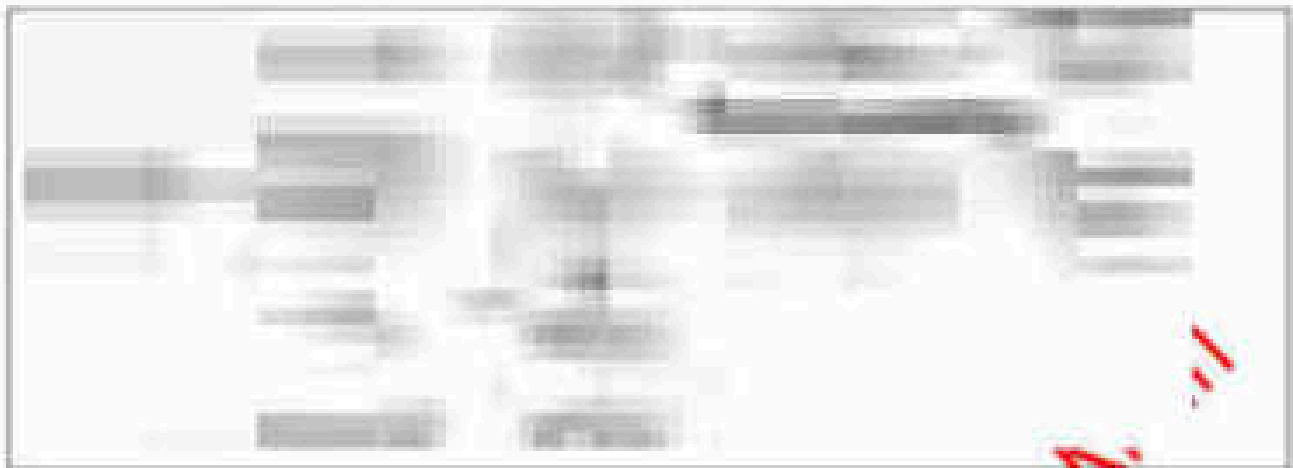
Ability of microorganisms to degrade toxic materials like oil, petroleum, plastic, etc. has opened a new field of research. E.g. *Pseudomonas*, *Acetivorus*. The biological pollution of water develops from the microorganisms that enters waste matters from various sources. Another aspect of microbiology of water pertains to natural bodies of microbes that serves as host of **biochemical transformations and are essential component of the food chain in these ecosystems.**

The microorganisms in air comes from aquatic and terrestrial environment. They may be a serious hazard to human as these must be controlled by using various methods such as UV radiation, dust control.

Issues related to microorganisms in bioprocessing

Bioprocess

Bioprocessing is used in the production of pharmaceuticals, foods, flavors, fuels and chemicals with the aid of a **biocatalyst** such as an enzyme, **microorganisms**, plant cell, or animal cell in a **bioreactor**. It also involves genetic engineering for the manipulation of plants, animals, and microorganisms such as yeasts, bacteria and fungi. **Downstream processing** is required to remove impurities, bulk-volume reduction and simultaneous concentration of the desired product from the bioreactor. Various steps deployed during **downstream processing** for recovering the final product in protein production. Protein recovery is sensitive to operating conditions because their function depends on the integrity of the delicate 3D **tertiary** structure.



Most pharmaceutical substances are manufactured in batch process by (a) chemical synthesis, (b) fermentation, (c) isolation and recovery from natural sources, and (d) a combination of the above. Fermentation broths are usually very dilute and contain many complex compounds. Because of the dilute and impure nature of the broths and sensitivity to operating conditions, MF, UF and NF are well-suited for downstream processes, i.e., separation, isolation, purification and recovery of the product. Several membrane applications in bioprocessing are including gas separation (GS). NF technology is used in the pharmaceutical and biotechnology industry to recover antibiotics from fermentation processes. A schematic flow diagram of NF separation for recovery of 6-APA, 6-Aminopenicillanic acid (6-APA, MW 216 Da) is an intermediate in the manufacture of synthetic penicillin and can be manufactured by an enzymatic process. A medium-sized plant produces 15–20 tons of product liquor per day. NF (solvent stable membrane is used) separates the 6-APA (in the retentate) from the other liquor at 60–80 kg/d and is recycled to the extracting unit, thereby minimizing product losses (recovery is 90–95%).

Bioprocessing – From Biotechnology to Biorefinery

Bioprocessing and bioproducts have gained commercial interest because of the perceived “green” advantages of using biomass rather than fossil energy for the production of chemicals and industrial products. Other key benefits in the move towards bioproducts include the sustainability of renewable biomass, replacing depleted fossil energy, and reducing greenhouse gas emissions from the present petroleum-based chemical and energy industries. Currently, only 10% of the one hundred million metric tons of fine, specialty, intermediate, and commodity

chemicals produced annually in the U.S. are bio based. Today, hydrocarbons still dominate the U.S. economy. Currently, plant biomass provides only about 7% (by weight) of organic chemical products and 3% of transportation fuels and power. However, with rising oil prices and advances in industrial biotechnology, the potential of biomass to replace petroleum-based chemicals and fuels is huge. The potential for new bioproducts with improved performance to move into new and non-conventional markets is also substantial.

Issues related to microorganisms in bioprocessing:

1. Bioprocessing

2. Issues of microorganism in bioprocessing

2.1 Issues in isolation

2.2 Issues in strain improvement

2.3 Issues of production strain

3. Conclusion

Bioprocessing

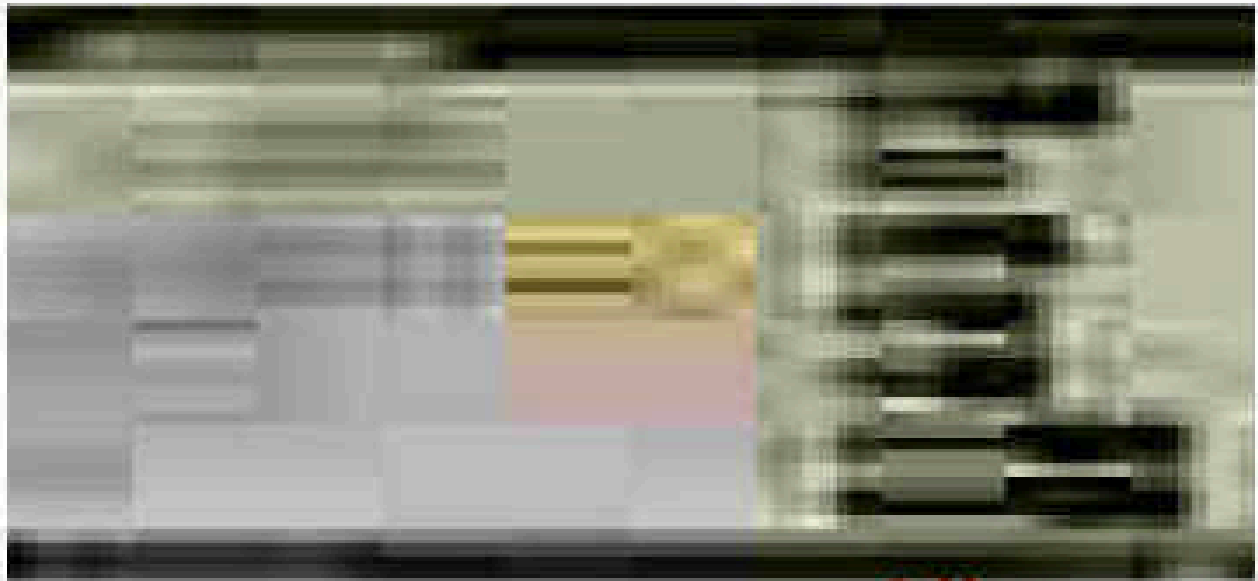
Application of natural or genetically manipulated whole cells/ tissues/ organs, or parts thereof, for the production of industrially or medically important products. It encompasses research, development, manufacturing, and commercialization of products prepared by using biological systems. There are two types of processing:

Upstream processing

Downstream processing

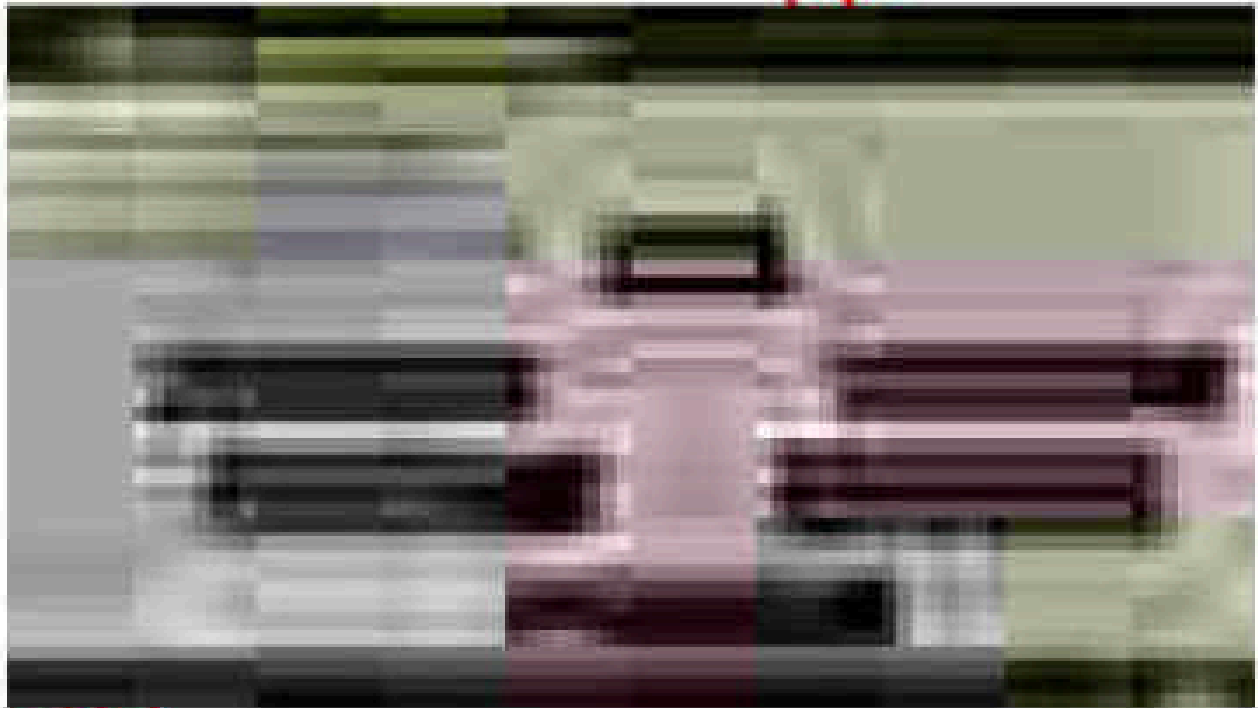
Issues of microorganisms in bioprocessing

Issues in isolation



Isle in Strain Improvement

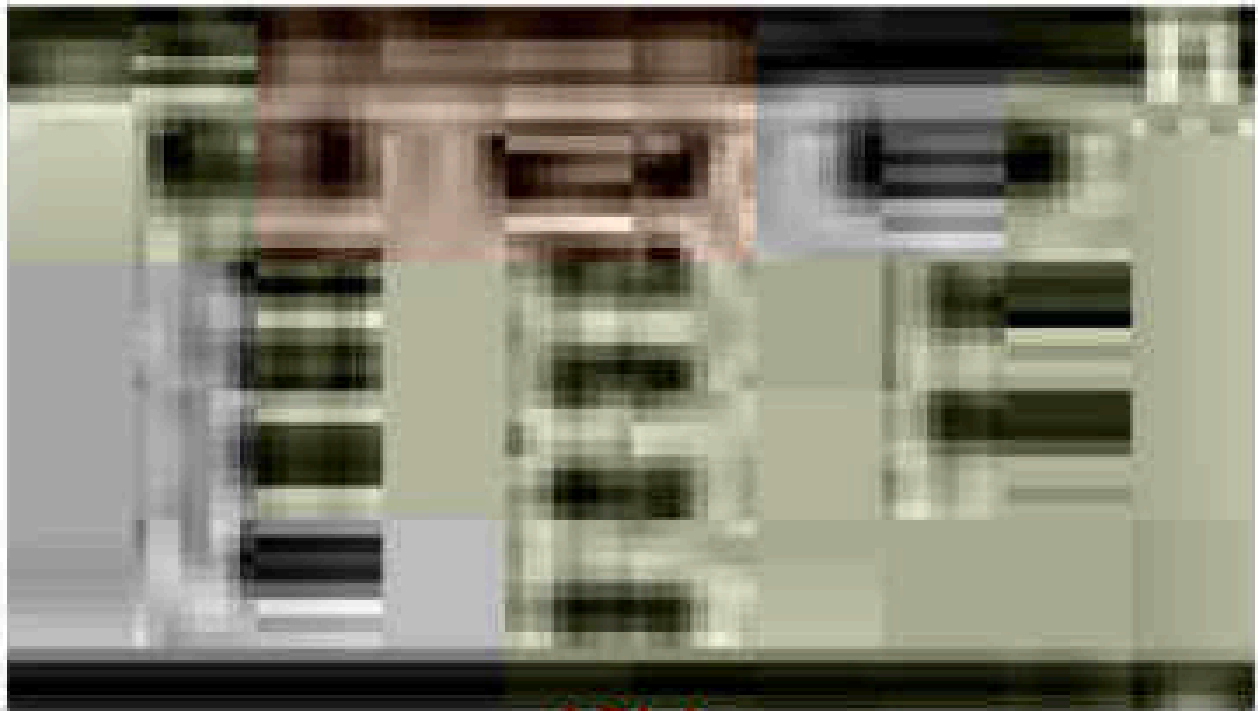
Ama



Wild type strains may have properties unique to the industry, but to fully exploit their potential, specific improvements are often required. In other cases it might be needed to reduce or eliminate an unwanted property. In addition, it can be of interest to improve strains which already have established industrial applicability. Recombinant DNA technology, due to its precision and versatility, would be an ideal technology to use to improve microbial performance.

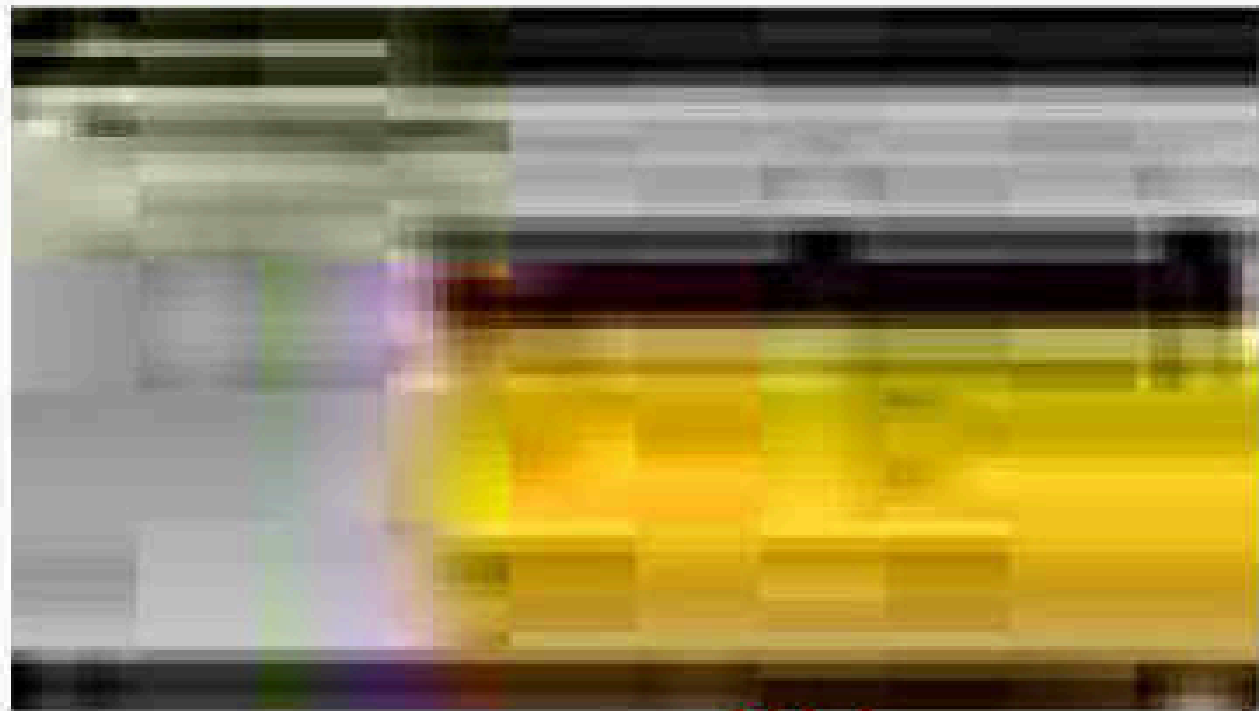
view it not for restrictive food legislation and consumer acceptance issues for genetically modified food ingredients. Thus, all efforts to improve strains for industrial application are, today, based on natural strategies for strain improvement (i.e. without the use of recombinant DNA technology) such as random mutagenesis, directed evolution and dominant selection. Random mutagenesis (classical strain improvement) has been used extensively in the food industry. This approach is based on the introduction of random mutations into the genome of interest, characterization of a large subset of variants, and selection of strains with the desired property for further use. Despite many successes, the method is generally hampered by the fact that, apart from the desired mutation, many unintended mutations which could have a negative impact on performance are introduced. Directed evolution (or adaptive evolution) is a technique in which a strain is slowly adapted to certain growth conditions reflecting an application parameter. In this case the population is enriched for strains with the desired property but, here too, there is a risk of accumulation of unintended mutations. Dominant selection is based on designing a selection scheme in which only strains with the desired property can grow. Success of such a method requires considerable insight into microbial physiology. If the selection is powerful enough, strains with single mutations can be obtained without using mutagenic agents. Furthermore, natural mechanisms like bacteriophage transduction, natural competence and conjugation can be mentioned as additional useful approaches since these are specifically excluded from the European Union's definition of recombinant DNA techniques provided that none of the strains involved are genetically modified organisms. Microbial strains overproducing commercially important metabolites are routinely obtained through mutagenesis and random screening and/or selection. Advances in recombinant DNA technology have made it possible for engineering improved microbial strains by specific addition or deletion of certain genes. The key to the genetic engineering approach, however, is the identification of genes controlling metabolic production. In recent years, innovative technologies have been developed to allow researchers to investigate the genetics and physiology of a microorganism on a global scale. Knowledge gained from these studies is beginning to modernize strain development processes. Fermentation processes must be constantly optimized in order to maximize the potential of each improved strain.

Issue in production strain



Physical factors:

Highly variable factor and important parameter of fermentation → directly affects growth rate of the microorganisms, and product composition.



Humidity

Humidity is the amount of moisture content in a media. Any change in optimal humidity will change the growth of microorganisms. Many enzymes work in aqueous environment due to change in water activity enzymes will not work effectively. It will also cause problem of dryness.

PH:

PH Based Classification of Microbes

Neutrophiles 6.5-7.5

Acidophiles 0.0-5.5

Alkaliphiles 9-11.5

Viscosity: Most important property of medium rheological or viscosity characteristics. Viscosity

is due to friction between neighboring particles in a fluid that are moving at different velocities. Estimation of viscosity of highly viscous fermentation media containing one or more solutes. It makes difficult to achieve proper and complete mixings. • Ultimately affect various mass transfer processes occurring in fermenter. • has a marked effect on many parameters and process

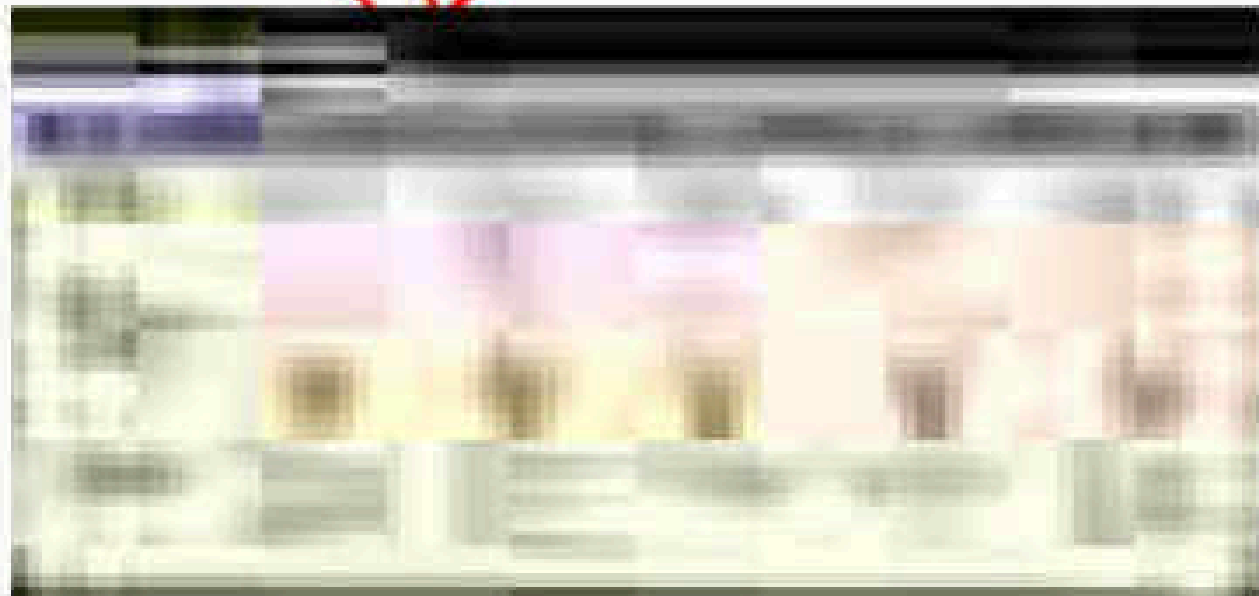
conditions within the bioreactor, often resulting in changes in microbial behavior and product formation.

Physiological Factors

- Morphology
- Metabolic by-products
- Shear Sensitivity
- Genetic Stability

Morphology: Morphology of microorganism is effected by: Mixing Aeration Pumping Media
and Carbon dioxide

- Effect of Mixing: Mixing is done for Homogenization of cultures present in a bioreactor. Maintenance of uniform concentration of microbial cells. Extensive mixing sometimes leads to whirlpool formation, causing mechanical damage to microbial cells. This ultimately alter the morphology of microbial colonies.
- Effect of aeration: Aeration is done to ensure the availability of oxygen for microbial growth. Sparger are used to achieve aeration. O_2 levels also determines morphology an organism will take. However, if cells are oxygenated rather than aerated then cell lysis takes place quickly, interfering with morphology. Oxygenated cell colonies are usually thicker.



- **Effect of Pumping:** Pumping is done for: Blending of mixtures, Achieving constant cell growth, Exchange of heat from the bioreactor. Centrifugal/Rotary Pumps. At higher pumping speed, morphological changes in microbial cell occur. At lower pumping speed, cell get trapped between walls of pump and impellers causing morphological changes. Cultivating filamentous microorganisms in a cyclone bioreactor: the influence of pumping on cell morphology.
- **Effect of Metal Ions:** Metal ions (Mn^{2+}) & chelators (EDTA) also interact with morphological patterns by Making pellets smaller and smooth rather than fuzzy i.e. *Aspergillus niger*. Altering pellet diameters. Mn^{2+} ions has alter cell wall, changing colony growth from pelleted to filamentous. This ultimately affect the biomass production after bioprocessing. Morphology.
- **Effect of CO_2 :** CO_2 is a by-product produce during aerobic respiration in fermenter. Elevated levels of CO_2 cause: intracellular pH changes, increased branching, swollen roots, hyphae, Low growth rates, Less production. This all will lead to morphological changes of microbial colonies.

Genetic stability

A measure of the resistance to change with time, of sequence of genes within a DNA molecule or of the nucleotide sequence within a gene. Issues:

- Can lose genomic DNA over the time.
- Biochemical changes to final products.
- Abnormal glycosylation.
- Abnormal gene translation modifications.

Shear stress

Shear stress: deformation of a material substance in which parallel internal surfaces slide past one another. Arises from the force vector component parallel to the cross section. -takes place by two ways

- By stirring devices.
- By fluid-mechanical stress from bursting gas bubbles.

Shear stress impact on the

- Disruption of cell membranes.
- The release of intracellular compounds.
- Alteration of aggregate size.
- Morphological variations.
- Lysis.

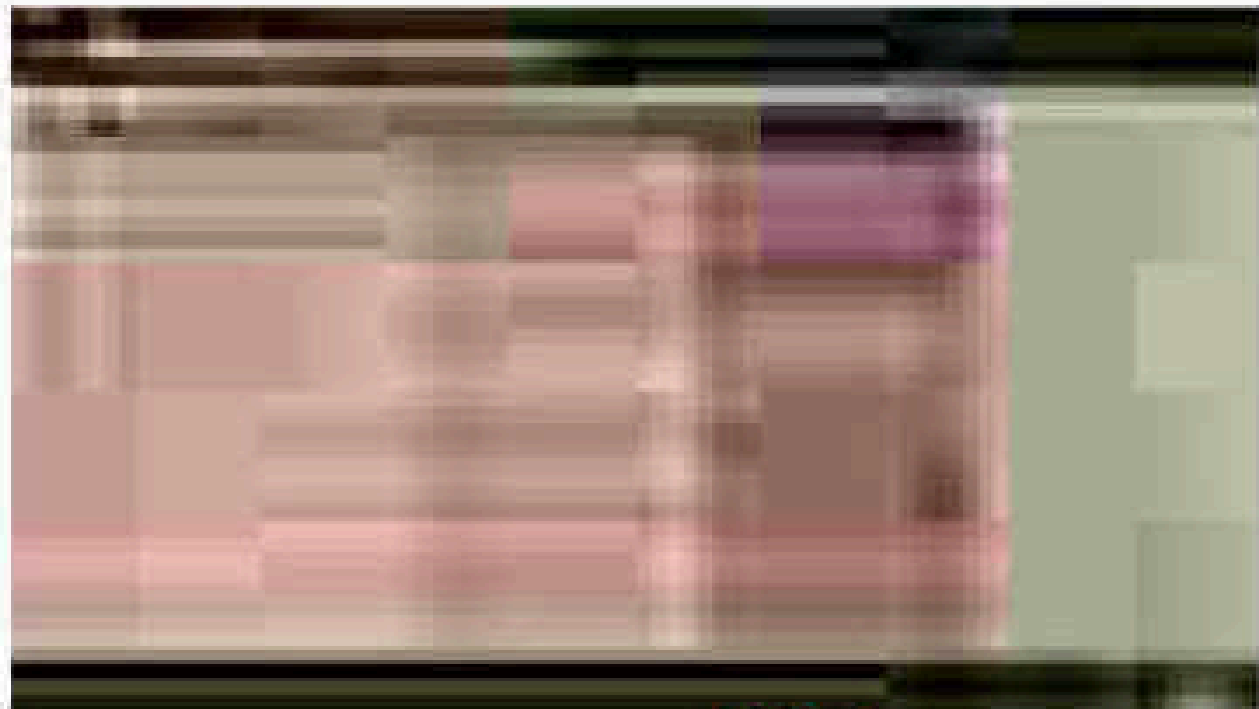
Metabolic by products

During the log phase microorganisms like yeast produce metabolites and metabolic by products as ethanol and carbon dioxide. Influenced by changing environment – maintaining redox balance and sugar concentration is crucial for the metabolic activity. — To ensure the redox

process, yeast can efficiently ferment sugar under aerobic condition. Yeast has the ability to grow at low pH but if there is high sugar content fermentation of aromatic compounds spoils beer by giving it vinegary taste. More acidic condition low level of oxygen can be overcome by increasing amount of oxygen in medium. Nutritional requirements can be determined from chemical composition of microorganisms. Obtain energy Metabolic activity New cellular component replicate grow

Nutritional factors

Issues: 1. improper nutrients will lead to the formation of abnormal molecules and hinders the growth of microorganisms; 2. The global structure of enzymes will get alter and create problem in product formation; 3. The required amount of energy will not produce to carry out fermentation. Thus, the optimization of conditions for isolation and for the biomass production of microorganisms is necessary. Any change regarding to their physiology or morphology can inhibit microorganism to produce the required product causing a lot of loss regarding time, cost and labor.



What are GMOs?

Genetically Modified Organisms (GMOs) are organisms, such as plants, animals and microorganisms whose genetic characteristics have been altered in a way that does not occur naturally by mating and/or natural recombination. Food and feed which contain, consist of or are produced from GMOs are called genetically modified (GM) food or feed.

Definition

A genetically modified organism is one whose genetic material has been whose genetic material has been altered using genetic engineering. A GMO (genetically modified organism) is the result of a laboratory process where genes from the DNA of one species are extracted and artificially forced into the genes of an unrelated plant or animal. The foreign genes may come from bacteria, viruses, insects, animals or even humans. Because this involves the transfer of genes, GMOs are also known as "transgenic" organisms. GMOs are commonly used in foods. GMOs are commonly used in foods and medicines. This has led to concern and medicines. This has led to

concern about the dangers they might cause is about the dangers they might cause to the environment, and to human health, the environment, and to human health.

How organisms are genetically modified?

Genetic engineering is the modification of an organism's phenotype by manipulating its genetic material. Some genetic engineering uses the principle of recombination. Recombination is the process through which a new gene is inserted into a bacterial DNA. "The plasmid" DNA needs to be cut with an enzyme called a restriction enzyme. The restriction enzyme used must have a specific shape that allows it to move along the DNA that is to be cut. The restriction enzyme looks for a specific point in the DNA sequence at which to cut the DNA. When the restriction enzyme cuts, it leaves a "Sticky end" which helps a new gene to attach at that point.

Another enzyme is used to attach the new DNA segment. This is called "DNA ligase".

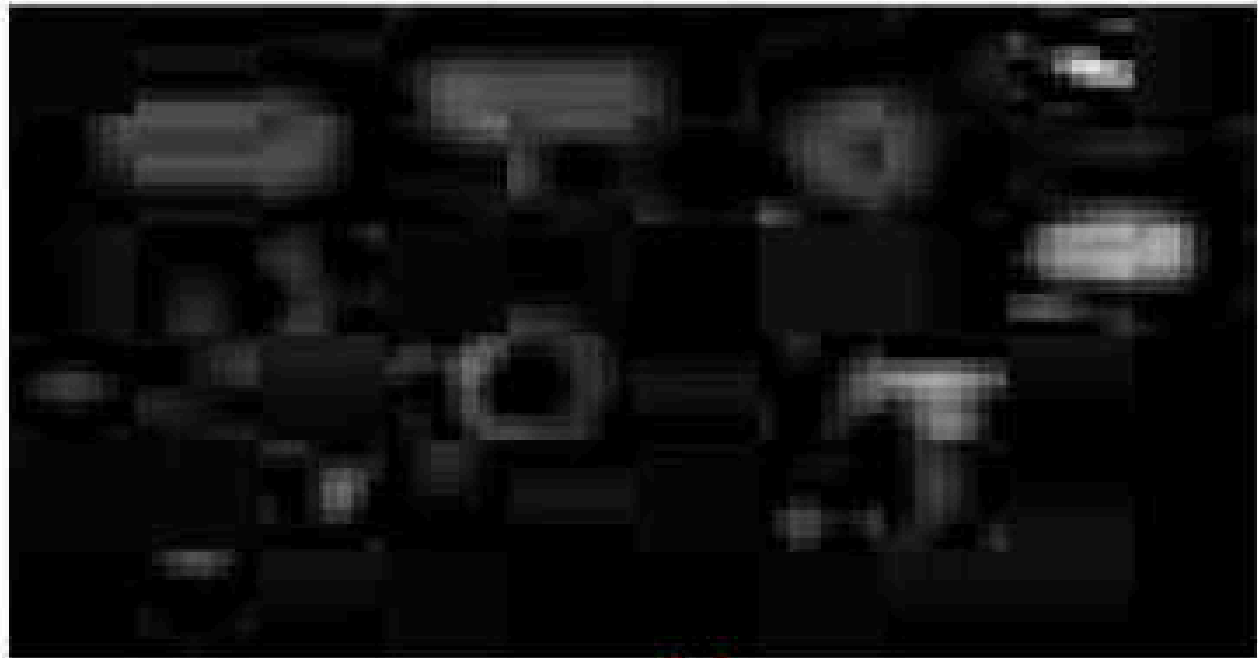
Genetically engineered bacterium is cultured and many new copies of the bacteria with the new gene are grown. Genetic modifications can be made to crops, plants, and animals.

Agrobacterium is bacteria that uses a Horizontal Gene Transfer (HGT). HGT is the transfer of DNA between different genomes (Pop. gen. genome is the complete set of genetic material present in an organism). HGT can occur in bacteria through transformation, conjugation and Transduction. However, it is also possible for HGT to occur between eukaryotes and bacteria though the mechanism for the transfer is not well understood.

Bacteria have three ways of transferring bacteria between cells:

1. **Transformation:** The uptake and incorporation of external DNA into the cell thereby resulting in the alteration of the genome.
2. **Conjugation:** The exchange of genetic material through cell-to-cell contact of two bacterial cells. A strand of plasmid DNA is transferred to the recipient cell and the donor cell then synthesizes DNA to replace the strand that was transferred to the recipient cell.
3. **Transduction:** A segment of bacterial DNA is carried from one bacterial cell to another by a bacteriophage. The bacteriophage infects a bacterial cell and takes up bacterial DNA. When this phage infects another cell, it transfers the bacterial DNA to the new cell. The bacteria can then become a part of the new host cell.

Agrobacterium also has the ability to transfer DNA between itself and plants and is therefore commonly used in genetic engineering.



Summary of process illustrated in the diagram above:

- The *agrobacterium* cell contains a bacterial chromosome and a Tumor inducing plasmid- "Ti Plasmid".
- The Ti plasmid is removed from the *agrobacterium* cell and a restriction enzyme cleaves the T-DNA restriction site.
- Next foreign DNA, which is also cleaved by the same enzyme, is inserted into the T-DNA at the site that was cleavage site.
- The recombinant plasmid is then reinserted in the *agrobacterium* and the bacterium inserts the T-DNA, which now carries a foreign gene into the plant cell.
- The plant cell is then cultured and results in a new plant that has the foreign DNA trait.

Environmental Effects

The issue of GMOs is a polarizing one. Those who are pro-GMO claim that GMOs are not only safe for us and the environment, but also potentially, a very beneficial development. Those who are anti-GMO argue that the risk of negative consequences to our environment is high and very difficult to predict. It is important to determine the magnitude of potential damage to our

environment due to the spread of GMO genes into wild plants and microbes. GM crops can cause short and long term effects on the environment. Different kinds of modifications are being bred into crops. The most common kinds of modification include: pesticide resistance, disease resistance, and drought tolerance. Other, more superficial changes can be made to crops we encounter regularly.

Some Potential Consequences to the Environment Include:

- Unintended selection
- Unintended change in gene expression
- Unintended effect on non-GM weeds, pests, or pathogens
- Survival & persistence beyond intended zone
- Production of toxic substance to non-target organisms
- "Horizontal Gene transfer "

Scientists have bred pesticide resistance into their crops, which causes controversy when pesticides, such as Roundup, are over sprayed. By using such quantities of chemicals to control weeds in the crops, the weeds begin to develop their own resistance to the pesticides thus creating 'super weeds.' This phenomenon is referred to as gene transfer. Seed dispersal and pollen transfer caused by wind, animals, and farming equipment, contribute to gene transfer.

There are many studies that evaluate the potential environmental and ecological risks of GMOs. These studies suggest that there is a heightened risk for hybridization among GMO crops. This undermines any claims scientists have over GMO crops and leads to the evolution of weed species that nullify the genetic modification (like pesticide resistance). When the weeds obtain pesticide resistance DNA from GMO crops, this defeats the purpose of modifying the crops at all. While there is some widely accepted evidence for these negative impacts against the environment and a lack of evidence proving otherwise—there are compelling statistics concerning potential benefits to the environment.

- A decreased use of pesticides & insecticides
- Reduced greenhouse gas emissions

Evidence has shown that with the introduction of GMO crops, there has been a significant decrease in the use of pesticides and insecticides. Brookes and Barfoot note a global trend toward reducing greenhouse gas emissions since GMO use has increased. They explain that pest-resistance and repellent traits that are bred into the crop's DNA lead to a reduced necessity for the use of pesticides and insecticides. This global decrease in the amount and frequency of pesticide use means fewer instances of pesticide spraying reduces the use of fuel and consequently reduced greenhouse gas emissions.

Risks and Benefits

Research studies present conflicting arguments as to whether consuming GM crops is beneficial or harmful to human health. With every alleged GMO health risk, there are counterarguments either opposing health risk claims or suggesting GMOs provide more benefits than harm. The most notable GMO risks to humans are the potential development of allergens to GM related crops and toxicity from GM crops. However, studies also show GM crops have benefits including the increased nutritional value in foods.

GMOs in Medicine and Research

GMOs have emerged as one of the mainstays of biomedical research since the 1980s. For example, GM animal models of human genetic diseases enabled researchers to test novel therapies and to explore the roles of candidate risk factors and modifiers of disease outcome. GM microbes, plants, and animals also revolutionized the production of complex pharmaceuticals by enabling the generation of safer and cheaper vaccines and therapeutics. Pharmaceutical products range from recombinant hepatitis B vaccine produced by GM baker's yeast to injectable insulin (for diabetics) produced in GM *Escherichia coli* bacteria and to factor VIII (for hemophiliacs) and tissue plasminogen activator (tPA, for heart attack or stroke patients), both of which are produced in GM mammalian cells grown in laboratory culture. Furthermore, GM plants that produce "edible vaccines" are under development. An edible vaccine is an antigenic protein that is produced in the consumable parts of a plant (e.g., fruit) and absorbed into the bloodstream when the parts are eaten. Once absorbed into the body, the protein stimulates the immune system to produce antibodies against the pathogen from which the antigen was derived.

Such vaccines could offer a safe, inexpensive, and painless way to provide vaccines, particularly in less-developed regions of the world, where the limited availability of refrigeration and sterile needles has been problematic for some traditional vaccines. Novel DNA vaccines may be useful in the struggle to prevent diseases that have proved resistant to traditional vaccination approaches, including HIV/AIDS, tuberculosis, and cancer.

Genetic modification of insects has become an important area of research, especially in the struggle to prevent parasitic diseases. For example, GM mosquitoes have been developed that express a small protein called SM1, which blocks entry of the malaria parasite, *Plasmodium*, into the mosquito's gut. This results in the disruption of the parasite's life cycle and renders the mosquito malaria-resistant. Introduction of these GM mosquitoes into the wild could help reduce transmission of the malaria parasite. In another example, male *Aedes aegypti* mosquitoes engineered with a method known as the sterile insect technique transmit a gene to their offspring that causes the offspring to die before becoming sexually mature. In field trials in a Brazil suburb, *A. aegypti* populations declined by 95 percent following the sustained release of sterile GM males.

Finally, genetic modification of humans via gene therapy is becoming a treatment option for diseases ranging from rare metabolic disorders to cancer. Coupling stem cell technology with recombinant DNA methods allows stem cells derived from a patient to be modified in the laboratory to introduce a desired gene. For example, a normal beta-globin gene may be introduced into the DNA of bone marrow-derived hematopoietic stem cells from a patient with sickle cell anemia; introduction of these GM cells into the patient could cure the disease without the need for a matched donor.



- Genetic modification involves the mutation, insertion, or deletion of genes. This can be accomplished artificially by: attaching the genes to a virus. Physically inserting the extra DNA into the nucleus of the intended host with a very small syringe using electroporation (that is, introducing DNA from one organism into the cell of another by use of an electric pulse), firing small particles from a gene gun



Step 1: Identify a trait of interest

In order to identify a desirable new trait, scientists most often look to nature. Successful discovery of a new genetic trait of interest is often a combination of critical thinking and luck. For example, if researchers are searching for a trait that would allow a crop to survive in a specific environment, they would look for organisms that naturally are able to survive in that specific environment. Or if researchers are aiming to improve the nutritional content of a crop, they would screen a list of plants that they hypothesize produce a nutrient of interest. An example of a trait currently in GMOs that was identified through this combination of luck and critical thinking is tolerance to the herbicide Roundup. Monsanto created "Roundup Ready" plants after finding bacteria growing near a Roundup factory that contained a gene that allowed them to survive in the presence of the herbicide. Although it is not on the market in the United States, Syngenta has designed Golden Rice with an increased amount of pro-vitamin A, which the human body may turn into the vitamin A. Researchers at Syngenta identified the gene sequence

that produces pro-vitamin A and compiled a list of plants to screen with that sequence. With a little luck, there was a plant in nature, maize, that contained a gene that would make Golden Rice produce pro-vitamin A at a level that could meet the nutritional needs of vitamin A deficient communities.

Step 2: Isolate the genetic trait of interest

Comparative analysis is used to decode what part of an organism's genetic makeup contains the trait of interest. The genomes of plants with the trait are compared to genomes in the same species without the trait, with the goal of identifying genes present only in the former. The genomes of different species with the same trait may also be compared in order to identify a gene, as was the case while developing Golden Rice. If there is no database of genetic information for comparison, scientists will purposefully delete, or "knock out," parts of the genome of interest until the desired trait is lost, thereby identifying the genes that lead to the trait. In order to expedite this process, Monsanto has developed and patented a method known as seed clipping. Through this method Monsanto shaves off parts of seeds for high-throughput genetic sequencing while leaving the rest of the seeds viable for planting. This creates a genetic database for plants before they are even grown, where a barcode system is used to match plants to their genotypes. Researchers may then use this database to identify new traits of interest as well as to optimize the desirable traits in a crop by selecting for the best genotypes based on plant phenotypes.

Step 3: Insert the desired genetic trait into a new genome

 Altering the genome of plant seeds is difficult due to their rigid structure. Many biotech companies use "gene guns" that shoot metal particles coated with DNA into plant tissue with a .22-caliber charge. Monsanto no longer uses gene guns, but instead takes advantage of bacteria, called *Agrobacterium tumefaciens*, that naturally invade seeds and alter plants by inserting pieces of their own DNA into a plant's genome. In biotechnology research it is common to genetically engineer bacteria to produce a desired protein. This is done by using enzymes to cut and paste a DNA strand of interest into a plasmid, which is a small

circular molecule of DNA [10]. Bacteria are then shocked using heat or electricity so that the cells accept the engineered plasmid. By modifying *A. tumefaciens*, which is easier to modify than plant seeds themselves, researchers may use the bacteria's naturally invasive behavior as a Trojan horse for inserting desirable traits into a crop's genome.

Step 4: Growing the GMO

After a genetic trait has been successfully inserted into an organism's genome, the modified organism must then be able to grow and replicate with its newly engineered genome. First, the genotype of the organisms must be checked so that researchers are only propagating organisms in which the genome was modified correctly. Biotech companies invest large sums into keeping these plants alive and reproducing once they have been successfully created. The companies use special climate-controlled growth chambers, and biologists often check on the plants by hand to make sure that they are growing as expected. During this process biotech companies will use automated machines, such as Monsanto's GenV planter, in order to track plants and calculate optimal seeding and growth conditions to create the best possible yields. GMO seeds often come with instructions on spacing and nutrition that result from these studies.

Examples of GMO plants

In agriculture, genetically engineered crops are created to possess several desirable traits, such as resistance to pests, herbicides, or harsh environmental conditions, improved product shelf life, increased nutritional value, or production of valuable goods such as drugs (pharming). • Plants, including corn, soybean, maize, and other plants have been genetically modified for use in producing fuel, known as biofuel. Genetically modified mammals are an important category of genetically modified organisms. Ralph L. Brinster and Richard Palmiter developed the techniques responsible for transgenic mice, rats, rabbits, sheep, and pigs in the early 1980s, and established many of the first transgenic models of human disease, including the first carcinoma caused by a transgene. The process of genetically engineering animals is a slow, tedious, and expensive process. However, new technologies are making genetic modifications easier and more precise. The first transgenic (genetically modified) animal was produced by injecting DNA into mouse embryos then implanting the embryos in female mice. Genetically modified animals

currently being developed can be placed into six different broad classes based on the intended purpose of the genetic modification:

- to research human diseases (for example, to develop animal models for these diseases);
- to produce industrial or consumer products (fibres for multiple uses);
- to produce products intended for human therapeutic use (pharmaceutical products or tissue for implantation);
- to enrich or enhance the animals' interactions with humans (hypo-allergenic pets);
- to enhance production or food quality traits (faster growing fish, pigs that eat less food more efficiently);
- to improve animal health (disease resistance)

Transgenic plants

Methods dependent on microbial biotechnology can increase the diversity of genes that can be incorporated into crop plants and dramatically shorten the time required for the production of new varieties of plants. It is now possible to transfer foreign genes into plant cells. Transgenic plants that are viable and fertile can be generated from these transformed cells, and the genes that have been introduced into these transgenic plants are as stable as other genes in the plant nuclei and show a normal pattern of inheritance. Transgenic plants are most commonly generated by exploiting a plasmid vector carried by *Agrobacterium tumefaciens*, a bacterium. Foreign DNA carrying from one to 20 genes can be introduced into plants in this manner, with the donor DNA originating from different plant species, animal cells, or microorganisms. Higher plants have genes whose expression shows precise temporal and spatial regulation in various parts of plants, such as roots, leaves, floral organs, and seeds that appear at specific times during plant development and/or at specific locations, or whose expression is regulated by light. Other plant genes respond to different stimuli, such as plant hormones, nutrients, lack of oxygen (anoxia), heat shock, and wounding. It is therefore possible to insert the control sequence(s) from such genes into transgenic plants to confine the expression of foreign genes to specific organelles or tissues and to determine the initiation and duration of such expression. Microorganisms that live on or within plants can be manipulated to control insect pests and fungal disease or to establish new symbioses, such as those between nitrogen-fixing bacteria and plants.

ABILITY TO GROW IN HARSH ENVIRONMENTS

Extending the habitat range for plants may be achieved by imparting traits such as cold, heat, and drought tolerance; ability to withstand high osmolarity or high salt concentrations; and resistance to iron deficiency in very alkaline soils. Tolerances toward environmental stresses are likely to be polygenic traits and as a consequence may be difficult to transfer from one kind of organism to another. However, there are some successes, as illustrated by the following example. Trehalose, a disaccharide of glucose, acts as a compatible solute that stabilizes and protects proteins and biological membranes in bacteria, fungi, and invertebrates from damage during desiccation. Except for highly desiccation-tolerant "resurrection plants," most plants do not accumulate detectable amounts of trehalose. *E. coli* genes *treA* and *treB* for trehalose biosynthesis were introduced into *indica* rice. An *mscA-stt3* fusion gene was generated so that only a single transformation event would be necessary and to achieve a higher catalytic efficiency of trehalose formation. To obtain either tissue-specific or stress-inducible expression, two different constructs were made. In one, the fusion gene, equipped with a transit peptide, was placed under the control of the promoter of the *psaA* gene encoding the small subunit of ribulose biphosphate carboxylase, to direct the gene product to the chloroplast. In the second, the gene was placed under the control of an osmotic acid-inducible promoter. Here, the OshA-OshB enzyme fusion remains in the cytosol. The constructs were introduced into rice using *Agrobacterium*-mediated gene transfer. Compared with non-transgenic rice, several independent transgenic lines showed improved plant growth under drought, salt, or low temperature stress conditions. The transgenic rice contained three- to nine-fold greater levels of trehalose than the non-transgenic rice. However, the striking finding was that the trehalose level did not exceed 1 mg/g wet weight of tissue under any conditions. Consequently, in rice, trehalose must exert its protective effect indirectly rather than primarily through affecting the bulk properties of water within the plant cells. A detailed analysis of the transgenic rice with each of the constructs showed less photo oxidative damage to photosystem II (allowing maintenance of higher capacity for photosynthesis), higher levels of soluble carbohydrate, and greater ability to control K⁺/Na⁺ balance in the roots under the stress conditions, than seen in non-transgenic rice controls. These results indicate that in rice, trehalose acts as a regulatory molecule that affects the expression of genes associated with carbon metabolism and those involved with ion uptake and possibly other processes as well. This example offers a valuable lesson. The presence of homologous genes in

widely diverged organisms that catalyze the synthesis of the same product offers no guarantee of a universal identical role for the product. Initial field trials on the transgenic rice are promising and offer the prospect of growing rice in saltier soils, or in areas where availability of water would depend on intermittent rainfall.

HERBICIDE TOLERANCE

Many otherwise effective broad-spectrum herbicides do not distinguish between weeds and crops. Crop plants can be modified to become resistant to particular herbicides. When applied to a weed-infested field of such genetically modified plants, these herbicides act as selective weed killers.

RESISTANCE TO INSECT PESTS

Certain strains of the bacterium *Bacillus thuringiensis* produce protein endotoxins that permeabilize the epithelial cells in the gut of the larvae of lepidopteran insects, moths, and butterflies. Genes encoding particular *B. thuringiensis* endotoxins have been transferred into and expressed into maize, cotton, and tomato. Infield tests, the transgenic tomato and tobacco plants were only slightly damaged by caterpillar larvae under conditions that led to total defoliation of control plants. Transgenic maize and cotton, containing *B. thuringiensis cry* genes that encode insecticidal proteins, accounted for over 26% of the global area of transgenic crops in 2003. A different approach to achieve the same end was to transfer a *B. thuringiensis* endotoxin gene into bacteria, such as *Corynebacterium xylem* subsp. *condensans*, which colonizes the interior of plants. This organism is generally found inside Bermuda grass plants but can reach population sizes in excess of 10⁸/gram of stem tissue if purposefully inoculated into other monocotyledonous species, such as corn. Recombinant *C. xylem* strains expressing the endotoxin show promise in controlling leaf- and stem-feeding lepidopteran larvae.

CONTROL OF PATHOGENIC BACTERIA, FUNGI, AND PARASITIC NEMATODES

The cell walls of many plant pests, such as insects and fungi, contain chitin (poly-*N*-acetyl glucosamine) as a major structural component. Many bacteria (e.g., species of *Serratia*, *Streptomyces*, and *Vibrio*) produce chitin-degrading enzymes (chitinases). The control of some fungal diseases by such bacteria has been correlated with the production of chitinases. Genes encoding chitinases from several different soil bacteria have been cloned into *Paraburtonia flauvirens*, an efficient colonizer of plant roots. The effectiveness of these recombinant strains in controlling fungal disease is not yet known.

BACILLUS SUBTILIS STRAINS AS BROAD-SPECTRUM MICROBIAL PESTICIDES

Selected *B. subtilis* strains are widely accepted as broad-spectrum microbial pesticides. Strains of the common soil bacterium *B. subtilis* secrete a broad array of compounds, which together display antifungal, antibacterial, and even insecticidal activities. These include two different classes of lipopeptides designated lipoic and plipostatic; a surfactant called surfactin; 2, 3-dihydroxyphenylglycine, an iron-chelating agent; and potent proteases with broad specificity. Lipoic lipopeptides consist of eight amino fatty acid and seven α -amino acids, whereas surfactin and plipostatic consist of six β -hydroxy fatty acid and seven and 10 α -amino acids, respectively.

Large amounts of a *B. subtilis* strain capable of producing this potent mixture of products can be obtained by solid-state fermentation with soybean curd residue as substrate. Under these cultivation conditions, it can produce greatly elevated levels of the lipopeptides. When the mixture of cells and metabolites obtained by the solid-state fermentation is directly applied to soil, it suppresses the growth of various plant pathogens.

RESISTANCE TO VIRAL DISEASES

Plant virus diseases are difficult to control. Research in the mid-1980s showed that transgenic tobacco expressing the coat protein (capsid) gene of tobacco mosaic virus (TMV) is resistant to TMV, and it was speculated that the resistance is the result of the interference with virus uncoating by the expressed coat protein. Similar coat protein transgene-mediated protection was reported for a number of other related plant RNA viruses, TMV, cucumber mosaic virus,

alfalfa mosaic virus, and several potato viruses. The protection is now known to be the result of RNA silencing, a cell-based sequence specific post transcriptional RNA degradation system that is programmed by the transgene-encoded RNA sequence. In Hawaii, papaya ranks as the second most important fruit crop. The crop was subject to severe damage caused by papaya ringspot virus (PRSV). The introduction in 1998 of transgenic papaya cultivars with a transgene that expressed a PRSV coat protein saved the Hawaiian papaya industry. In recent years, transgenic plants have been engineered with a variety of other sequences, encoding either viral proteins or RNAs that confer virus resistance.

NITROGEN FIXATION

Product Starting material Fermenting organisms Beer B barley and hops *Saccharomyces carlsbergensis* Cheeses Milk Various Cider Apples *Saccharomyces* spp. Kraut Cabbage Lactic acid bacteria Olives Green olives *Lactobacillus plantarum* *Pediococcus dextrinicus* Pickles Cucumbers *Lactobacillus plantarum* *Pediococcus dextrinicus* Vinegar Cider or wine *Acetobacter* spp. Whisky Corn, rye *Saccharomyces cerevisiae* Wine Grapes *Saccharomyces* spp. Yogurt Milk *Lactobacillus bulgaricus* *Streptococcus thermophilus* Leguminous plants, including important crops such as soybeans, form symbiotic associations with species of *Rhizobium*, *Bradyrhizobium*, and *Frankia* that fix atmospheric nitrogen. Free-living rhizobia are found in the soil. Natural infection of host plant by the bacteria leads to formation of root nodules within which the rhizobia proliferate. It has been a practice for almost a hundred years to add commercially produced rhizobia to soil as separate inoculants to reduce the need for nitrogenous fertilizers. No adverse effects of such applications have been observed. Consequently, no adverse consequences should attend large-scale applications of genetically engineered strains of rhizobia. Strains of *Bradyrhizobium japonicum* and *Rhizobium meliloti*, engineered to increase the expression of certain genes important to nitrogen fixation, and were shown to give greater biomass increases of their respective host plants under greenhouse conditions compared with the wild-type bacterial strains. Because of the very high population of free-living rhizobia in the soil, newly introduced strains have to be introduced at very high concentrations to overcome competition from the resident bacteria. This leads to high inoculant cost. Studies of the mechanism of infection and of the biochemical determinants of *Rhizobium* competitiveness may reveal ways of resolving this difficulty. Transfer of the genes for nodule formation to *Agrobacterium* enables the recombinant

organisms to initiate nodulation on non-legumes, which suggests that it may be possible to extend nitrogen fixation to non-leguminous plants. This goal will require manipulation of the host plant as well as of the bacterial genes. Exploitation of microbial biotechnology in agriculture is driven by the realization that agricultural practices that rely heavily on expensive nitrogenous fertilizers and widespread use of pesticides are no longer sustainable.

Transgenic animal

A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. The foreign gene is constructed using recombinant DNA technology. In addition to the gene itself, the DNA usually includes other sequences to enable it

- to be incorporated into the DNA of the host and
- to be expressed correctly by the cells of the host.

Transgenic sheep and goats have been produced that express foreign proteins in their milk.

Transgenic chickens are now able to synthesize human proteins in the "white" of their eggs.

These animals should eventually prove to be valuable sources of proteins for human therapy.

In July 2000, researchers from the team that produced Dolly reported success in producing transgenic lambs in which the foreign gene had been inserted at a specific site in the genome and functioned well.

Transgenic model animals provided the tools for exploring many biological questions.

An example:

Normal mice cannot be infected with polio virus. They lack the cell-surface molecule that, in humans, serves as the receptor for the virus. So normal mice cannot serve as an inexpensive, easily-manipulated model for studying the disease. However, transgenic mice expressing the human gene for the polio virus receptor

- can be infected by polio virus and even

- Develop paralysis and other pathological changes characteristic of the disease in humans.

Two methods of producing transgenic mice are widely used:

- **transferring embryonic stem cells** (ES cells) growing in tissue culture with the desired **DNA**.
- Injecting the desired gene into the **pronucleus** of a fertilized mouse egg.

The Embryonic Stem Cell Method (Method 1)

Embryonic stem cells (ES cells) are harvested from the **inner cell mass (ICM)** of mouse blastocysts. They can be grown in culture and retain their full potential to produce all the cells of the mouse animal, **including its gametes**.



1. Make your DNA

Using recombinant DNA methods, build molecules of DNA containing

- the gene you desire (e.g., the insulin gene).

- **vector** DNA to enable the molecules to be inserted into host DNA molecules.
- **Promoter and enhancer sequences** to enable the gene to be expressed by host cells.

2. Transform ES cells in culture

Expose the cultured cells to the DNA so that some will incorporate it.

3. Select for successfully transformed cells

4. Inject these cells into the inner cell mass (ICM) of mouse blastocysts.

5. Embryo transfer

- Prepare a **pseudopregnant** mouse (by mating female mouse with a **vasectomized** male). The stimulus of mating elicits the hormonal changes needed to make her uterus receptive.
- Transfer the embryos into her uterus.
- Hope that they **implant** successfully and develop into healthy pups (no more than one-third will).

6. Test her offspring

- Remove a small piece of tissue from the tail and examine its DNA for the desired gene. No more than 10-20% will have it, and they will be heterozygous for the gene.

7. Establish a transgenic strain

- Mate two heterozygous mice and screen their offspring for the 1 in 4 that will be **homozygous** for the transgene.
- Mating these will found the transgenic strain.

The Pronuclear Method (Method "2")

1. Prepare your DNA as in Method 1

2. Transform fertilized eggs

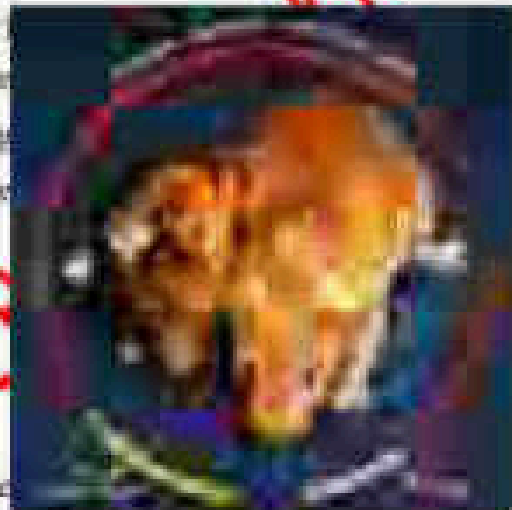
- Harvest freshly fertilized eggs before the sperm head has become a pronucleus.
- Inject the male pronucleus with your DNA.
- When the pronuclei have fused to form the diploid zygote nucleus, allow the zygote to divide by mitosis to form a 2-cell embryo.

3. Implant the embryos in a pseudopregnant foster mother and proceed as in Method 1.

An Example

This image (courtesy of R. L. Brinster and R. E. Hammer) shows a transgenic mouse (right) with a normal littermate (left). The giant mouse developed from a fertilized egg transformed with a recombinant DNA molecule containing:

- the gene for [human growth hormone](#)
- a strong mouse gene [promoter](#)



The levels of growth hormone in the serum of some of the transgenic mice were several hundred times higher than in control mice.

Transgenic Sheep and Goats

Until recently, the transgene introduced into sheep inserted randomly in the genome and often worked poorly. However, in July 2000, success at inserting a transgene into a specific gene locus was reported. The gene was the human gene for [alpha₁-antitrypsin](#), and two of the animals expressed high quantities of the human protein in their milk.

Then it was done,

Sheep fibroblasts ([correction: tissue](#) cells) growing in tissue culture were treated with a [vector](#) that contained these segments of DNA:

1. 2 regions homologous to the sheep [COL1A1](#) gene. This gene encodes Type I collagen. (Its absence in human causes the inherited disease [osteogenesis imperfecta](#).)

This locus was chosen because fibroblasts secrete large amounts of collagen and thus one would expect the gene to be easily accessible in the chromatin.

2. A aminoglycoside-resistance gene to aid in isolating those cells that successfully incorporated the vector.

3. The human gene encoding alpha₁-antitrypsin.

Some people inherit two non- or poorly-functioning genes for the protein, resulting in low level or absence produces the disease **Alpha₁-Antitrypsin Deficiency (A1AD or Alpha₁)**. The main symptoms are damage to the lungs (and sometimes to the liver).

4. Enhancer sites from the beta-lactoglobulin gene. These promote hormone-driven gene expression in milk-producing cells.

5. Boiling sites for ribosomes for efficient translation of the beta-lactoglobulin mRNA.

Successfully-transformed cells were then

- fused with enucleated sheep eggs and
- implanted in the uterus of a ewe (female sheep).
- Several embryos survived until their birth, and two young lambs lived over a year.
- When treated with hormones, these two lambs secreted milk containing large amounts of alpha₁-antitrypsin (200 µg/ml, 50 times higher than previous results using random insertion of the transgenes).

On June 10, 1993, the company doing this work abandoned it because of the great expense of building a facility for purifying the protein from sheep's milk. Purification is important because even when 99.9% pure, human patients can develop antibodies against the tiny amounts of sheep proteins that remain.

However, another company, GTC Biotherapeutics, has persevered and in June of 2006 won preliminary approval to market a human protein, antithrombin, in Europe. Their protein — the first made in a transgenic animal to receive regulatory approval for human therapy — was secreted in the milk of transgenic goats.

WHAT ARE THE ADVANTAGES OF GMOs

1. HELPING REDUCE GREENHOUSE GAS EMISSIONS

As herbicide-resistant corn and soybeans were introduced in the U.S.—conservation tillage practices become more widely adopted. GM or genetically modified crops with herbicide-resistant traits can allow farmers to manage weeds without relying on the traditional method of tilling fields. Generally, less tillage results in lower tractor-related emissions.

2. PROTECTING THE FOOD SUPPLY

Insects cause billions in crop damage every year. Using bacteria found in soils around the world, GM corn can fight harmful pests like the corn borer or bollworm. According to the USDA, when GM corn is planted, even nearby farms benefit. This genetically modified crop controls insect pest populations so effectively, that neighboring fields experience less pressure from corn borers.

3. DEFENDING PLANTS FROM DISEASE

In the 1990s, papaya farming in Hawai was on the verge of extinction. A rampant outbreak of the ringspot virus had engulfed the island. In response, the local government and universities used genetic modification to impart viral resistance to local papayas. Today, this remains the only effective method of preventing this devastating virus. This success story also offers hope for addressing pathogens affecting potatoes, citrus trees, and cassava.

4. MAINTAINING BIODIVERSITY

GM crops, paired with advanced breeding techniques, can help increase productivity without expanding the footprint of a farm. In 2015, had GM crops not been available, more than 48 million additional acres would have been needed to meet the demand for corn, soy, cotton,

and canola. In total, this land mass would roughly be the size of New York state. By helping reduce the need to expand farmland, GM crops can help preserve native habitat.

SPREADING PROSPERITY TO GROWING NATION

Since the introduction of herbicide-resistant maize, farmers in South Africa have experienced notable gains in income and efficiency. GM crops have helped improve yields and allowed growers (on smallholder farms in particular) to adopt less labor intensive practices—like hand weeding. If this trend continues, GM crops will help create more economic security for farmers in developing nations.

Sketching our Environmental Footprint

GMOs enable farmers to be better stewards of the environment, allowing farmers large and small to grow more crops on less land while using fewer pesticides and less water.

Lowering the Price of Food

- Because they require fewer pesticides, land and water, GMOs help keep food production costs down resulting in lower prices for consumers. GM technology helps reduce the price of crops used for food, such as corn, soybeans and sugar beets by as much as 15–30%.

Alleviating Hunger and Malnutrition around the World

- GMOs have helped feed more than 300 million Americans and a global population of 7 billion – of which 1 in 8 suffer from hunger and malnutrition

In General

- Reduce use of pesticide and other Toxic chemicals.
- Desired characteristics of food are achieved and in a shorter time.
Improves nutritional value.
- Many people rely on GM food for medicines.
- Gene technology is the best solution to the problem of world hunger.
- GMOs is an effective way to provide farmers a larger profit while spending less time & resources.
- Economically beneficial because they are used to repel pests, which prevents the need for pesticides to be used.
- This is also known to decrease food prices due to enhanced crops and lower cost. GMOs will certainly help families that cannot afford to buy the food they need for everyday living.
- Less starvation in the world due to the fact that it cuts food prices.
- It is more nourishing to the body, which is proven to be effective.
- The precise evaluation and testing of GMOs crops and other products. In fact, according to research, it is safer as compared to traditional crops.

Disadvantages of GMOs

1. GMO crops can contaminate other fields.

The crops may be genetically modified, but they still grow the same way as any other crop. That means pollination is required for the crop to produce the "fruit" that is being grown. Bees do much of the pollination work, which means they are exposed to the genetic changes of the plant. Seeds are produced by these GMO crops as well in many instances, which can be spread to other

fields and contaminate them. If cross-pollination occurs, there is no predictable outcome for both fields, though soybeans are an exception since they don't cross-pollinate.

2. Animal proteins could be affected by GMO crops.

The vast majority of the core crops in the US that are grown are GMO crops. These crops are then fed to livestock, aquaculture, and apiculture, which impacts groceries that are based on animal protein. GMO ingredients can be found in milk, seafood, eggs, and animal muscle tissues. Even honey can have GMO ingredients when bees are pollinating genetically modified crops to produce it.

3. Genetic Contamination/Introbreeding

Introduced GMOs may interbreed with the wild-type or closely compatible relatives. The novel trait may disappear in wild types unless it confers a selective advantage to the recipient. However, tolerance abilities of wild types may also develop, thus altering the native species' ecological relationship and behaviour.

4. Competition with Natural Species

Faster growth of GMOs can enable them to have a competitive advantage over the native organisms. This may allow them to become invasive, to spread into new habitats, and cause ecological and economic damage.

5. Increased Selection Pressure on Target and Nontarget Organisms

Pressure may increase on target and nontarget species to adapt to the introduced changes as if to a geological change or a natural selection pressure causing them to evolve distinct resistant populations.

6. Ecosystem Impacts

The effects of changes in a single species may extend well beyond to the ecosystem. Single impacts are always joined by the risk of ecosystem damage and destruction.

7. Impossibility of Followup

Once the GMOs have been introduced into the environment and some problems arise, it is impossible to eliminate them. Many of these risks are identical to those incurred with plans to the introduction of naturally or conventionally bred species. But still this does not suggest that GMOs are safe or beneficial, nor that they should be less scrutinized.

8. Horizontal Transfer of Recombinant Genes to Other Microorganisms

One risk of particular concern relating to GMOs is the risk of horizontal gene transfer (HGT). HGT is the acquisition of foreign genes (via transformation, transduction, and conjugation) by organisms in a variety of environmental situations. It occurs especially in response to changing environments and provides organisms, especially prokaryotes, with access to genes other than those that can be inherited. HGT of an introduced gene from a GMO may confer a novel trait in another organism which could be a source of potential harm to the health of people or the environment.

Some of the important potential impacts of HGT from GMOs include the following

Adverse Effects on the Health of People or the Environment

These include enhanced pathogenicity, emergence of a new disease, pest or weed, increased disease burden if the recipient organism is a pathogenic microorganism or virus, increased weed or pest burden if the recipient organism is a plant or invertebrate, and adverse effects on species, communities, or ecosystems.

Unpredictable and Unintended Effects

HGT may transfer the introduced genes from a GMO to potential pests or pathogens and many yet to be identified organisms. This may alter the ecological niche or ecological potential of the recipient organism and even bring about unexpected changes in structure or function. Furthermore, the gene transferred may insert at variable sites of the recipient gene, not only introducing a novel gene but also disrupting an endogenous gene, causing unpredictable and unintended effects.

Loss of Management Control Measures

Regulatory approvals for field trials of GMOs often require measures to limit and control the release in space and time. With the spread of the introduced gene(s) to another species by HGT, a new GMO is created. This new GMO may give rise to adverse effects which are not controlled by management measures imposed by the original license or permit.

Long-Term Effects

Sometimes the impact of HGT may be quite severe in the long term. Even under relatively strong selection pressure, it may take thousands of generations for a recipient organism to become the dominant form in the population [23]. In addition, other factors such as timing of appropriate biotic or abiotic environmental conditions and additional changes in the recipient organism could delay adverse effects.

Ethical Concerns

Various ethical issues associated with HGT from GMOs have been raised including perceived threats to the integrity and intrinsic value of the organisms involved, to the concept of natural order and integrity of species, and to the integrity of the ecosystem in which the genetically modified organism occurs.

GMOs could be dangerous to some insects.

1. This is because the new genes of the crops can be deadly to certain insects. But sometimes that are not actually dangerous to crops.
2. The people who oppose GMOs aren't sure 'good' as compared to 'naturally produced' crops.
3. It doesn't need enough pesticide, because the crop itself is dangerous to some insects.
4. The tariff, quota and trade issues may become a problem in Europe and other countries.
5. Critics claim that GMOs can cause particular disease or illnesses.
6. As the major producer of GMOs, it could start an issue in some countries. They are not agree in American way.
7. Genetically modified crops may also cause a threat to the environment. This is because it is not a natural way to plant and cultivate crops.
8. Possible good or self-indulgence of the manufacturers and companies of GMOs. This is due to the profit that can be acquired.

Microbes in need for microbial research

Microbial diversity is an immense natural resource that deserves greater attention. Too small to be seen, too large means too small to be studied or valued. Microbial diversity encompasses the spectrum of variability among all types of microorganisms (bacteria, fungi, viruses and many more) in the natural world and as altered by human environments. Microorganisms are essential for the earth to function. They play many roles both on land and in water, including being the first to colonize and ameliorate effects of naturally occurring and man-made disturbed environments. Learning more about these microorganisms will be of value for the following reasons: microorganisms are important sources of knowledge about the strategies and limits of life, microorganisms are of critical importance to the sustainability of life on our planet, the untapped diversity of microorganisms is a resource for new genes and organisms of value to biotechnology, diversity patterns of microorganisms can be used for monitoring and predicting environmental change, microorganisms play a role in conservation and restoration biology of higher organisms, and microbial communities are excellent models for understanding biological interactions and evolutionary history. Several initiatives are underway or are being proposed that seek to inventory biota of the world. Although microorganisms are known to make up the bulk of the biota in both natural and managed ecosystems, they are mentioned only in passing in these initiatives.

Importance of microbes

Microbes can do anything they want, wherever they want and without microbes, humans couldn't be alive.

Definition

Microbes are organisms that we need a microscope to see. The lower limit of our eye's resolution is about 0.1 to 0.2 mm or 100 – 200 μm (microns). Most microbes range in size from about 0.2 μm to the 200 μm upper limit, although some fruiting bodies of fungi can become much larger. Microbes include the bacteria, algae, fungi, and protozoa.

Evolution

There are two major groups of bacteria, the "eubacteria" and the relatively recently discovered "archaebacteria". The eubacteria contain most of the common bacteria such as *E. coli* (a common bacterium in the human gut) and the cyanobacteria (blue-green algae). The archaebacteria are found mainly in the deep ocean near hydrothermal vents. What is striking from the standpoint of the divergence of genetic material (the order and sequences of genes), is that these two groups of bacteria are more different from one another than are animals and plants. In other words, these two groups of bacteria have evolutionarily diverged further from one another than animals have diverged from plants through evolutionary history.

Important Aspects of Microbes on Ecosystems

Generate Oxygen in the Atmosphere

Almost all of the production of oxygen by bacteria on Earth today occurs in the ocean by the cyanobacteria or "blue-green algae". And over time, most of the oxygen produced in Earth's history has been done by bacteria.

Recycle nutrients stored in organic matter to an inorganic form.

Decomposition releases the mineral nutrients (e.g., N, P, K (potassium)) bound up in dead organic matter in an inorganic form that is available for primary producers to use. Without the recycling of inorganic nutrients, primary productivity on the globe would stop. On land, most of the decomposition (also called 'mineralisation') of dead organic matter occurs at the soil surface, and the rate of decomposition is a function of moisture and temperature (too little or too much of either reduces the rate of decomposition). Fungi are important in terrestrial systems, but not in aquatic. They are present even before the leaves and twigs enter the soil and so decomposition starts in the living or senescent plant material. Fungi are the most important decomposers of structural plant compounds (cellulose and lignin – but note that lignin is not broken down when oxygen is absent). The fungi invade the organic matter in soils first and are then followed by bacteria.

In water, the decomposition of organic matter is mostlyoxic in streams and in the ocean and anoxic in the bottoms of lakes or in swamps. As shown in the table above, oxic decomposition proceeds faster (produces higher energy yields for the bacteria) than decomposition in environments where there is no oxygen. In the open ocean, the water is so deep (average 3900 m) and contains so much oxygen, that most of the algal-derived organic matter at the surface decomposes aerobically before it reaches the bottom. For example, only 2% of the primary productivity in the upper ocean sinks to a depth of 3500 m. Most of the world is ocean, and most of the ocean is deep, so most of the aquatic decomposition must be aerobic. But in shallow waters, coastal oceans, lakes and estuaries, 25-60% of the organic matter produced may settle out of the upper waters rapidly and be decomposed anaerobically (without oxygen). Of course another important impact of decomposition besides generating inorganic nutrients is to produce CO_2 and CH_4 that is released to the atmosphere.

Fix nitrogen from the Atmosphere into a Usable Form

The only organisms capable of removing N_2 gas from the atmosphere and "fixing" it into a usable nitrogen form, ammonia and ammonium (NH_3 , NH_4^+), are bacteria. The specific bacteria that can perform N fixation are scattered throughout the groups including the cyanobacteria. All organisms that fix nitrogen use the same mechanisms and the same enzymes – this ability probably evolved only once and early in the history of life. Symbiotic N_2 fixation costs the plant photosynthate to support the fixation and the NH_3 assimilation; this cost could be from 15-30%

of the total carbon assimilated by the plant. In fact, to fix one molecule of N_2 requires about 25 molecules of ATP, so it is expensive from the bacterial standpoint, and that means that the plant must support that energy requirement. In return the plant receives nitrogen, which may otherwise be a limiting nutrient for the plant.

Another difficulty for the bacteria is that one of the enzymes necessary for nitrogen fixation is destroyed by oxygen (which is necessary for efficient ATP formation). One solution to this problem is to form symbiotic relationships with other organisms that can provide carbohydrates; these include diatoms, the fungi of certain lichens, shipworms, termites, and certain plants especially in nodules of the roots. These symbiotic relationships are often inside nodules surrounded by thick tissues, which limit the diffusion of oxygen and keep the bacterial enzymes from being inactivated by oxygen.

Allow Herbivores to Consume Poor Quality Food

In the ocean, most of the primary productivity is consumed by herbivores. In contrast, in terrestrial systems most of the primary productivity is not consumed by the herbivores. The reasons for this difference are: (1) animals lack digestive enzymes capable of using cellulose and lignin and other structural plant compounds; (2) plants often have anti-grazing toxins, aromatic resins, or thorns; (3) most land plant tissue is poor in mineral nutrients (especially N and P) compared to the tissue in the herbivore. In a ruminant animal (cattle, deer, giraffe) the ingested food, possibly regurgitated and re-chewed, passes into the rumen together with saliva (60-100 liters produced per day). The rumen is really a continuous fermenter where the complex carbohydrates of the plant are fermented by bacteria into methane, carbon dioxide, and fatty acids. The biota of the rumen are found in about equal biomasses of bacteria (10^{11} cells / mL), protozoans (10^7 / mL to 10^9 / mL), and fungi (poorly known biomass). About 60-65% of the total energy removed from the plant food that is ingested by the animal comes from rumen fermentation. Plant tissues passing from the rumen undergo secondary fermentation in the caecum and large intestine where an additional 8-30% of the total energy is provided.

In addition, many termites contain protozoans and bacteria in their guts that perform similar operations. The protozoans are capable of digesting cellulose, and bacteria in the gut generate CH_4 from the organic compounds released from the cellulose degradation. Finally, some termites

also have bacteria in their guts that are capable of fixing nitrogen from the atmosphere, providing a useable nitrogen source for the termite.

Give Plant Roots Access to Nutrients in the Soil

As plant roots scavenge the soil they create a zone of nutrient depletion around themselves. To have access to new sources of nutrients, a plant can either grow more roots and small root hairs (some as small as 10 μm) or form an association with a fungus whose hyphae provide an even longer and more efficient absorptive structure. Most vascular plants can form such associations, which are called "mycorrhizae". Mycorrhizal fungi include those living on the surface of plants (ectotrophic or sheathing) and those which enter the host (endotrophic or vesicular-arbuscular or simply "V-A"). The added advantage to the plant of forming these relationships with fungi is that the hyphae can secrete enzymes that break down organic molecules and make inorganic nutrients available. While the plants gain nutrients, the fungi gain carbohydrate food from the plant. There is also a cost to the plant in this association; one study reported that mycorrhizal

Decaying process

Many bacteria help decompose dead organisms and animal wastes into chemical compounds such as ammonia. In sewage treatment plant bacteria are used to break down the complex animal and plant matter into simple compounds.

Digestion

One of the main components of plant is cellulose. Cellulose is hard to digest by the animal. How they digest the cellulose...? Cellulose can be digested by enzyme named cellulase. How they can get cellulase? Herbivorous depend on bacteria that live in their stomach to digest cellulose. This bacteria secretes cellulase into the stomach.

Medicine

Some type of microorganism produce important drugs called antibiotics. Functions of antibiotics
Weaken, Destroy other microorganism *Penicillium notatum* is a green mould. It produces antibiotic called penicillin.

Genetically engineered bacteria

Some bacteria have been genetically engineered why? To produce certain vaccines that are used to prevent infectious diseases Insulin, produced to treat diabetes.

Agriculture

Bacteria play important roles to promote the plant growth. Bacteria break down dead plants and dead animals by releasing ammonia. Ammonia adds nitrogen into the soil. The nitrogen fixing bacteria in the soil change the ammonia into nitrates. The nitrates will be used by plants.

Importance of microbes to human life

Microbes make the oldest form of life on earth. Humans and microbes rely on these interactions to grow and stay healthy. Different species of microbes live at different places in and on human body and they are adapted to the conditions in these places. Human host and its microbial flora constitute a complex ecosystem whose equilibrium serves as a remarkable example of reciprocal adaptation. Normally, microbe is responsible for resistance to colonization by exogenous pathogenic microorganisms. However, sometimes potential pathogenic bacteria come in close contact with the host and are responsible for opportunistic infections in immune-compromised hosts. Some microbes that colonize in human are commensal that is they co-exist without harming human, whereas others have a mutualistic relationship with their human host that is both are beneficial to each other (symbiotic approach).

Conversely, some non-pathogenic microbes can harm human host via the metabolites produced by them viz. trimethylamine, indole sulphate, trimethylamine-N-oxide. Certain microbes perform specific tasks known to be useful to the human host. However, role of most resident microorganisms is not well understood. Moreover, based on evidences, now scientists are convinced that modern trends of diet, excessive use of antibiotics, obsession with cleanliness, caesarean deliveries etc. are disrupting the delicate balance contributing to some of the most perplexing ailments including asthma, allergies, obesity, diabetes, autoimmune diseases, cancer and perhaps even autism.

Microbes on Skin

Skin is the largest human organ which is the point of contact with the world. The most diverse population of microbes present in human live on the skin. Here we can find at least 1,000 different species of bacteria, fungi, viruses and other microbes which live on the skin. Most of which are harmless or even beneficial to human host. Colonization of skin is highly variable depending on endogenous host factors, topographical location and exogenous environmental factors. Symbiotic microorganisms occupy a wide range of skin niches and protect against invasion by more pathogenic or harmful organisms. One example of bacteria that protects the skin is *Bacillus subtilis*. It produces bacitracin on the skin surface that helps it in fighting with other microbes. The property of bacitracin to act as an anti-bacterial agent has been exploited to use it as antibiotics. Skin microflora also has a role in educating the billions of T cells, priming them to respond to similarly matched pathogen. Primary bacterial colonizers are *Staphylococcus epidermidis* and other coagulase-negative *Staphylococci*. Other microorganisms that are generally regarded as skin colonizers are species of *Corynebacterium*, *Propionibacterium* and *Brevibacterium*. The most commonly isolated fungal species is *Malassezia* sp. which is especially prominent in areas of the skin enriched in sebaceous glands.

Microflora in nasal cavity

However, virulent type *Streptococci* are sparsely present in the nasal cavity. On the other hand, species of *Corynebacterium*, *Aureobacterium*, *Rhodococcus* and *Staphylococcus* have been found to be present dominantly. Here data suggested that microbiota present in the nasal cavity of adult humans are strikingly different from that of the pharynx. The scientist examined 194 nasal cavities of 97 young healthy persons and showed that all had aerobic bacteria. However,

only 76.5% cavities had anaerobic bacteria. Key showed that most common aerobic bacteria found was *Staphylococcus epidermidis* in 79% cases, whereas diphtheroids were on second number (in 41% cases) and *Staphylococcus aureus* on third number (in 34% cases). There also found *Haemophilus influenzae* in 5% cases and *Streptococcus pneumoniae* in 0.5% case. The anaerobic bacteria found were *Propionibacterium acnes* in 74.5% cases and *Peptococcus magnus* in 3.5% cases.

Microbes in Mouth/Oral Cavity

The oral cavity or mouth includes several distinct microbial habitats such as nose, gingival sulcus, gingiva, tongue, cheek, lip, gingival sulcus hard palate, and soft palate. Microorganisms found in the human oral cavity are called as the oral microflora or microbiota or oral microbiome. The oral microbiome includes the species of *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Chloroflexus*, *Euryarchaeota*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Spinobacteres*, *Streptococcus*, *Synergistetes* and *Tenericutes*. Scientists have found the agonist as well as antagonist interactions between these microbes. For example, interaction between *Streptococcus gordonii* and *Actinomyces naeslundii* are both agonist and antagonist in nature. Both these microbes are involved in biofilm formation.

Microbes in Human Gut

Human gut serves two major functions: nutrition and defense. It digests food, absorbs nutrients, and assists with waste excretion. At the same time, intestine serves as house for enormous population of microbes that help in digestion and guard against pathogenic microbes. The intestinal microbiota of newborns is characterized by low diversity and a relative dominance of the phyla *Actinobacteria* and *Acetivibacteriota*. Initial microbial colonization of the gut in infants appears to be dependent on delivery mode that is vaginally delivered babies acquire microbiota similar to those of their mother's vagina (dominated by *Lactobacillus* and *Prevotella*) and babies delivered via caesarian section acquire microbiota similar to those typically associated with the skin which has *Staphylococcus*, *Propionibacterium* and *Corynebacterium*.

Microbes and biotechnology

- Microorganisms are present everywhere on earth which includes humans, animals, plants and other living creatures, soil, water and atmosphere.
- Microbes can multiply in all three habitats except in the atmosphere. Together their numbers far exceed all other living cells on the planet.
- Microorganisms are relevant to all of us in a multitude of ways. The influence of microorganisms in human life is both beneficial as well as detrimental also.
- For example microorganisms are required for the production of bread, cheese, yogurt, alcohol, wine, beer, antibiotics (e.g. penicillin, streptomycin, Chloramphenicol), vaccines, vitamins, enzymes and many more important products.
- Microorganisms are indispensable components of our ecosystem. Microorganisms play an important role in the recycling of organic and inorganic material through their roles in the C, N and S cycles, thus playing an important part in the maintenance of the stability of the biosphere.
- They are also the source of nutrients at the base of all ecological food chains and webs. In many ways all other forms of life depend on the microorganisms.
- Microorganisms also have harmed humans and disrupted societies over the millennia. Microbial diseases undoubtedly played a major role in historical events such as decline of the Roman Empire and conquest of the new world.
- In addition to health threat from some microorganisms many microbes spoil food and deteriorate materials like iron pipes, glass lenses, computer chips, jet fuel, paints, concrete, metal, plastic, paper and wood plings.
- The scope in this field is immense due to the involvement of microbiology in many fields like medicine, pharmacy, dairy, industry, clinical research, water industry, agriculture, chemical technology and nanotechnology.
- **Genetics:** Mainly involves engineered microbes to make hormones, vaccine, antibiotics and many other useful products for human being.
- **Agriculture:** The influence of microbes on agriculture: the prevention of the diseases that mainly damage the useful crops.
- **Food science:** It involves the prevention of spoilage of food and food borne diseases and the uses of microbes to produce cheese, yogurt, pickles and beer.
- **Immunology:** The study of immune system which protect the body from pathogens.

- **Medicine:** deals with the identification of plans and measures to cure diseases of humans and animals which are infectious to them.
- **Industry:** it involves use of microbes to produce antibiotics, steroids, alcohol, vitamins and amino acids etc.

Agricultural microbiology – try to combat plant diseases that attack important food crops, work on methods to increase soil fertility and crop yields etc. Currently there is a great interest in using bacterial or viral insect pathogens as substitute for chemical pesticides.

Microbial ecology – biogeochemical cycles – bioremediation to reduce pollution effects

Food and dairy microbiology – try to prevent microbial spoilage of food and transmission of food borne diseases such as botulism and salmonellosis. Use microorganisms to make foods such as cheese, yogurt, pickles and beers.

Industrial microbiology – used to make products such as antibiotics, vaccines, steroids, alcohols and other solvents, vitamins, amino acids and enzymes.

Microbial physiology and Biochemistry – study the synthesis of antibiotics and toxins, microbial energy production, microbial nitrogen fixation, effects of chemical and physical agents on microbial growth and survival etc.

Microbial genetics and Molecular biology – nature of genetic information and how it regulated the development and function of cells and organisms. Development of new microbial strains that are more efficient in synthesizing useful products.

Genetic engineering – arisen from work of microbial genetics and molecular biology. Engineered microorganisms are used to make hormones, antibiotics, vaccines and other products. New genes can be inserted into plants and animals.

The environment:

- Microbes are responsible for the cycling of carbon, nitrogen phosphorus (geochemical cycles)
- Maintain ecological balance on earth
- They are found in association with plants in symbiotic relationships, maintain soil fertility and may also be used to clean up the environment of toxic compounds (bioremediation).
- Some are devastating plant pathogens, but others act as biological control agents against these diseases.

Medicine:

- Disease causing ability of some microbes such as
- Small Pox (Variola virus)
- Cholera (Vibrio cholera)
- Malaria (Plasmodium, protozoa) etc.
- They have also provided us with the means of their control in the form of antibiotics and other medically important drugs.

Food:

- Microorganisms have been used to produce food, from brewing and wine making, through cheese production and bread making, to manufacture of soy sauce.
- Microbes are also responsible for food spoilage.

Biotechnology:

- Commercial applications include the synthesis of acetone, organic acids, enzymes, alcohols and many drugs.
- Genetic engineering – bacteria can produce important therapeutic substances such as insulin, human growth hormone, and interferon.

Research:

- Because of their simple structure they are easier to study most life processes in single unicellular organisms than in complex multicellular ones.
- Millions of copies of the same single cell can be produced in large numbers very quickly and at low cost to give plenty of homogeneous experimental material.
- Because they reproduce very quickly, they are useful for studies involving the transfer of genetic information.

Role of Bacteria in Food Production

A microorganism is a microscopic organism that is either unicellular, or composed of cell clusters. This includes organisms such as bacteria, fungi and yeast. While some microorganisms can be harmful, and may tend to give others a bad name, there are plenty of microorganisms that are very beneficial and can be put to good use. One example of this is the use of microorganisms in food and beverage production. Microorganisms have been used in the production of certain foods for thousands of years now. They are used in many processes including brewing, winemaking, baking, pickling as well as the fermentation of dairy and other products. Different types of microorganisms are used in the production of different types of foods. Many dairy

products are made as a result of the fermentation of certain microorganisms found in milk and the products of milk. Buttermilk is made as the result of the soured of low fat milk by lactic acid. Buttermilk's unique flavor comes from substances such as diacetyl and acetaldehyde. These are produced by different species of *Streptococcus*, *Lactococcus* and *Lactobacillus* as they grow. In addition to dairy products, other foods such as sauerkraut and pickles are produced through fermentation. Bacteria are not the only microorganisms that take part in the production of foods. Fungi can play a big role in food production as well. One type of food that fungi help to produce is bread. The fungi used in bread making are actually the yeast. The yeast that is used most often in the baking of bread is *Saccharomyces cerevisiae*.

This yeast ferments the carbohydrates in the dough to produce carbon dioxide. In this process, warm water brings the yeast cells to action. They begin to eat and digest the sugars in the bread and flour, which is when the carbon dioxide and small amounts of ethanol alcohol is released. The carbon dioxide produced causes the dough to rise when the bubbles become trapped in the bread dough, and creates the bread's soft texture. The yeast normally needs a few hours for this process to occur in order to sufficiently cause the dough to rise. Many different types of microorganisms are used in the making of cheese depending on which type of cheese is being made. Besides being a part of bread and cheese making, yeast is also used in the production of beer and other alcoholic beverages. Depending on the type of beer, different yeast may be used for different purposes and the process of making the beer is slightly different. Other foods that are made using microorganisms include coffee, chocolate, soy sauce, olives, meat products such as salami and bologna, and also certain food additives such as MSG and citric acid. The truth is that microorganisms make up a huge part of our diets.

Role of bacteria in food spoilage:

As we all know, microorganisms are small living forms of life that cannot be seen with the naked eye. • Bacteria, yeasts and molds are three types of microorganisms. • Some people often confuse, and almost always misunderstand, their functions, • but they are just as real and alive as you are. They eat and grow. They reproduce and die. Bacteria, yeasts and molds can be found everywhere. Scientists have gathered them from clouds above mountain tops and in the deepest parts of the ocean. They are present on animals, people and even in the air we breathe. Food

spoilage means the original nutritional value, texture, flavor of the food are damaged, the food become harmful to people and unsuitable to eat.

Microbial spoilage

There are three types of microorganisms that cause food spoilage -- yeasts, moulds and bacteria.

- Yeasts growth causes fermentation which is the result of yeast metabolism. There are two types of yeasts true yeast and false yeast. True yeast metabolizes sugar producing alcohol and carbon dioxide gas. This is known as fermentation. False yeast grows as a dry film on the surface, such as on pickle brine. False yeast occurs in foods that have a high sugar or high acid environment.
- Moulds grow in filaments forming a fuzzy mass which is visible as 'mould growth'. Moulds form spores which, when dry, float through the air to the suitable conditions where they can start the growth cycle again. Mould can cause illness especially if the person is allergic to moulds. Usually though, the main symptoms from eating mouldy food will be nausea or vomiting from the bad taste and smell of the mouldy food.

Bacteria are round, rod or spiral shaped microorganisms. Bacteria may grow under a wide variety of conditions. There are many types of bacteria that cause spoilage. They can be divided into spore forming and non-spore-forming. Bacteria generally prefer low acid foods like vegetables and meat. In order to destroy bacteria spores in a relatively short period of time, low acid foods must be processed for the appropriate length of time at 116°C (240°F) in a pressure canner. Eating spoiled food caused by bacteria can cause food poisoning.

Role Of Microorganisms in Fermented products:

Nature uses microorganisms to carry out fermentation processes, and for thousands of years mankind has used yeasts, moulds and bacteria to make food products such as bread, beer, wine, vinegar, yogurt and cheese, as well as fermented fish, meat and vegetables.

Preservation of foods by fermentation is a widely practiced and ancient technology. Fermentation ensures not only increased shelf life and microbiological safety of a food but also may also make some foods more digestible. Lactic acid bacteria because of their unique metabolic characteristics are involved in many fermentation processes of milk, meats, cereals and vegetables. Main categories of micro-organisms:

- Micro-organism responsible for the majority of food fermentations are classified into 3 categories:
- Yeasts
- Moulds
- Bacteria

The Role of Bacteria in Fermentation:

Lactic bacteria include a great variety of micro-organisms used in the food industry. They are primarily used in the dairy industry but can also be used in a number of other applications. Fermenting bacteria have characteristic sugar fermentation patterns, i.e., they can metabolize some sugars but not others. Industrial usage: The lactobacilli are present almost everywhere in nature and are widely used in the food industry where they assure a consistent production of high-quality food products. Such as • Dairy industry • Meat industry • Bread industry • Agricultural industry....etc Wine industry: In the wine industry, it is well known that yeasts are responsible for the alcoholic fermentation. Lactic bacteria also play an important role in wine making.

Meat Industry

Microorganisms are tiny living creatures that are present everywhere in nature and in human environments, including our food supply. People often refer to microorganisms as germs. Bacteria, yeasts, molds and viruses are the most common microorganisms, however, they are too small to be seen with the naked eye. Some microorganisms are useful for the production of specialty meat products, while others may cause spoilage or lead to foodborne illness. Fermented meat products such as summer sausage and pepperoni are made by adding selected microorganisms to meat in the form of starter cultures. This practice is similar to adding yeast to flour mixtures to produce bread. In both cases, microorganisms are purposely added to food to create a product that will exhibit certain desired characteristics. The tangy flavor typically associated with fermented meat products is the result of acid-producing bacteria that are found in starter cultures. Some microorganisms cause meat to spoil. Meat should be stored in the coldest part of your refrigerator, or be stored frozen. The temperature of your refrigerator should be kept between 30 and 40F. Although refrigeration will slow the growth of many microorganisms, it does not stop microbial growth entirely. Ground beef, for example, can be safely stored refrigerated for 1 to 2 days; if the meat will not be cooked and consumed within this time period, frozen storage is recommended. Spoiled red meat and poultry generally smells bad, is discolored and may be slimy to the touch. In addition, spoiled poultry may exhibit darkening at the wing tip, plus be sticky under the wing and on the upper surface of the tail. All spoiled meat should be

discarded and not eaten. Some microorganisms are pathogenic which means that they have the ability to cause foodborne illness and make people sick. Pathogens are not intentionally added to meat. Depending on the species, pathogens may be found in soil, in the gastrointestinal tract of domestic animals and humans, in water, and in many other locations. *Staphylococcus aureus* bacteria can be found in the nose and throat and on the hair and skin of more than half the healthy human population. Because this is such a common pathogen, food can become easily contaminated with the organism. Good hygiene practices are extremely important to prevent

Staphylococcus contamination in meat and other foods, in addition to proper handling, cooking and cooling practices. It is important for you to be aware that microorganisms exist and that they are found in our food supply. You can minimize your risk of exposure to foodborne illness by handling meat safely.

FOOD TECHNOLOGY

PREPARATION OF FERMENTED FOODS

The use of microorganisms to produce fermented foods has a very long history. Microbial fermentation is essential to production of many foods, including cheeses, lactic iders, salami, sauerkraut, and many more. The metabolic end products produced by the microorganisms flavor fermented foods. For example, mold-ripened cheeses owe their distinctive flavors to the mixture of aldehydes, ketones, and short-chain fatty acids produced by the fungi. Lactic acid bacteria are widely used to produce fermented foods. These organisms are also of particular importance in the food fermentation industry because they produce peptides and proteins (bacteriocins) that inhibit the growth of undesirable organisms that cause food spoilage and the multiplication of foodborne pathogens. The latter include *Clostridium botulinum* (the cause of botulism) and *Listeria monocytogenes* (which produces meningococcalitis, meningitis, perinatal septicemia, and other disorders in humans).

Nisin, an antimicrobial peptide produced by strains of *Lactococcus lactis*, is widely used as a preservative at low concentrations (up to 250 ppm in the finished products) primarily in heat-processed and low pH foods. Nisin inhibits the growth of a wide range of Gram-positive bacteria, including *Listeria*, *Clostridium*, *Bacillus*, and enterococci, but is not effective against Gram-negative bacteria, yeasts, and molds. The antibacterial activity of nisin is the combined outcome of its high-affinity interaction with lipid II at the outer leaflet of the bacterial

cytoplasmic membrane and permeabilization of the membrane through pore formation. Nisin is designated as a Generally Regarded as Safe (GRAS) food preservative in the United States and in many other countries around the world. It is used in many food products, including pasteurized cheese spreads with fruits, vegetables, or meats; liquid egg products; dressings and sauces; fresh and recombined milk; some beers; canned foods; and frozen dessert.

LACTOBACILLUS SALIVARIUS: A PROMISING BIOPRESERVATIVE

L. salivarius, a psychrophilic, lactic acid bacterium, was first isolated from a Chinese rice beer that is produced partly by lactic acid fermentation. Subsequently, *L. salivarius* strains were found to dominate the spontaneous fermentation of meat in the manufacture of salami and other dry fermented sausages. Such strains are also major components of the microbial flora of processed food products stored at cold temperature. *L. salivarius* cultures have come to be widely used in the manufacture of fermented meats, and this organism has been shown to prevent the growth of spoilage organisms and pathogens. A number of other lactic acid bacteria are either transient or permanent members of the human gastrointestinal flora, including *Lactobacillus acidophilus*. In that setting, these organisms, called probiotic species, stimulate the immune response and suppress the growth of potentially pathogenic bacteria. Recently, the genome of *L. salivarius* 23K, isolated from a French sausage, was completely sequenced and was 43% identical to *L. acidophilus*. There is much interest in using such bacteria as biopreservatives, and for the various reasons outlined above, *L. salivarius* is an excellent candidate. The availability of the complete genome of *L. salivarius* 23K allows one to formulate testable hypotheses as to the attributes of this organism that enable it to flourish on fresh meat and to survive stressful conditions it encounters the human gut during meat fermentation and storage. Such challenges include high levels of oxidative stress, high salt, and low temperatures. The *L. salivarius* genome codes for four proteins predicted to be involved in cell-cell interaction and in binding to collagen exposed on the surface of meat. Such proteins are absent from other lactobacilli. Two other gene clusters are predicted to function in the production of surface polysaccharides that may contribute to the attachment of the bacterium to the meat surface. These protein and polysaccharide surface components might mediate the aggregation of *L. salivarius* and formation of a biofilm on the meat surface that would exclude other microorganisms.

Meat undergoes auto proteolysis on aging with release of amino acids. *L. salivarius* is auxotrophic for all amino acids (except glutamic and aspartic), and thus the meat surface is an

excellent ecological niche. Meat storage frequently requires refrigeration and salts (up to 9% NaCl). *L. sakei* is well adapted to both low temperature and the osmotic stresses encountered at high salt concentrations. It has a larger number of putative cold stress proteins than other lactobacilli. It also has uptake systems for the efficient accumulation of osmo- and cryoprotective solutes such as betaine and carnitine. *L. sakei* is also well equipped with enzymes that detoxify reactive oxygen species such as superoxide or organic hydro peroxides generated during meat processing. Finally, *L. sakei* requires and takes up both heme and iron from the meat. The competition for iron may represent yet another important factor in the ability of *L. sakei* to exclude other organisms from the meat surface.

Significance of microbes in food production

Microorganisms, particularly the bacteria and fungi, have served humans since hundreds of years for the purpose of food drugs, and other high-value chemical products. The use of microbes for fermentation is known since Neolithic age. Microbes not only give a good taste, texture and smell to the foods, but also produce certain inhibitory compounds that help in stopping food spoilage thus increasing the storage and safety of food. Bacteria are used to make a wide range of food products. The most important bacteria in food manufacturing are *Lactobacillus* species, also referred to as lactic bacteria.

Dairy Industry

- It would be impossible to make **cheese** without a starter culture
- The production of cheese utilizes starter cultures being added to milk with the aim of producing soft and hard cheeses
- The starter cultures produce lactic acid, resulting in a pH decrease in the milk and promoting curd synthesis

In **yoghurt** and other fermented milk products, the culture is responsible for the taste and texture of the final product. Depending on the acidity, the product will have either a mild or strong taste, and the viscosity depends on the quantity of polysaccharides – chains of sugar molecules – that are produced.

- In recent years, **probiotic cultures** have become popular in dairy products because of their health benefits.
- These cultures are all very carefully selected strains, and there is good evidence that they help improve digestion, safeguard the immune system, and keep the body's intestinal flora in balance.

Meat Industry

Meat starter cultures are used to make dried, fermented products such as salami, pepperoni, chorizo and dried ham. Lactic bacteria develop the flavor and color of the products. In addition, a wide variety of molds are used to ripen the surface of sausages, preserving the natural quality of the product and controlling the development of flavour. Fermented meat products such as summer sausage and pepperoni are made by adding selected microorganisms to meat in the form of starter cultures. This practice is similar to adding yeast to flour mixtures to produce bread. In both cases, microorganisms are purposely added to food to create a product that will exhibit certain desired characteristics. The tangy taste typically associated with fermented meat products is the result of acid-producing bacteria that are found in starter cultures.

Wine Industry

Yeasts are responsible for the fermentation process which produces alcohol in wine. However, lactic bacteria also play an important role, as they convert the unstable malic acid that is naturally present in wine into the stable lactic acid. This conversion gives the stability that is characteristic of high-quality wines that improve on storage.

MIDTERM SYLLABUS



All living organisms get the energy required to perform cellular functions by respiration. In most of the animals, respiration occurs in the presence of oxygen (aerobically). Some organisms like bacteria, yeast, etc. can produce energy in the absence of oxygen (anaerobically).

In general Fermentation is an enzyme catalyzed metabolic process whereby organisms convert starch or sugar to alcohol or an acid anaerobically releasing energy. The science of fermentation is called "zymology". Fermentation in food processing is the process of converting carbohydrates to alcohol or organic acids using microorganism's yeasts or bacteria under anaerobic conditions. Fermentation usually implies that the action of microorganisms is desired. The term fermentation sometimes refers specifically to the chemical conversion of sugars into ethanol, producing alcoholic drinks such as wine, beer, and cider. However, similar processes take place in the leavening of bread (CO_2 produced by yeast activity), and in the preservation of sour foods with the production of lactic acid, such as in sauerkraut and yogurt. Other widely consumed fermented foods include vinegar, olives, and cheese. Many localized foods prepared

by fermentation may also be based on beans, grain, vegetables, fruit, honey, dairy products, fish, meat, or tea.

• Fermentative enzymes occur within the stomachs of animals, including humans.

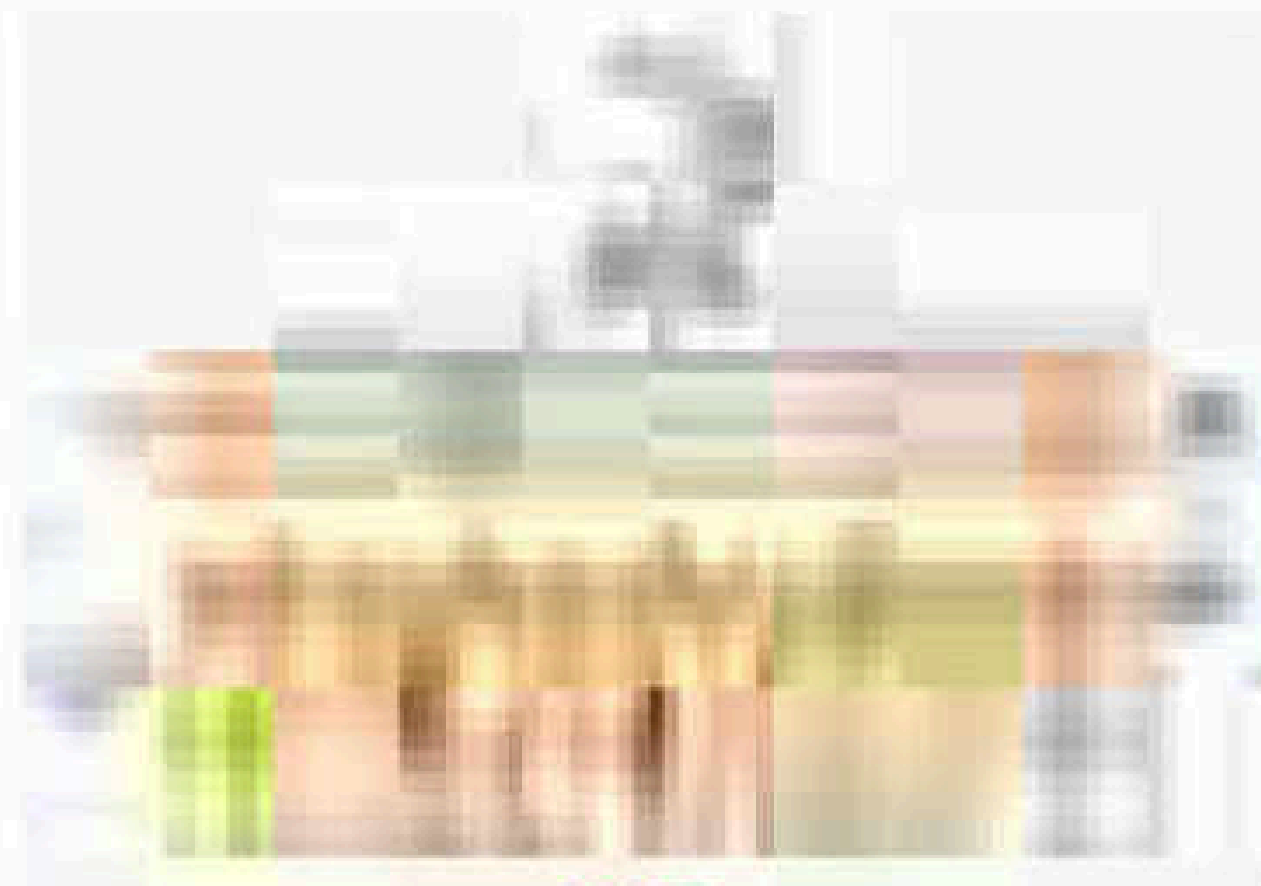
History of fermentation

The process was originated in 1856 when French chemist Louis Pasteur demonstrated that fermentation was caused by yeast. Fermentation occurs in certain types of **bacteria** and **fungi** that require an oxygen-free environment to live (known as **obligate anaerobes**), in facultative anaerobes such as yeast, and also in **muscle** cells when oxygen is in short supply (as in strenuous exercise). The processes of fermentation are valuable to the food and beverage industries, with the conversion of sugar into ethanol used to produce alcoholic beverages, the release of CO₂ by yeast used in the leavening of bread, and with the production of organic acids to preserve and flavor vegetables and dairy products.

Advantages of Fermentation

Fermented foods are rich in probiotics, beneficial microorganisms that help maintain a healthy gut so it can extract nutrients from food.

- Probiotics aid the immune system because the gut produces antibiotic, anti-tumor, antiviral, and antifungal substances, and pathogens don't do well in the acidic environment fermented foods create.
- Fermentation also helps neutralize anti-nutrients like phytic acid, which occurs in grains, nuts, seeds, and legumes and can cause mineral deficiencies. Phytates also make starches, proteins, and fats less digestible, so neutralizing them is extremely beneficial.
- Fermentation can increase the vitamins and minerals in food and make them more



Types of Fermentation

Batch Fermentation:

A batch fermentation is a closed culture system, because initial and limited amount of sterilized nutrient medium is introduced into the fermenter. The medium is inoculated with a suitable microorganism and incubated for a definite period for fermentation to proceed under optimal physiological conditions. Oxygen in the form of air, an antifoam agent and acid or base, to control the pH, are being added during the course of fermentation process. During the course of incubation, the cells of the microorganism undergo multiplication and pass through different phases of growth and metabolism due to which there will be change in the composition of culture medium, the biomass and metabolites. The fermentation is run for a definite period or until the nutrients are exhausted. The culture broth is harvested and the product is separated.

Batch fermentation may be used to produce biomass, primary metabolites and secondary metabolites under cultural conditions supporting the fastest growth rate and maximum growth would be used for biomass production. The exponential phase of growth should be prolonged to get optimum yield of primary metabolite, while it should be reduced to get optimum yield of

secondary metabolites. The used medium along with cells of microorganism and the product is drawn out from the fermenter. When the desired product is formed in optimum quantities, the product is separated from the microorganisms and purified later on.

Continuous Fermentation:

It is a closed system of fermentation, run for indefinite period. In this method, fresh nutrient medium is added continuously or intermittently to the fermenter and equivalent amount of used medium with microorganisms is withdrawn continuously or intermittently for the recovery of cells or fermentation products.

As a result, volume of the medium and concentration of nutrients at optimum level are being maintained. This has been operated in an automatic manner. The continuous fermenter has its maximum use that take long time to reach high productivity, reduces down time and lowers the operating costs. In continuous mode, starting medium and inoculum are added to the fermenter. After the culture is grown the fermenter is fed with nutrients and broth is withdrawn at the same rate maintaining a constant volume of broth in the fermenter. In continuous mode with cell cycle, the cell mass is retained to the fermenter using micro filaments with bacteria or screens with fungal mycelium.

A continuous fermentation is generally carried out in the following ways:

- (a) Single stage fermentation
- (b) Recycle fermentation
- (c) Multiple stage fermentation

Feed Batch Fermentation:

It is a modification to the batch fermentation. In this process substrate is added periodically in instalments as the fermentation progresses, due to which the substratum is always at an optimal concentration. This is essential as some secondary metabolites are subjected to catabolic repression by high concentration of either glucose, or other carbohydrate or nitrogen compounds present in the medium. For this reason, the critical elements of the nutrient medium are added in low amount in the beginning of the fermentation and these substrates continue to be added in small doses during the production phase. This method is generally employed for the production of substances such as penicillin. Yoshida (1973) introduced this term for the first time for

feeding the substrates to the medium as the nutrients are exhausted, so as to maintain the nutrients at an optimum level.

The fed-batch fermentation may be of three types:

(i) Variable Volume Fed Batch Culture:

The same medium is added resulting in an increase in volume.

(ii) Fixed Volume Fed Batch Culture:

A very concentrated solution of the limiting substrate is added at a very little amount resulting in an insignificant increase in the volume of medium.

(iii) Cyclic Fed Batch Culture:

As it is not possible to measure the substrate concentration by following direct methods during fermentation, which is necessary for controlling the feeding process, generally indirect methods are employed. For example – in the production of organic acids, the pH value may be used to determine the rate of glucose utilization.

Fermentation can be surface culture or static and submerged.

Surface Fermentations:

Surface fermentations are those where the substratum may be solid or liquid. The organism grows on the substratum and draws the nutrients from the substratum. These types of fermentations are desirable where the products are based on sporulation. But it has several disadvantages such as it exposes the organism to unequal conditions, both oxygen and nutrients.

Submerged Fermentations:

Submerged Fermentations are those in which the nutrient substratum is liquid and the organism grows inside the substratum. The culture conditions are made uniform with the help of spargers and impeller blades. Most of the industrial fermentations are of this type. The substratum which is in a liquid state and such medium is also called as broth.

Solid Substrate/State Fermentation:

Solid state (substratum) fermentation (SSF) is generally defined as the growth of the microorganism on moist solid materials in the absence or near the absence of free water. In recent years SSF has shown much promise in the development of several bioprocesses and products. SSF has been unambiguously used as solid-state fermentation or solid-substrate fermentation.

However, it is proper to distinguish between two processes. Solid substrate fermentation should be used to define only those processes in which the substrate itself acts as carbon source occurring in absence or near absence of free water. On the other hand, the solid state fermentation is that fermentation which employs a natural substrate as above or an inert substrate used as solid support.

Lactic acid fermentation

It is a metabolic process by which glucose and other six-carbon sugars (also, the chlorides of six-carbon sugars, e.g. sucrose or lactose) are converted into cellular energy and the metabolic lactate. It is an anaerobic fermentation reaction that occurs in some bacteria and animal cells, such as muscle cells. If oxygen is present in the cell, many organisms will bypass fermentation and undergo cellular respiration; however, facultative anaerobic organisms will both ferment and undergo respiration in the presence of oxygen. Sometimes even when oxygen is present and aerobic metabolism is happening in the mitochondria, if pyruvate is building up faster than it can be metabolized, the fermentation will happen anyway. Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD⁺. In homolactic fermentation, one molecule of glucose is ultimately converted to two molecules of lactic acid. Heterolactic fermentation, in contrast, yields carbon dioxide and ethanol in addition to lactic acid, in a process called the phosphoketolase pathway. Our muscles sometimes cry out from the strain we put on them, trying to work hard to meet our body's demands. When we race too fast or work too hard, the oxygen supply can't keep up with the need. That is when our muscles switch from aerobic respiration to lactic acid fermentation.

Lactic acid fermentation is the process by which our muscle cells deal with pyruvate during anaerobic respiration. When our cells need energy, they break down simple molecules like glucose. The process for breaking down glucose anaerobically is called glycolysis. Glycolysis takes place in the cytosol of the cell and does not involve oxygen. The cells turn pyruvate, the products of glycolysis, into lactic acid. When glycolysis is complete, two pyruvate molecules are left. Normally, those pyruvates would be changed and would enter the mitochondrion. Once in the mitochondrion, aerobic respiration would break them down further, releasing more energy. However, there are times when our muscle cells don't receive the oxygen they need to perform aerobic respiration. This occurs when we work them too hard. They still need energy, so they

perform glycolysis, but they cannot then perform aerobic respiration. This is when they turn to lactic acid fermentation.

Alcoholic Fermentation

Bread, beer, and Bordeaux: most of us love some or all of these! But they would not exist if not for yeast, a eukaryotic microorganism that can metabolize sugars anaerobically through a pathway called alcohol fermentation. Humans have been using yeasts to make these products for thousands of years, but only learned of their existence in the last two hundred years. How exactly do these tiny creatures make these delicious food and drink items? Alcohol fermentation, also known as ethanol fermentation, is the anaerobic pathway carried on by yeasts in which simple sugars are converted to ethanol and carbon dioxide. Yeasts typically function under aerobic conditions, or in the presence of oxygen, but are also capable of functioning under anaerobic conditions, or in the absence of oxygen. When no oxygen is readily available, alcohol fermentation occurs in the cytosol of yeast cells. Let's explore the process of alcohol fermentation then see what it means for yeasts and for humans.

The Process of Alcohol Fermentation

The basic equation for alcohol fermentation shows that yeast starts with glucose, a type of sugar, and finishes with carbon dioxide and ethanol. However, to better understand the process, we need to take a look at some of the steps that take us from glucose to the final products. The process of alcohol fermentation can be divided into two parts. In the first part, the yeast breaks down glucose to form 2 pyruvate molecules. This part is known as glycolysis. In the second part, the 2 pyruvate molecules are converted into 2 carbon dioxide molecules and 2 molecules of ethanol, carbon dioxide known as alcohol. This second part is called fermentation. The main purpose of alcohol fermentation is to produce ATP, the energy currency for cells, under anaerobic conditions. So from the yeast's perspective, the carbon dioxide and ethanol are waste products. That's the basic overview of alcohol fermentation.

Role of microorganisms in Industries

1. Beverages:

Microbes especially yeast have been used from time immemorial for the production of beverages like Wine, Beer, Whisky, Brandy or Rum. For this purpose, the yeast *Saccharomyces cerevisiae* (commonly called Brewer's yeast) is used for fermenting malted cereals and fruit juices to produce ethanol. Among these beverages, Wine and Beer are produced without distillation whereas whisky, brandy and rum are distilled beverages.

2. Antibiotics:

Antibiotics produced by microbes are regarded was one of the most significant discoveries of the twentieth century and have made major contributions towards the welfare of human society. Many antibiotics are produced by microorganisms, predominantly by Actinomycetes in the genus *Streptomyces* (e.g. Tetracycline, Streptomycin and Actinomycin D) and by filamentous fungi (e.g. Penicillin, Cephalosporin).

3. Organic acids:

Microbes are also used for the commercial and industrial production of certain organic acids. These compounds can be produced directly from glucose (e.g. gluconic acid) or formed as end products from pyruvate or ethanol. Examples of acids producers are *Aspergillus Niger* (a fungus) of Citric acid, *Acetobacter aceti* (a bacterium) of acetic acid, *Lactobacillus* (a bacterium) of lactic acid and many others.

4. Amino Acids:

Amino acids such as Lysine and Glutamic acid are used in the food industry as nutritional supplements in bread products and as flavor enhancing compounds such as Monosodium Glutamate (MSG). In early days, monosodium glutamate (MSG) was extracted from the vegetable proteins (wheat and soy). Amino acids are generally synthesized as primary metabolites by microbes. However, when the rate and amount of synthesis of some amino acids exceed the cell's need for protein synthesis, then cell excrete them into the surrounding medium.

5. Enzymes:

Many microbes synthesise and excrete large quantities of enzymes into the surrounding medium. Using this feature of these tiny organisms, many enzymes have been produced commercially. These include Amylase, Cellulase, Protease, Lipase, Pectinase, Streptokinase and many others.

Enzymes are extensively used in food processing and preservation, washing powders, Leather Industry, Paper Industry and in scientific research.

6. Vitamins:

Vitamins are some organic compounds which are capable of performing many life-sustaining functions inside our body. These compounds cannot be synthesised by humans, and therefore, they have to be supplied in small amounts in the diet. Microbes are capable of synthesising these compounds and hence they can be used for the commercial production of many of the Vitamins e.g. thiamine (Vitamin B1), riboflavin (Vitamin B2), pyridoxine (Vitamin B6), folic acid, pantothenic acid (Vitamin B5), biotin (Vitamin B7), Vitamin B12, ascorbic acid (Vitamin C).

7. Biofuels:

Organic solvents such as ethanol, acetone, butanol and glycerol are some very important

chemicals that are widely used in petrochemical industries. These chemicals can be commercially produced by using microbes and low-cost raw materials (e.g. wood, cellulose, starch). Brazil was the first country to produce ethanol in large scale by yeast fermentation, utilising sugarcane and Corn. Yeast (*Saccharomyces cerevisiae*) is used for commercial production of ethanol. Ethanol is used as motor fuel and is often referred to as green petrol.

8. Single Cell Protein (SCP):

Single Cell Protein (SCP) can serve as an alternate source of energy when a larger portion of the world is suffering from hunger and malnutrition. SCPs are microbial cells that are rich in protein, minerals, fats, carbohydrate and vitamins and can be used as food supplements for humans and animals. Microbes like *Spirulina* can be grown easily on materials like waste water from potato processing plants (containing starch), straw, molasses, animal manure and even sewage, to produce large quantities.

9. Steroids:

Steroids are a very important group of chemicals, which are used as anti-inflammatory drugs,

28

and as hormones such as estrogens and progesterone, which are used in oral contraceptives. Producing steroids from animal sources or chemically synthesizing them is difficult, but microorganisms can synthesize steroids from sterols or from related compounds.

10. Vaccines:

Vaccines are a product of Industrial Microbiology. Many antiviral vaccines are now produced in chicken eggs or cell cultures. The production of vaccines against bacterial diseases usually requires the growth of large amounts of the bacteria. Recombinant DNA technology is increasingly important in the development and production of viral vaccines.

11. Pharmaceutical Drugs:

Many pharmaceutical drugs are also produced by microbes e.g. Cyclosporin A that is used as an immunosuppressive agent in organ-transplant surgery is produced by the fungus *Trichoderma polysporum*. Statins produced by the yeast *Monascus purpureus* have been commercialized as blood-cholesterol-lowering agents. It acts by competitively inhibiting the enzyme responsible for the synthesis of cholesterol.

Microorganisms, which are used or represented only in environmental DNA samples, constitute the natural resource base of microbial biotechnology. Numerous prokaryotic and fungal genomes have been completely sequenced and the functions of many genes established. For a newly sequenced prokaryotic genome, functions for over 60% of the open reading frames can be previously assigned by sequence homology with genes of known function. Knowledge of the ecology, genetics, physiology, and metabolism of thousands of prokaryotes and fungi provides an indispensable complement to the sequence database. This is an era of explosive growth of analysis and manipulation of microbial genomes as well as of invention of many new, creative ways in which both microorganisms and their genetic endowment are utilized. Microbial biotechnology is riding the crest of the wave of genomics. The umbrella of microbial biotechnology covers many scientific activities, ranging from production of recombinant human

hormones to that of microbial insecticides, from mineral leaching to bioremediation of toxic wastes. In this chapter, we sketch the complex terrain of microbial biotechnology. The purpose of this chapter is to convey the impact, the extraordinary breadth of applications, and the multidisciplinary nature of this technology. The common denominator to the subjects discussed is that in all instances, prokaryotes or fungi provide the indispensable component.

Vaccines

A vaccine is a biological preparation that provides active acquired immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as a threat, destroy it, and recognize and destroy any of these microorganisms that it later encounters.

History

During the late 1760s whilst serving his apprenticeship as a surgeon Edward Jenner learned of the story, common in rural areas, that dairy workers would never have the often-fatal or disfiguring disease smallpox. Because they had already had cowpox, which has a very mild effect in humans. Edward Jenner In 1796, Jenner took pus from the hand of a milkmaid with cowpox, scratched it into the arm of an 8-year-old boy. Six weeks later inoculated the boy with smallpox, afterwards observing that he did not catch smallpox. Jenner extended his studies and in 1798 reported that his vaccine worked in children and adults. The second generation of vaccines was introduced in the 1880s by Louis Pasteur who developed vaccines for chicken cholera and anthrax. From the late nineteenth century vaccines were considered a matter of national prestige, and compulsory vaccination laws were passed.

HUMAN THERAPEUTICS PRODUCTION OF HETEROLOGOUS PROTEINS

One of the most dramatic and immediate impacts of genetic engineering was the production in bacteria of large amounts of proteins encoded by human genes. In 1982, insulin, expressed from human insulin genes on plasmids inserted into *Escherichia coli*, was the first genetically engineered therapeutic agent to be approved for clinical use in humans. Bacterially produced insulin, used widely in the treatment of diabetes, is indistinguishable in its structure and clinical effects from natural insulin. Human growth hormone (hGH), a protein made naturally by the pituitary gland, was the second such product. Inadequate secretion of hGH in children results in

disorder. Before the advent of recombinant DNA technology, tGH was prepared from pituitaries removed from human cadavers. The supply of such preparations was limited and the cost prohibitive. Furthermore, there were dangers in their administration that led to withdrawal from the market. Some patients treated with injections of pituitary tGH developed a disease caused by a contaminating slow virus, Jakob-Creutzfeldt syndrome, which leads to dementia and death. tGH can be produced in genetically engineered *E. coli* in large amounts, at relatively little cost, and free from such contaminants. Human tissue plasminogen activator (tPA), a serine protease (a "serine" protease) with an affinity for fibrin clots, is another therapeutic agent made available in large amounts as a consequence of recombinant DNA technology. At the surface of fibrin clots, tPA cleaves a single peptide bond in plasminogen to form active serine protease, plasmin, which then degrades the clots. This clot-degrading property of tPA makes it a life-saving drug in the treatment of patients with acute myocardial infarction (damage to heart muscle due to arterial blockage). Recombinant human insulin and tGH offered impressive proof of the clinical efficacy and safety of human proteins made by cultured microorganisms.

DNA VACCINES

In the early 1990s, attention focused on the potential wide-ranging opportunities offered by DNA vaccines. DNA vaccines consist of appropriately engineered plasmid DNA prepared on a large scale in *E. coli*. The obvious advantages of DNA plasmid vaccines are that they are not infectious, do not replicate, and encode only the protein(s) of interest. Unlike other types of vaccines, there is no protein component, and hence induction of an immune response against subsequent immunisation is minimised. A vaccine plasmid includes the following major components: a strong promoter system for expression in eukaryotic cells of an antigenic protein (e.g., a viral coat protein), the immediate early promoter of cytomegalovirus is frequently used; a cloning site for the insertion of the gene encoding the antigenic protein; and an appropriately located polyadenylation termination sequence. Most eukaryotic mRNAs contain a polyadenylate (polyA) tail at the 3' end that appears to be important to the translation efficiency and the stability of the mRNA. The plasmid also includes a prokaryotic origin of replication for its production in *E. coli* and a selectable marker, such as the ampicillin resistance gene, to allow selection of bacterial cells that contain the plasmid. DNA vaccines are generally introduced by intramuscular injection. It is still not known how cells internalise the DNA after the injection. The encoded antigen is then expressed *in situ* in the cells of the vaccine recipient and elicits an immune

response. Such vaccines have attractive features. The immunizing antigens may be derived from viruses, bacteria, parasites, or tumors. Antigens can be expressed singly or in multiple combinations. In one case, the DNA vaccine contained multiple variants of a highly mutable gene, for example, the gene encoding gp120, a glycoprotein located on the external surface of HIV.

In other vaccines, the entire genome of the infectious microorganism was introduced into a common plasmid backbone by "shotgun cloning." DNA vaccines induce both humoral responses (the appearance of serum antibodies against the antigen) and cellular response (activation of various like all T cells, these responses have been documented in animal models of disease in which protection is mediated by such responses. Important issues remain to be resolved before DNA vaccines can take a regular place alongside other types of vaccines. In clinical trials, vaccines for malaria, hepatitis B, HIV, and influenza elicited only moderate response in human volunteers. An assessment of DNA vaccines encoding certain highly conserved influenza virus proteins concluded that there is a need for considerable enhancement of the immune response to DNA immunization before such vaccines become a promising approach for humans.

SOURCES OF DRUGS

Microorganisms produce a large number of small molecular weight compounds that are broadly described as *secondary metabolites*. A traditional approach to the discovery of new, naturally occurring bioactive compounds utilizes "screens." A screen is an assay procedure that allow testing of numerous compounds for a particular activity. Tens of thousands of secondary metabolites and other compounds have been examined for biological activity in various organisms and many have proved valuable as *antibacterial* or *antifungal* agents, *anticancer* drugs, *immunomodulators*, *herbicides*, *tools for research*. Genetically modified microorganisms have been engineered to produce such compounds in large amounts. Among these, antibiotics are the **secondary metabolites considered among the most important to human therapeutics, and the most** extensive use of screens is in the search for compounds with selective toxicity for bacteria, fungi, or protozoa. It is estimated that natural microbial antibiotics provide the starting point for over 75% of marketed antimicrobial agents.

AVERMECTINS

Many microorganisms indigenous to the soil, especially actinomycete bacteria and many fungi, produce biologically active secondary metabolites. Intensive screening of culture supernatants (usually called "fermentation broths"), rich in secondary metabolites, has led to the discovery of numerous clinically valuable antibiotics, with penicillin as the most famous example, but of many other types of valuable compounds as well. The structures of newly characterized compounds with herbicidal, insecticidal, and nematocidal activities from soil microorganisms are described in the scientific literature at a rate of several hundred each year. The avermectins were discovered in the early 1980s as a result of a deliberate search for antihelminthic compounds produced by soil microorganisms. Helminths are parasitic worms that infect the intestines of any animal unfortunate enough to ingest their eggs. There were two particularly notable features of the screening program. First, the microbial fermentation broths were tested by being administered in the diet to mice infested with the nematode *Nippostrongylus brasiliensis*. Nematodes are a subclass of helminths that includes roundworms and pinworms. Although such an *in vivo* assay was expensive, it simultaneously tested for efficacy of the preparation against the nematode and toxicity to the host. Second, to increase the chance of discovering new types of compounds, the selection of microorganisms for testing was biased toward those with unusual morphological traits and nutritional requirements.

Types of Vaccine

There are several different types of vaccines. Each type is designed to teach your immune system how to fight off certain kinds of germs — and the serious diseases they cause.

There are 4 main types of vaccines:



- Live-attenuated vaccines
- Inactivated vaccines
- Subunit, recombinant, polysaccharide, and conjugate vaccines
- Toxoid vaccines

Live, Attenuated Vaccines

Live, attenuated vaccines contain a version of the living microbe that has been weakened in the lab so it can't cause disease. Because a live, attenuated vaccine is the closest thing to a natural infection, these vaccines are good "teachers" of the immune system. They elicit strong cellular and antibody responses and often confer lifelong immunity with only one or two doses. Despite the advantages of live, attenuated vaccines, there are some drawbacks. It is the nature of living things to change, or mutate, and the organisms used in live, attenuated vaccines are no different. The remote possibility exists that an attenuated microbe in the vaccine could revert to a virulent form and cause disease. Also, not everyone can safely receive live, attenuated vaccines. For their own protection, people who have damaged or weakened immune systems—because they've undergone chemotherapy or have HIV, for example—cannot be given live vaccines. Another limitation is that live, attenuated vaccines usually need to be refrigerated to stay potent. If the vaccine needs to be shipped overseas and stored by healthcare workers in developing countries that lack widespread refrigeration, a live vaccine may not be the best choice. Live, attenuated vaccines are relatively easy to create for certain viruses. Vaccines against measles, mumps, and chickenpox, for example, are made by this method. Viruses are simple microbes containing a small number of genes, and scientists can therefore more readily control their characteristics. Viruses often are attenuated through a method of growing generations of them in cells in which they do not reproduce very well. This hostile environment takes the fight out of viruses. As they evolve to adapt to the new environment, they become weaker with respect to their natural host, human beings. Live, attenuated vaccines are more difficult to create for bacteria. Bacteria have thousands of genes and thus are much harder to control. Scientists working on a live vaccine for a bacterium, however, might be able to use recombinant DNA technology to remove several key genes. This approach has been used to create a vaccine against the bacterium that causes cholera, *Vibrio cholerae*, although the live cholera vaccine has not been licensed in the United States.

Inactivated Vaccines

Scientists produce inactivated vaccines by killing the disease-causing microbe with chemicals, heat, or radiation. Such vaccines are more stable and safer than live vaccines. The dead microbes can't mutate back to their disease-causing state. Inactivated vaccines usually don't require refrigeration, and they can be easily stored and transported in a freeze-dried form, which makes them accessible to people in developing countries. Most inactivated vaccines, however, stimulate

a weaker immune system response than do live vaccines. So it would likely take several additional doses, or booster shots, to maintain a person's immunity. This could be a drawback in areas where people don't have regular access to health care and can't get booster shots on time.

Subunit Vaccines

Instead of the entire microbe, subunit vaccines include only the antigens that have stimulate the immune system. In some cases, these vaccines use epitopes—the very specific parts of the antigen that antibodies or T cells recognize and bind to. Because subunit vaccines contain only the relevant antigens and not all the other molecules that make up the microbe, the chances of adverse reactions to the vaccine are lower.

Subunit vaccines can contain anywhere from 1 to 20 or more antigens. Of course, identifying which antigens best stimulate the immune system is a tricky, time-consuming process. Once scientists do that, however, they can make subunit vaccines in one of two ways:

- They can grow the microbe in the laboratory and then use chemicals to break it apart and gather the important antigens.
- They can manufacture the antigen molecules from the microbe using recombinant DNA technology. Vaccines produced this way are called "recombinant subunit vaccines."

A recombinant subunit vaccine has been made for the hepatitis B virus. Scientists inserted hepatitis B genes that code for important antigens into common baker's yeast. The yeast then produced the antigens, which the scientists collected and purified for use in the vaccine. Research is continuing on a recombinant subunit vaccine against hepatitis C virus.

Toxoid Vaccines

For bacteria that secrete toxins, or harmful chemicals, a toxoid vaccine might be the answer. These vaccines are used when a bacterial toxin is the main cause of illness. Scientists have found that they can inactivate toxins by treating them with formalin, a solution of formaldehyde and distilled water. Such "denatured" toxins, called toxoids, are safe for use in vaccines. When the immune system receives a vaccine containing a harmless toxoid, it learns how to fight off the

neutral toxin. The immune system produces antibodies that lock onto and block the toxin. Vaccines against diphtheria and tetanus are examples of toxoid vaccines.

Conjugate Vaccines

If a bacterium possesses an outer coating of sugar molecules called polysaccharides, as many harmful bacteria do, researchers may try making a conjugate vaccine for it. Polysaccharide coatings disguise a bacterium's antigens so that the immature immune systems of infants and younger children can't recognize or respond to them. Conjugate vaccines, a special type of subunit vaccine, get around this problem. When making a conjugate vaccine, scientists link antigens or toxins from a microbe that an infant's immune system can recognize to the polysaccharides. The linkage helps the immature immune system react to polysaccharide coatings and defend against the disease-causing bacterium. The vaccine that protects against *Haemophilus influenzae* type B (Hib) is a conjugate vaccine.

DNA Vaccines

Once the genes from a microbe have been analyzed, scientists could attempt to create a DNA vaccine against it. Still in the experimental stages, these vaccines show great promise, and several types are being tested in humans. DNA vaccines take immunization to a new technological level. These vaccines dispense with both the whole organism and its parts and get right down to the essence: the microbe's genetic material. In particular, DNA vaccines use the genes that code for those all-important antigens. Researchers have found that when the genes for a microbe's antigens are introduced into the body, some cells will take up that DNA. The DNA then instructs those cells to make the antigen molecules. The cells secrete the antigens and display them on their surfaces. In other words, the body's own cells become vaccine-making factories, creating the antigens necessary to stimulate the immune system. A DNA vaccine against a microbe would evoke a strong antibody response to the free-floating antigen secreted by cells, and the vaccine also would stimulate a strong cellular response against the microbial

antigens displayed on cell surfaces. The DNA vaccine couldn't cause the disease because it wouldn't contain the microbe, just copies of a few of its genes. In addition, DNA vaccines are relatively easy and inexpensive to design and produce.

So-called naked DNA vaccines consist of DNA that is administered directly into the body. These vaccines can be administered with a needle and syringe or with a needle-less device that uses high-pressure gas to shoot microscopic gold particles coated with DNA directly into cells. Sometimes, the DNA is mixed with molecules that facilitate its uptake by the body's cells. Naked DNA vaccines being tested in humans include those against the viruses that cause influenza and herpes.

Recombinant Vector Vaccines

Recombinant vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. "Vector" refers to the virus or bacterium used as the carrier.

In nature, viruses latch on to cells and inject their genetic material into them. In the lab, scientists have taken advantage of this process. They have figured out how to take the empty genomes of certain harmless or attenuated viruses and insert portions of the genetic material from other microbes into them. The carrier viruses then ferry that microbial DNA to cells. Recombinant vector vaccines closely mimic a natural infection and therefore do a good job of stimulating the immune system.

Attenuated bacteria also can be used as vectors. In this case, the inserted genetic material causes the bacteria to display the antigens of other microbes on its surface. In effect, the harmless bacterium mimics a harmful microbe, provoking an immune response.

✓✓

Types of Vaccine

There are several different types of vaccines. Each type is designed to teach your immune system how to fight off certain kinds of germs — and the serious diseases they cause.

There are 4 main types of vaccines:

- Live-attenuated vaccines
- Inactivated vaccines
- Subunit, recombinant, polysaccharide, and conjugate vaccines
- Toxoid vaccines



Live, Attenuated Vaccines

Live, attenuated vaccines contain a version of the living microbe that has been weakened in the lab so it can't cause disease. Because a live, attenuated vaccine is the closest thing to a natural infection, these vaccines are good "teachers" of the immune system. They elicit strong cellular and antibody responses and often confer lifelong immunity with only one or two doses. Despite the advantages of live, attenuated vaccines, there are some downsides. It is the nature of living things to change, or mutate, and the organisms used in live, attenuated vaccines are no different. The remote possibility exists that an attenuated microbe in the vaccine could revert to a virulent form and cause disease. Also, not everyone can safely receive live, attenuated vaccines. For their own protection, people who have damaged or weakened immune systems—because they're undergoing chemotherapy or have HIV, for example—cannot be given live vaccines. Another limitation is that live, attenuated vaccines usually need to be refrigerated to stay potent. If the vaccine needs to be shipped overseas and stored by healthcare workers in developing countries that lack widespread refrigeration, a live vaccine may not be the best choice. Live, attenuated vaccines are relatively easy to create for certain viruses. Vaccines against measles, mumps, and chickenpox, for example, are made by this method. Viruses are simple microbes containing a small number of genes, and scientists can therefore more readily control their characteristics. Viruses often are attenuated through a method of growing generations of them in cells in which they do not reproduce very well. This hostile environment takes the fight out of viruses. As they evolve to adapt to the new environment, they become weaker with respect to their natural host, human beings. Live, attenuated vaccines are more difficult to create for bacteria. Bacteria have thousands of genes and thus are much harder to control. Scientists working on a live vaccine for

a bacterium, however, might be able to use recombinant DNA technology to remove several key genes. This approach has been used to create a vaccine against the bacterium that causes cholera, *Vibrio cholerae*, although the live cholera vaccine has not been licensed in the United States.

Inactivated Vaccines

Scientists produce inactivated vaccines by killing the disease-causing microbe with chemicals, heat, or radiation. Such vaccines are more stable and safer than live vaccines. The dead microbes can't mutate back to their disease-causing state. Inactivated vaccines usually don't require refrigeration, and they can be easily stored and transported in a freeze-dried form, which makes them accessible to people in developing countries. Most inactivated vaccines, however, stimulate a weaker immune system response than do live vaccines. So it would likely take several additional doses, or booster shots, to maintain a person's immunity. This could be a drawback in areas where people don't have regular access to health care and can't get booster shots on time.

Subunit Vaccines

Instead of the entire microbe, subunit vaccines include only the antigens that best stimulate the immune system. In some cases, these vaccines use *splines*—the very specific parts of the antigen that antibodies or T cells recognize and bind to. Because subunit vaccines contain only the essential antigens and lack all the other molecules that make up the vaccine, the chances of adverse reactions to the vaccine are lower.

Subunit vaccines can contain anywhere from 1 to 20 or more antigens. Of course, identifying which antigens best stimulate the immune system is a tricky, time-consuming process. Once scientists do that, however, they can make subunit vaccines in one of two ways:

- They can grow the microbe in the laboratory and then use chemicals to break it apart and gather the important antigens.
- They can manufacture the antigen molecules from the microbe using recombinant DNA technology. Vaccines produced this way are called "recombinant subunit vaccines."

A recombinant subunit vaccine has been made for the hepatitis B virus. Scientists inserted hepatitis B genes that code for important antigens into common baker's yeast. The yeast then produced the antigens, which the scientists collected and purified for use in the vaccine. Research is continuing on a recombinant subunit vaccine against hepatitis C virus.

Toxoid Vaccines

For bacteria that secrete toxins, or harmful chemicals, a toxoid vaccine might be the answer. These vaccines are used when a bacterial toxin is the main cause of illness. Scientists have found that they can inactivate toxins by treating them with formalin, a solution of formaldehyde and distilled water. Such "denatured" toxins, called toxoids, are safe for use in vaccines. When the immune system receives a vaccine containing a harmless toxoid, it learns how to fight off the natural toxin. The immune system produces antibodies that lock onto and block the toxin. Vaccines against diphtheria and tetanus are examples of toxoid vaccines.

Conjugate Vaccines

If a bacterium possesses an outer coating of sugar molecules called polysaccharides, as many harmful bacteria do, researchers may try making a conjugate vaccine for it. Polysaccharide coatings disguise a bacterium's antigens so that the immature immune systems of infants and younger children can't recognize or respond to them. Conjugate vaccines, a special type of subunit vaccine, get around this problem. When making a conjugate vaccine, scientists link antigens or toxins from a microbe that an infant's immune system can recognize to the polysaccharides. The linkage helps the immature immune system react to polysaccharide coatings and defend against the disease-causing bacterium. The vaccine that protects against *Haemophilus influenzae* type B (Hib) is a conjugate vaccine.

DNA Vaccines

Once the genes from a microbe have been analyzed, scientists could attempt to create a DNA vaccine against it. Still in the experimental stages, these vaccines show great promise, and several types are being tested in humans. DNA vaccines take immunization to a new technological level. These vaccines dispense with both the whole organism and its parts and get right down to the essentials: the microbe's genetic material. In particular, DNA vaccines use the genes that code for those all-important antigens. Researchers have found that when the genes for a microbe's antigens are introduced into the body, some cells will take up that DNA. The DNA then instructs those cells to make the antigen molecules. The cells secrete the antigens and display them on their surfaces. In other words, the body's own cells become vaccine-making factories, creating the antigens necessary to stimulate the immune system. A DNA vaccine against a microbe would evoke a strong antibody response to the free-floating antigen secreted by cells, and the vaccine also would stimulate a strong cellular response against the microbial antigens displayed on cell surfaces. The DNA vaccine couldn't cause the disease because it wouldn't contain the microbe, just copies of a few of its genes. In addition, DNA vaccines are relatively easy and inexpensive to design and produce.

So-called naked DNA vaccines consist of DNA that is administered directly into the body. These vaccines can be administered with a needle and syringe or with a needle-less device that uses high-pressure gas to shoot microscopic gold particles coated with DNA directly into cells. Sometimes, the DNA is mixed with molecules that facilitate its uptake by the body's cells. Naked DNA vaccines being tested in humans include those against the viruses that cause influenza and herpes.

Recombinant Vector Vaccines

Recombinant vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. "Vector" refers to the virus or bacterium used as the carrier.

In nature, viruses latch on to cells and inject their genetic material into them. In the lab, scientists have taken advantage of this process. They have figured out how to take the empty genomes of certain harmless or attenuated viruses and insert portions of the genetic material from other microbes into them. The carrier viruses then ferry that microbial DNA to cells. Recombinant

vector vaccines closely mimic a natural infection and therefore do a good job of stimulating the immune system.

Attenuated bacteria also can be used as vectors. In this case, the inserted genetic material causes the bacteria to display the antigens of other microbes on its surface. In effect, the harmless bacterium mimics a harmful microbe, provoking an immune response.

in Khan

Types of Vaccine

There are several different types of vaccines. Each type is designed to teach your immune system how to fight off certain kinds of germs — and the serious diseases they cause.

There are 4 main types of vaccines:

- Live-attenuated vaccines
- Inactivated vaccines
- Subunit, recombinant, polysaccharide, and conjugate vaccines
- Toxoid vaccines

Live, Attenuated Vaccines

Live, attenuated vaccines contain a version of the living microbe that has been weakened in the lab so it can't cause disease. Because a live, attenuated vaccine is the closest thing to a natural infection, these vaccines are good "teachers" of the immune system. They elicit strong cellular and antibody responses and often confer lifelong immunity with only one or two doses. Despite the advantages of live, attenuated vaccines, there are some downsides. It is the nature of living things to change, or mutate, and the organisms used in live, attenuated vaccines are no different. The remote possibility exists that an attenuated microbe in the vaccine could revert to a virulent form and cause disease. Also, not everyone can safely receive live, attenuated vaccines. For their own protection, people who have damaged or weakened immune systems—because they've undergone chemotherapy or have HIV, for example—cannot be given live vaccines. Another

Limitation is that live, attenuated vaccines usually need to be refrigerated to stay potent. If the vaccine needs to be shipped overseas and stored by healthcare workers in developing countries that lack widespread refrigeration, a live vaccine may not be the best choice. Live, attenuated vaccines are relatively easy to create for certain viruses. Vaccines against measles, mumps, and chickenpox, for example, are made by this method. Viruses are simple microbes containing a small number of genes, and scientists can therefore more readily control their characteristics. Viruses often are attenuated through a method of growing generations of them in cells in which they do not reproduce very well. This hostile environment takes the fight out of viruses. As they evolve to adapt to the new environment, they become weaker with respect to their natural host, human beings. Live, attenuated vaccines are more difficult to create for bacteria. Bacteria have thousands of genes and thus are much harder to control. Scientists working on a live vaccine for a bacterium, however, might be able to use recombinant DNA technology to remove several key genes. This approach has been used to create a vaccine against the bacterium that causes cholera, *Vibrio cholerae*, although the live cholera vaccine has not been licensed in the United States.

Inactivated Vaccines

Scientists produce inactivated vaccines by killing the disease-causing microbe with chemicals, heat, or radiation. Such vaccines are more stable and safer than live vaccines. The dead microbes can't mutate back to their disease-causing state. Inactivated vaccines usually don't require refrigeration, and they can be easily stored and transported in a freeze-dried form, which makes them accessible to people in developing countries. Most inactivated vaccines, however, stimulate a weaker immune system response than do live vaccines. So it would likely take several additional doses, or booster shots, to maintain a person's immunity. This could be a drawback in areas where people don't have regular access to health care and can't get booster shots on time.

Subunit Vaccines

Instead of the entire microbes, subunit vaccines include only the antigens that best stimulate the immune system. In some cases, these vaccines use epitopes—the very specific parts of the antigen that antibodies or T cells recognize and bind to. Because subunit vaccines contain only

the essential antigens and just all the other molecules that make up the vaccine, the chance of adverse reactions to the vaccine are lower.

Subunit vaccines can contain anywhere from 1 to 20 or more antigens. Of course, identifying which antigens best stimulate the immune system is a tricky, time-consuming process. Once scientists do that, however, they can make subunit vaccines in one of two ways:

- They can grow the microbe in the laboratory and then use chemicals to break it apart and gather the important antigens.
- They can manufacture the antigen molecules from the microbe using recombinant DNA.

Subunit vaccines produced this way are called "recombinant subunit vaccines."

A recombinant subunit vaccine has been made for the hepatitis B virus. Scientists inserted hepatitis B genes that code for important antigens into common baker's yeast. The yeast then produced the antigens, which the scientists collected and purified for use in the vaccine. Research is continuing on a recombinant subunit vaccine against hepatitis C virus.

Toxoid Vaccines

For bacteria that secrete toxins, or harmful chemicals, a toxoid vaccine might be the answer. These vaccines are used when a bacterial toxin is the main cause of illness. Scientists have found that they can inactivate toxins by treating them with formalin, a solution of formaldehyde and sterilized water. Such "killed" toxins, called toxoids, are safe for use in vaccines. When the immune system receives a vaccine containing a harmless toxoid, it learns how to fight off the natural toxin. The immune system produces antibodies that lock onto and block the toxin. Vaccines against diphtheria and tetanus are examples of toxoid vaccines.

Conjugate Vaccines

If a bacterium possesses an outer coating of sugar molecules called polysaccharides, as many harmful bacteria do, researchers may try making a conjugate vaccine for it. Polysaccharide coatings disguise a bacterium's antigens so that the immature immune systems of infants and younger children can't recognize or respond to them. Conjugate vaccines, a special type of subunit vaccine, get around this problem. When making a conjugate vaccine, scientists link

antigens or toxins from a microbe that an infant's immune system can recognize to the polysaccharides. The linkage helps the immature immune system react to polysaccharide coatings and defend against the disease-causing bacterium. The vaccine that protects against *Haemophilus influenzae* type B (Hib) is a conjugate vaccine.

DNA Vaccines

Once the genes from a microbe have been analyzed, scientists could attempt to create a DNA vaccine against it. Still in the experimental stages, these vaccines show great promise, and several types are being tested in humans. DNA vaccines take immunization to a new technological level. These vaccines dispense with both the whole organism and its parts and get right down to the essential: the microbe's genetic material. In particular, DNA vaccines use the genes that code for those all-important antigens. Researchers have found that when the genes for a microbe's antigens are introduced into the body, some cells will take up that DNA. The DNA then instructs those cells to make the antigen molecules. The cells secrete the antigens and display them on their surfaces. In other words, the body's own cells become vaccine-making factories, creating the antigens necessary to stimulate the immune system. A DNA vaccine against a microbe would evoke a strong antibody response to the free-floating antigen secreted by cells, and the vaccine also would stimulate a strong cellular response against the microbial antigens displayed on cell surfaces. The DNA vaccine couldn't cause the disease because it wouldn't contain the microbe, just copies of a few of its genes. In addition, DNA vaccines are relatively easy and inexpensive to design and produce.

So-called naked DNA vaccines consist of DNA that is administered directly into the body. These vaccines can be administered with a needle and syringe or with a needle-less device that uses high-pressure gas to shoot microscopic gold particles coated with DNA directly into cells. Sometimes, the DNA is mixed with molecules that facilitate its uptake by the body's cells. Naked DNA vaccines being tested in humans include those against the viruses that cause influenza and herpes.

Recombinant Vector Vaccines

Recombinant vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. "Vector" refers to the virus or bacterium used as the carrier.

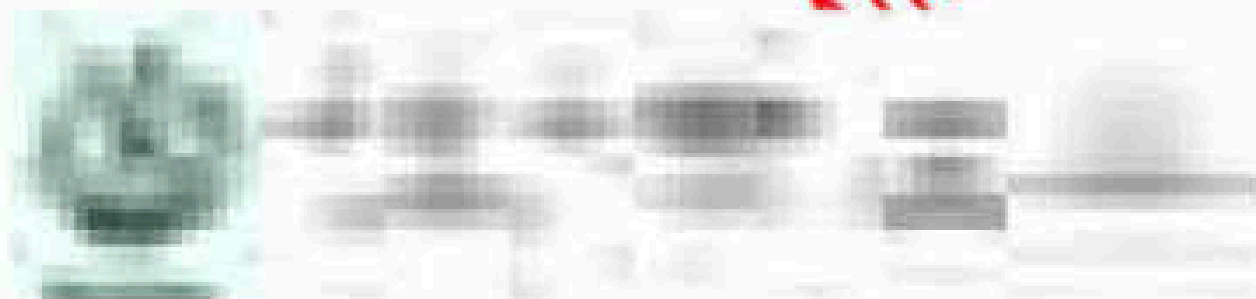
In nature, viruses latch on to cells and inject their genetic material into them. In the lab, scientists have taken advantage of this process. They have figured out how to take the coding portions of certain harmless or attenuated viruses and insert portions of the genetic material from other microbes into them. The carrier viruses then ferry that microbial DNA to cells. Recombinant vector vaccines closely mimic a natural infection and therefore do a good job of stimulating the immune system.

Attenuated bacteria also can be used as vectors. In this case, the inserted genetic material causes the bacteria to display the antigens of other microbes on its surface. In effect, the harmless bacterium mimics a harmful microbe, provoking an immune response.

What Is a Vaccine Adjuvant?

An adjuvant is a substance that is formulated as part of a vaccine to enhance its ability to induce protection against infection. The word "adjuvant" comes from the Latin *adjuvare* and means "to help." Adjuvants help activate the immune system, allowing the antigen—pathogen components that elicit an immune response—in vaccines to induce long-term protective immunity. An effective vaccine stimulates both arms of the immune system: innate immunity and adaptive immunity. Innate immunity occurs within hours, as immune cells recognize a pathogen. Subsequently, the adaptive immune response develops over several days and involves coordination and expansion of adaptive immune cells called T and B cells. This leads to immune memory, when cells highly specific to the pathogen are retained for later use in case of re-infection. Adjuvants are important for activating the innate immune response, resulting in improved adaptive immunity with enhanced activation of T and B cells.

The first human vaccines were based on weakened or killed pathogens that cannot cause disease. These vaccines contain naturally occurring adjuvants and antigens from the encapsulated pathogens and can elicit strong protective immune responses. Many of these types of vaccines are still widely used. For example, the seasonal flu shot contains killed influenza virus and the nasal spray flu vaccine includes weakened virus, which is unable to cause flu illness. Most vaccines developed today include only the antigens that best stimulate the immune system, such as proteins, rather than the entire virus or microbe. For example, the egg-free flu vaccine contains an influenza virus protein that is produced in cell culture. Although this design makes vaccines safer and easier to produce, it often requires the incorporation of adjuvants to elicit a strong protective immune response, because the antigens alone are not sufficient to induce adequate immunity and long-term protection.



The egg-free flu vaccine contains haemagglutinin (HA), the surface protein that binds influenza virus to the cell being infected, rather than killed influenza virus. HA antigen is produced using recombinant DNA technology. The HA gene is placed into baculovirus, a carrier virus that infects insect cells and is harmless to humans. Baculovirus-infected insect cells produce HA, which scientists harvest and purify from cell culture for inclusion in the vaccine.

Adjuvants are generally classified into two groups: vehicles and immunostimulants with some components sharing both properties. Vehicles are usually of particulate nature (mineral salts, e.g. aluminium hydroxide, emulsions, liposomes). They afford the display of selected antigens in multiple copies, mimicking natural display by microorganisms and are used to deposit the antigen at the site of administration or to increase its delivery into APCs (micro- and nanoparticles). In contrast, the immunostimulants (TLR agonists, saponins, cytokines...) directly target and activate cells of the immune system and increase the immune response to antigens.

(Figure 2). Within this group of adjuvants, those targeting the pathogen recognition receptors (PRRs) of the innate immune system have received considerable attention over the last decade.

Principal properties of an adjuvant:
Enhance speed, strength, and duration of the immune response
Affords broadening of antibody cross-reactivity
Select, or modulate cell-mediated versus humoral immunity
Favors MHC class I or II presentation
Direct through T helper cell stimulation
Elicit both T helper and cytotoxic lymphocytes
Decrease the amount of antigen needed for protection (antigen dose sparing)
Increase immune response to vaccines in immunologically immature, immunosuppressed or senescent individuals
Increase the immunogenicity of weakly immunogenic antigen
Modulate antibody isotype, subclass, quantity and specificity

PRRs allow innate immune cells to recognize microbial or infectious components, known as pathogen associated molecular patterns (PAMPs) and components released from injured or dying cells, known as damage associated molecular patterns (DAMPs). These PAMPs and DAMPs binding to PRRs, induce inflammatory response that will in turn enhance the adaptive immune response. There are four classes of PRRs: Toll-like receptors (TLRs), Nod-like receptors (NLRs), RIG-I like receptors (RLRs) and C-type lectin Receptors (CLRs). Nearly all members of the PRR family are considered to be receptors for adjuvants. A third group of adjuvants formed by the combination of these immunostimulants with particulate delivery vehicles and referred to as "adjuvant system" or "adjuvant combinations" is emerging as a group comprising some of the most potent adjuvants. This group comprises the combination of TLR agonists with Alum, emulsions or liposomes.

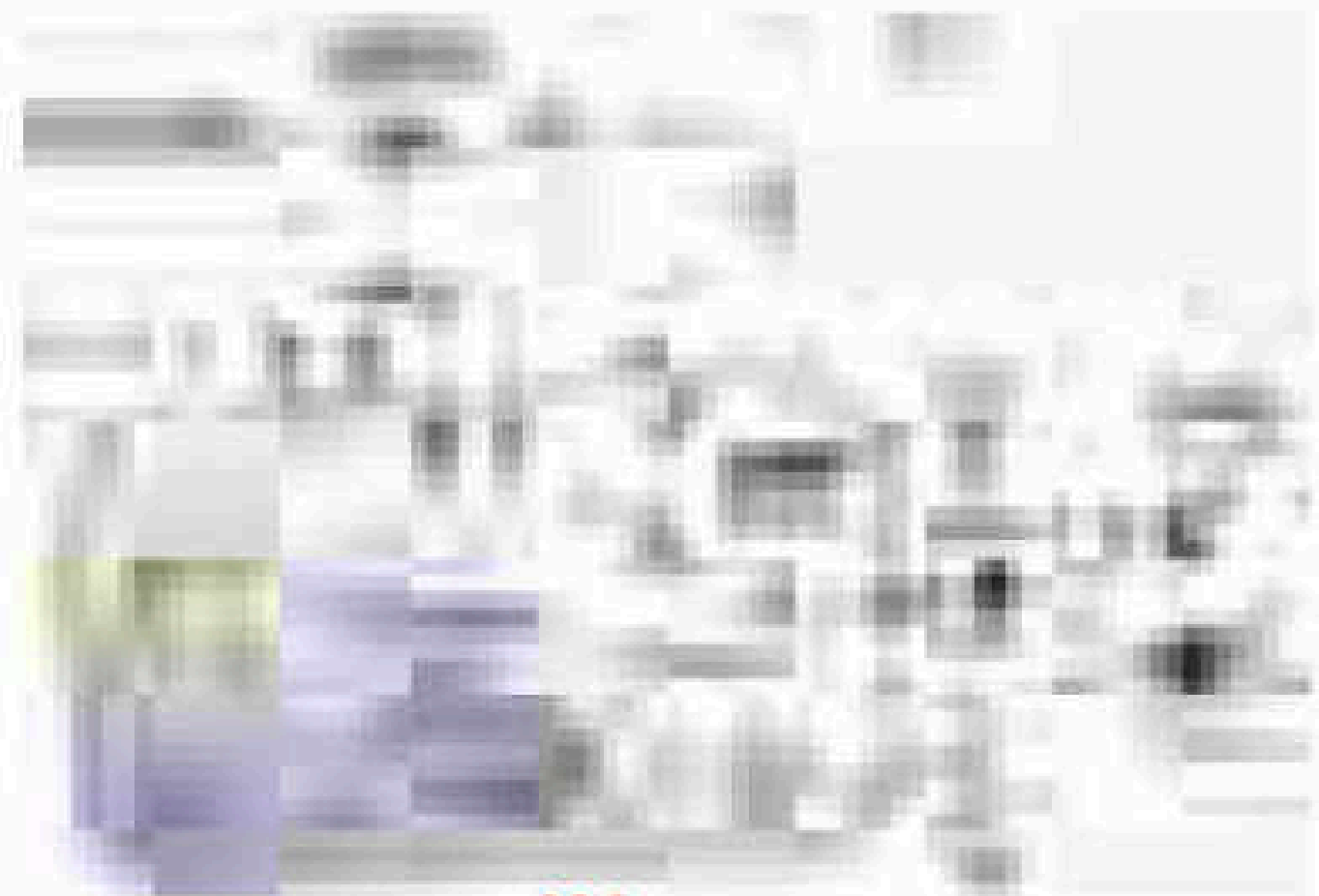


Figure 2. Adjuvanted antigen activation of APC.

FREUND'S ADJUVANTS

Incomplete Freund's Adjuvant (IFA) = strong Th2 immune response + low Th1 response

Complete Freund's Adjuvant (CFA) = Strong Th1 immune response

IFA is Incomplete Freund's Adjuvant (IFA) and **CFA** is Complete Freund's Adjuvant (CFA) are water-in-oil emulsions consisting in mixtures of mineral oil and an emulsifier in a ratio of 85% v/v oil and 15% v/v emulsifier. Contrary to the Complete Freund's Adjuvant (CFA), IFA does not contain heat-killed mycobacteria (*Mycobacterium tuberculosis*). IFA and CFA have been used extensively in experimental immunology and IFA has been used for decades in practical veterinary vaccination. Importantly, Freund's adjuvants are not pre-formed emulsions and thus they must be mixed with an equal volume of aqueous solution of antigens and subsequently emulsified prior to use. Even if the mechanisms of action of oil emulsions are still

poorly understood, some evidences suggest a partial requirement for NOD2. Moreover, these entities are prone to cause cellular damage upon injection and thus, endogenous signals released during necrotic cell death may also contribute to their adjuvant activity. Immune response directed by CFA is dramatically enhanced by the presence of the mycobacterial component that attracts macrophages and immune cells at the site of injection. CFA induces principally a Th1 response and can cause granulomas and an intense inflammatory reaction at the inoculation site. Indeed, the use of CFA should be used responsibly and with care in order to avoid or minimize the adverse effects of excessive inflammation. As opposed to CFA that induces principally a Th1 response, IFA that lacks mycobacterial components induces a Th2 response^[10]. For most applications, CFA is usually only necessary for the initial immunization, while IFA is the adjuvant of choice for subsequent immunization. CFASol and CFASolVax adjuvants are designed to provide continuous release of antigen necessary for stimulating strong persistent immune response.

ALUMINIUM ADJUVANT (EFFECT ON IMMUNE RESPONSE)

Alum is the most common adjuvant used in approved prophylactic vaccines because of its excellent safety profile and ability to enhance protective humoral immune response (Th2). It consists of precipitates of aluminum phosphate and / or aluminum hydroxide to which antigens are adsorbed through hydrophobic and electrostatic interactions or entrapped. [Alum/As hydroxide](#) is positively charged at a physiological pH of 7.4 and binds acidic proteins. [Alum/As phosphate](#) on the other hand is negatively charged and therefore binds basic proteins. The association of antigen with Alum favors a high local antigen concentration and an improved uptake by APC. Thus early observations described that Alum enhances the response to antigen by extending the time during which the antigen is available. Furthermore, it is now admitted that Alum like many other adjuvants, acts by direct activation of the immune cells. In mice, Alum induces a profoundly polarized Th2 immune response which is characterized by the production of IL-4 and IL-5 and the strong induction of antibodies production as immunoglobulin IgG and

IgG1 [12-13]. Consequently, Alum is very effective against pathogens that require Th2 humoral-mediated immunity. In parallel, Alum alone can activate the NLRP3 inflammasome to produce mature IL-1 creating a favorable environment for the immune response. Nonetheless, Alum fails to induce Th1 responses associated with the induction of INF- γ and cytotoxic T lymphocytes which are required to clear the body of intracellular infections.

Different aluminum salts are contained in numerous licensed vaccines (see table below) and their safety profiles have been well established through the use of billions of doses of aluminum-containing vaccines administered to individuals from infants to elderly.

Aluminum Salts	Vaccines
$Al(OH)_3$ – aluminum hydroxide and/or	Pertussis, Diphtheria
$AlPO_4$ – aluminum phosphate	Tetanus, HBV + HAV
$(Al)_2PO_4SO_4OH$ – aluminum hydroxyphosphate sulphate	HPV

HBV, Hepatitis B virus; HAV, Hepatitis A virus; HPV, human papillomavirus

SQUALENE EMULSION (STRONG IMMUNE RESPONSE – BALANCE TH1/TH2 RESPONSE)

[Squal/Vax](#) is an oil-in-water emulsion made of squalene droplets in a continuous aqueous phase. Squalene droplets are stabilized by the addition of two non-ionic surfactants that are widely used as emulsifiers in food cosmetics and pharmaceuticals. It is fully biodegradable, which is an important advantage over alternative oils used in emulsion adjuvants, like Freund's adjuvant that contains mineral oil (paraffin oil) and thus has long term persistence in the organisms.

Squalene emulsion induces local stimulation and recruitment of DCs and granulocytes, differentiation of monocytes into DCs and increased uptake of antigen by APCs. The emulsion acts more specifically on macrophages present at the site of injection. A local increase in chemokines release also influences the recruitment of immune cells from the blood to the site of vaccination, creating an amplification loop. This type of formulation enhances differentiation of monocytes towards a mature phenotype, thereby promoting migration of antigen-loaded cells to the draining lymph node. Compared to aluminum salts, a stronger immune response is elicited (e.g. higher antibody and T-cell response) with a mixed and more balanced Th1/Th2 cell

phenotype. Squalene emulsions are present in licensed seasonal and pandemic influenza vaccines. They enhance immune response in a challenging population such as elderly and can facilitate immune response against specific drift variants of the seasonal influenza virus not included in the vaccine.

Vaccine production

Vaccines are produced in large scale as they need to be administered to large populations of children and adults to be effective as a public health tool. This large scale production is often a challenge.

Stages of vaccine production

Vaccine production has several stages. Process of vaccine manufacture has the following steps:

- Inactivation – This involves making of the antigen preparation
- Purification – The isolated antigen is purified
- Formulation – The purified antigen is combined with adjuvants, stabilisers and preservatives to form the final vaccine preparation.

Generating the antigen from the microbe

The first step in order to produce a vaccine is generating the antigen that will trigger the immune response. For this purpose the pathogen's proteins or DNA need to be grown and harvested using the following mechanisms. For this the virus or microbe is grown either on primary cells such as chicken eggs (e.g. in influenza) or on cell lines or cultured human cells (e.g. Hepatitis A). Bacteria against which the vaccines are developed may be grown in bioreactors (e.g. Haemophilus influenzae type b). The antigen may also be a toxin or toxin from the organism (e.g. Diphtheria or tetanus) or it may be part of the microorganism as well. Proteins or parts from the organism can be generated in yeast, bacteria, or cell cultures. Bacteria or viruses may be weakened using chemicals or heat to make the vaccine (e.g. polio vaccine).

Release and isolation of the antigen

The aim of this second step is to release as much virus or bacteria as possible. To achieve this, the antigen will be separated from the cells and isolated from the proteins and other parts of the growth medium that are still present.

Purification

In a third step the antigen will need to be purified in order to produce a high purity/quality product.

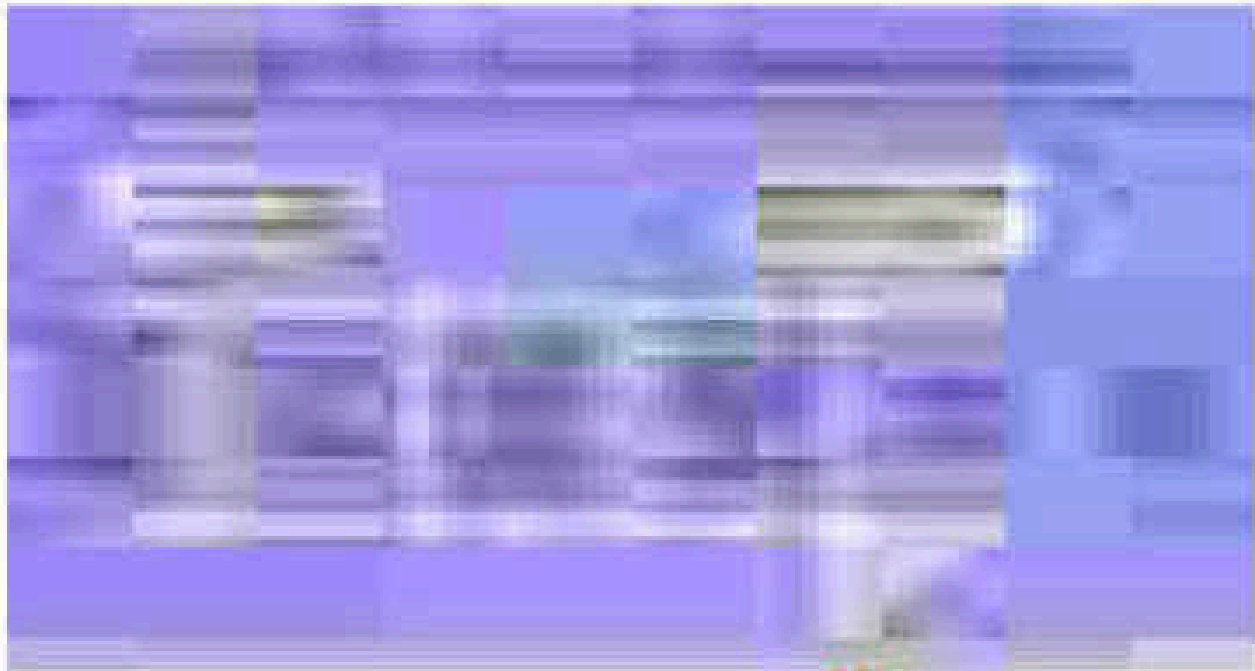
This will be accomplished using different techniques for protein purification. For this purpose several separation steps will be carried out using the differences in for instance protein size, physico-chemical properties, binding affinity or biological activity.

Addition of other components

The fourth step may include the addition of an adjuvant, which is a material that enhances the recipient's immune response to a supplied antigen. The vaccine is then formulated by adding stabilisers to prolong the storage life or preservatives to allow multi-dose vials to be used safely as needed. Due to potential incompatibilities and interactions between antigens and other ingredients, combination vaccines can be more challenging to develop. Finally, all components that constitute the final vaccine are combined and mixed uniformly in a single vial or syringe.

Packaging

Once the vaccine is put in recipient vessel (either a vial or a syringe), it is sealed with sterile stoppers. All processes described above will have to comply with the standards defined for Good Manufacturing Practices that will involve several quality controls and an adequate infrastructure and separation of activities to avoid cross-contamination, as shown in the diagram below. Finally, the vaccine



Mercurial mining

Environmental contamination by heavy metals from anthropogenic and industrial activities has caused considerable irreparable damage to aquatic ecosystems. Sources include the mining and smelting of ores, effluent from storage batteries and automobile exhaust, and the manufacturing and inadequate use of fertilizers, pesticides, and many others. The metals and metalloids that contaminate waters and are most commonly found in the environment include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold, and nickel. These metals are the subject of concern due to their high toxicity. Apart from being hazardous to human health, they also have an adverse effect on the fauna and flora, and they are not biodegradable in nature. Thus, there is a need to seek new approaches in developing treatments to minimize or even eliminate metals present in the environment.

Several different physicochemical and biological processes are commonly employed to remove heavy metals from industrial wastewaters before their discharge into the environment. Conventional physicochemical methods such as electrochemical treatment, ion exchange, precipitation, ion-exchange, evaporation, and sorption are not cost-effective, and some of them are not environmentally friendly. On the other hand, bioremediation processes show promising results for the removal of metals, even when present in very low concentrations where physicochemical

removal methods fail to operate. Furthermore, this is an eco-compatible and economically feasible option. The bioremediation strategy is based on the high metal binding capacity of biological agents, which can remove heavy metals from contaminated sites with high efficiency. In this regard, microorganisms can be considered as a biological tool for metal removal because they can be used to concentrate, remove, and recover heavy metals from contaminated aquatic environments. Several studies have been conducted using microorganisms for the uptake of heavy metals in polluted waters as an alternative strategy to conventional treatments. Bioremediation by microorganisms is very useful due to the action of microorganisms on pollutants, even when they are present in very dilute solutions, and they can also adapt to extreme conditions. Although the mechanisms associated with metal bio sorption by microorganisms are still not well understood, studies show that they play an important role in the uptake of metals and that this action involves accumulation or resistance.

The first microorganisms appeared on Earth 3.7 billion years ago at a time when there was no free oxygen and the atmosphere was composed of methane, carbon dioxide, ammonia and hydrogen. They have since evolved to inhabit almost all parts of the globe, developing many systems to deal with life at the extremes. Some microorganisms can grow in strongly acidic conditions, withstand levels of heavy metals fatal to most other life forms, live in temperatures above the boiling point of water and withstand radiation levels much greater than we can tolerate. Many microorganisms make significant contributions to environmental cycles, such as the fixation of nitrogen, the cycling of carbon and the transformation of a range of metals such as iron, manganese, gold, copper and uranium. Microorganisms are used in a vast number of industrial processes, ranging from chocolate production to gold extraction. Since ancient times we have relied on them in the manufacture of alcohol, bread and cheese. Microorganisms can catalyze reactions that, in energy terms, are only marginally favorable. We have the technology to track what microorganisms in the environment do and to apply it to our industrial processes, encompassing all of those billions of years of evolutionary refinement into simple solutions for seemingly complex problems.

Bioremediation

Around 3000 BCE, mining along the banks of Spain's Rio Tinto River began. Miners sourced ore and placed it in beds along with water from the local river. Unbeknown to them, the

microorganisms in the river water were breaking down the sulfide-containing ores to extract copper. The river is very acidic and rich in heavy metals, so no vegetation or animals live along its shores. Not until the 1940s was it recognized that microorganisms there played a role in the dissolution of metals from their ores, a process termed "bioleaching". The microorganisms commonly associated with bioleaching and bio-oxidation (see below) are chemolithoautotrophs. As the name suggests, their energy is obtained by oxidizing inorganic iron and/or sulfur and using the freed electrons in energy production and using carbon dioxide from the atmosphere as their source of carbon. Generally, the pH of bioleaching operations is within the range of 1.2–2.0, and hence these microorganisms are acidophilic. As a result of the oxidation of iron and/or sulfur at low pH by these bioleaching microorganisms, minerals trapped in the ore become soluble. These solubilized metals can then be processed using traditional methods, such as solvent extraction and electro winning. By the end of the 19th century, Rio Tinto was a full-scale mining operation and was providing materials to fuel the development of Europe. When the English took over the mine, they switched from smelting to modern metal-processing techniques such as smelting. These changes caused a vast amount of pollution from the mine to leak into the environment. The main problem was the emission of sulfur dioxide gases, which affected anybody within 15–20 km. The people of the area demonstrated against the mine, calling for better health and safety conditions. The military retaliated against the people and at least 200 men, women and children were killed. Soon after this, the Rio Tinto mine closed down, but the method of bioleaching was not about to make its way out of Europe. In 1959 the first commercial bioleaching operation began at the Bingham Canyon Mine in Utah, USA. Since that time it has been an expanding industry driven heavily by the benefits of using the technology over traditional mining processing methods. Scientists have since been able to use their knowledge to expand the application of these microorganisms to the extraction of many types of metals. Currently it is estimated that ore processed using microorganism's accounts for 20% of the world's copper supply. A number of other metals have now been extracted using bioleaching, including gold, silver, uranium, nickel, zinc, lead and cobalt.

Bio-oxidation

Bio-oxidation is another method in which microorganisms help to extract a metal of interest. However, during bio-oxidation the metal of interest remains in an insoluble form. Hence, bio-oxidation is often used as a pretreatment method prior to metal extraction, such as the extraction

of ores containing gold. After bio-oxidation, the gold is extracted by a more traditional method such as cyanide extraction. Bio-oxidation is a relatively new technique, with the first commercial operation opening in the 1960s in Utah. During the past 20 years the method has expanded rapidly, specifically for the treatment of refractory gold ores. BIOXIM is the most commonly used bio-oxidation process, and has been used since 1986 to recover gold from sulfide-containing ores. During this process, flotation concentrate is mixed with nutrients to promote microbial growth. This is then added to a series of reactors where a selected culture of microorganisms break down the sulfide within the ore. The product is then cleaned, leached by traditional metal extraction methods, such as cyanidation. Finally the waste products are pH-adjusted and made safe for disposal in a tailings dam. Stirred tank bio-oxidation is the latest method of bioleaching but, due to higher costs, is only appropriate for large operations and is currently being used at ten different sites across the world.

ENVIRONMENTAL APPLICATIONS OF MICROORGANISMS

Microorganisms mitigate a multitude of impacts that result from human use of the natural resources of the planet. First and foremost, the essential role of microorganisms in the treatment of wastewater is critical to the well-being of life on Earth. Bioremediation, biotining, and microbial desulfurization of coal are other large-scale processes in which important positive environmental outcomes are achieved by directly exploiting the combined metabolic capabilities of naturally occurring communities of microorganisms. In such applications, the functioning of a particular microbial community can be influenced through the manipulation of conditions (e.g., nutrients, oxygen levels, temperature, agitation).

WASTEWATER TREATMENT

Living organisms consist of about 70% water. A human being, for instance, has to consume an average of 1.5L/day to survive. Fresh water represents only about 2.5% of the water on the planet and is now a scarce resource in many parts of the world. The volume of water being contaminated and the need to reclaim wastewater are increasing with the growth in population and industrial use. Wastewater originates from four primary sources: sewage, industrial effluents, agricultural runoff, and storm water and urban runoff. Treatment of wastewater is essential to prevent contamination of drinking water and the entry of pathogens and contaminants into the

food chain. Given the number and variety of the substantial contaminants, the success of some of the current treatments for the reclamation of pure water is little short of amazing. Primary treatment of sewage consists of removal of suspended solids. The secondary treatment of sewage reduces the biochemical oxygen demand. This is accomplished by lowering the organic compound content of the effluent from the primary treatment through microbial oxidation by an incompletely characterized community of microorganisms in "activated sludge." Bacteria of *Zooploea* species play an important role in the aerobic secondary stage of sewage treatment. These organisms produce abundant extracellular polysaccharide and, as a result, form aggregates called flocs. Such aggregates efficiently adsorb organic matter, part of which is then metabolized by the bacteria. The flocs settle out and are transferred to an anaerobic digester, where other bacteria complete the degradation of the adsorbed organic matter. The microbial communities in a water treatment plant convert organic carbon to carbon dioxide, water and sludge, converting 80% of the ammonia and nitrate to molecular nitrogen, remove some soluble phosphate through incorporation into the sludge, 60% as polyphosphate granules within bacterial cells or as struvite (crystalline $MgNH_4PO_4$), and remove pathogenic bacteria. However, serious challenges in wastewater treatment have yet to be fully addressed. The level of residual fixed nitrogen compounds and phosphorus in the effluent is still high enough to pose risks of eutrophication in the receiving bodies of water. Residues of many widely used pharmaceuticals present in municipal wastewater are incompletely removed and emerge in the effluent. Some of these compounds are biologically active at nanograms per liter and have demonstrable undesirable environmental effects. The chemical industry uses thousands of synthetic organic compounds in huge amounts, and many of these (or their degradation products) pose a similar concern to the pharmaceutical ones. Finally, wastewater treatment consumes energy but converts much of the ammonia and nitrate to nitrogen gas, and a significant amount of the phosphate remains in the effluent. Alternative processes that would recover the fixed nitrogen compounds and phosphate would offset the energy and economic costs of manufacturing the corresponding amount of chemical fertilizer and lessen the acute environmental problems associated with elevated nutrient levels in aquatic ecosystems.

REFERENCES

Bioremediation depends on the activities of living organisms to clean up pollutants dispersed in the environment. Physical or chemical treatments, such as vaporization, extraction, or adsorption, relocate rather than remove pollutants. In contrast, there are many instances in which biodegradation converts organic pollutants to harmless inorganic products, including carbon dioxide, water, and halide ions. Other advantages are that bioremediation is generally inexpensive and causes little disturbance to the environment. Naturally occurring consortia, frequently dominated by bacteria, have the capacity to degrade a wide variety of environmental pollutants. Notably, such consortia are responsible for the cleanup of major oil spills. There is a long list of oil spills with serious environmental impact.

Following are three of many examples of this type of widely dispersed problem. In March 1989, some 41 million liters (~10.5 million gallons) of crude oil escaped from the tanker *Exxon Valdez* and contaminated more than 2000 km (~1250 miles) of rocky, mountain coastline in Alaska. In 1991, during the Gulf War, huge amounts of oil were released into the marine environment, with devastating impact on marine life. In 1997, more than 100 tons of heavy oil leaked from the Russian tanker *Malheur*, which ran aground and sank in the Sea of Japan. The oil contaminated more than 500 km (~310 miles) of the coastline. Over time, in all of these cases, the endogenous microbial community largely degraded the oil. In the case of the *Exxon Valdez*, the activity of the naturally occurring hydrocarbon-degrading bacteria at the spill site was enhanced by the addition of fertilizer containing organic nitrogen compounds and inorganic phosphorus compounds. Many thousands of organic and inorganic compounds are used daily around the world in hundreds of thousands of products. These compounds are introduced either accidentally or on purpose into the soil and groundwater. The seriousness of the problem posed by the introduction of human-made contaminants into the environment is highlighted by the following pronouncement by the

World Health Organization (2003). The government's new reporting goal with regard to chemicals is that by 2020, there should no longer be any products or goods on the market containing chemicals with particularly problematic health or environmental impacts. Among such pollutants, highly chlorinated compounds have received particular attention because of their known and potential adverse environmental and health impacts. One class of such compounds includes highly chlorinated aliphatics such as trichloroethylene, tetrachloroethene, 1,1,1-trichloroethane, and carbon tetrachloride, which are used as dry cleaning fluids and degreasing solvents. Another class is represented by highly chlorinated aromatics such as pentachlorophenol (wood preservative), polychlorobiphenyls (insulators, heat exchangers), and dioxins (combustion

byproducts). These compounds are either fully or partially degraded by the combined activities of various endogenous microorganisms under aerobic or anaerobic conditions. By and large, the natural attenuation of chlorinated organic compounds at many different sites by the action of endogenous microbial populations, whether under aerobic or anaerobic conditions, is slow, is incomplete, and, in some cases, has resulted in the formation of toxic products. Cleanup of sites contaminated by radionuclides poses an exceptionally challenging problem of great importance. A U.S. Department of Energy (DOE) report summarizes the situation succinctly. With the end of the Cold War threat in the early '90s and the subsequent shutdown of all nuclear weapons production reactors in the United States, DOE has shifted its emphasis to remediation, decommissioning, and decontamination of the immense volumes of contaminated water and soils, and the over 7,000 structures spread over 120 sites (7,280 square kilometers) in 36 states and territories. DOE's environmental legacy includes

1.7 trillion gallons of contaminated ground water in 5,700 affected plumes, 40 million cubic meters of contaminated soil and debris, and 3 million cubic meters of waste buried in landfills, trenches, and spill areas.

Subsurface bioremediation of such sites has attracted much attention. A key objective is to stabilize the buried wastes in place to prevent leaching and widespread contamination of groundwater. The most common radioactive components in these wastes are uranium (U), strontium (Sr), plutonium (Pu), cesium (Cs), and technetium (Tc).

BIOLEACHING FOR METAL EXTRACTION USING MICROORGANISMS

Bioleaching utilizes naturally occurring prokaryotic communities. Here, microorganisms are used to leach metals, especially copper but also nickel and zinc, from low-grade sulfide- and/or iron-containing ores. The process exploits the energy metabolism of various acidophilic chemolithoautotrophic, but utilize inorganic compounds as energy sources and CO_2 as the source of carbon. These organisms use either ferrous iron or sulfide as an electron donor and oxygen as an electron acceptor with the formation of ferric iron or sulfuric acid. In the first case, the subsequent reaction of Fe^{3+} with insoluble metal sulfides yields soluble metal sulfates; in the second, metal sulfides are oxidized directly to metal sulfates. The metals are readily recovered from the leachate by electrolytic procedures, and the residual solution is recycled. Gold is leached by microbial action. However, bioleaching of sulfidic gold-containing ores under acidic conditions

opens up the interior of the ore particles to solvent. After bioleaching, the ore is rinsed with water and the gold is solubilized with a cyanide solution. Current research on bioleaching is directed to improving understanding of the microbiology of the leaching process and to exploring the use of microbes that grow at high temperatures.

MICROBIAL DESULFURIZATION OF COAL

Coal contains substantial amounts of sulfur, both in pyrite (FeS_2) and in organic sulfur compounds (predominantly thiophene derivatives). The composition of coal varies considerably depending on the source. For example, Texas lignite coal contains 0.4% pyrite S and 0.8% organic S, whereas Illinois coal contains 1.2% pyrite S and 3.2% organic S by weight. When coal is burned, most of this sulfur is converted to SO_2 . The SO_2 combines with moisture in the atmosphere to form sulfuric acid (H_2SO_4), a major component of acid smog and acid rain. Microbial desulfurization of coal, by converting the pyrite to ferrous sulfate and leaching it out of the coal (see "Bioleaching" earlier), provides one way of combating this problem. As much as one or two weeks are required to complete the desulfurization, and large areas of land are required for the leach heaps and the storage of coal.

MICROBIOLOGICAL DEGRADATION OF XENOBIOTICS

Natural biological and geochemical processes produce enormous quantities of organic compounds with a great diversity of structures. Nearly every one of these compounds can be utilized by some microorganism as a source of energy and/or of cell building blocks. However, many of the tens of thousands of organic compounds produced artificially by chemical synthesis for industrial or agricultural purposes have no obvious counterparts in the natural world. Such synthetic compounds are called *xenobiotics* (from the Greek "foreign"), and many of these compounds are stable in the environment both under aerobic and anaerobic conditions. The ever-growing list of xenobiotics released into the environment on a large scale includes numerous halogenated aliphatic and aromatic compounds, nitroaromatics, phthalate esters, and polycyclic aromatic hydrocarbons. These compounds enter the environment through many different paths. Some, as components of fertilizers, pesticides, and herbicides, are distributed by direct application. Others, such as the polycyclic aromatic hydrocarbons, dibenzo-p-dioxins, and dibenzofurans, are released by combustion processes. And of course many kinds of xenobiotics

are found in the waste effluents produced by the manufacture and consumption of all the commonly used synthetic products. Various xenobiotics are found in particular environments in concentrations ranging from parts per thousand (ppt) to parts per billion (ppb). The local concentration depends on the amount of the compound released, the rate at which it is released, the extent of dilution in the environment, the mobility of the compound in a particular environment (e.g., in soils), and its rate of degradation, both biological and nonbiological. Many toxic xenobiotics present in the environment in parts per billion levels at which toxicity cannot be demonstrated, are nevertheless strictly regulated. Such regulation is necessary when a compound becomes progressively more concentrated in each link of a food chain – a process called *biomagnification*. The first study to measure biomagnification was carried out in Clear Lake in northern California. In 1949, Clear Lake had been treated with the persistent pesticide dichlorodiphenylchloroethane (DDD), a close relative of DDT, at 0.01 to 0.02 ppm of water to control the gnat *Culiseta inornata*. By 1954, western grebe, duck like birds, began dying around the lake. The body fat levels of DDD in the grebes were found to be 1600 ppm, some 100,000 times higher than the DDD concentration in the water.

Biobank

A **biobank** is defined as any bank whose primary objective is to store biological samples for research. This definition serves to make our understanding of biobanks as clear as possible, so let's unpack it a bit.

Biological Carbon Fixation

Carbon fixation is a process that takes inorganic carbon (in the form of things like CO_2) and converts it into organic compounds. In other words, any process that converts carbon dioxide into a molecule that would be found in a living organism is carbon fixation. If this process occurs in a living organism, it is referred to as biological carbon fixation.

Fuel

The next part of the definition of a biobank involves fuel. A fuel is nothing more than something from which we humans can get energy. Carbon fixation can lead to a number of different

compounds, like proteins, fats, and alcohols (just to name a few). If any of these molecules can be used to provide energy in a mechanical setting, we call it a fuel.

The Real Definition of a Biofuel and the Practical Definition

A biofuel is a hydrocarbon that is made by or from a living organism that we humans can use to power something. The definition of a biofuel is rather formal. In practical consideration, any hydrocarbon fuel that is produced from organic matter (living or once living material) in a short period of time (days, weeks, or even months) is considered a biofuel. This contrasts with fossil fuels, which take millions of years to form and with other types of fuel which are not based on hydrocarbons (nuclear fission, for instance).

What makes biofuels tricky to understand is that they need not be made by a living organism, though they can be. Biofuels can also be made through chemical reactions, carried out in a laboratory or industrial setting, that use organic matter (called biomass) to make fuel. The only real requirements for a biofuel are that the starting material must be CO_2 that was fixed (turned into another molecule) by a living organism and the final fuel product must be produced quickly and not over millions of years.

Biomass

Biomass is simply organic matter. In other words, it is dead material that was once living. Kernels of corn, roots of sugar beets, and stalks of sugar cane are all biomass. Before global warming related to burning fossil fuels became a major factor in determining where energy came from, the major concern was that fossil fuels, which are considered limited in supply, would run out over the next century. It was thought that if we could produce hydrocarbons another way, and quickly, then we could meet our energy demands without much problem. This leads to one of the major separating factors between a biofuel and a fossil fuel - renewability. Biomass refers to anything that is living or was once living. Bioenergy feedstock came from wide range of sources: household waste, woodchips with proportion coming from agriculture - such as extensive use of straw in Denmark, the use of energy crop in UK and production of energy crops for biogas in Austria. The majority of biofuel come from food crops, either sugars or oils, what are called first generation biofuel. There are incentives in many countries for increased use of energy crop and

in stationary and transport biomass which put pressure on availability of land for food production and negative environment consequences.

Ethanol is a type of alcohol that can be produced using any feedstock containing significant amounts of sugar, such as sugar cane or sugar beet, or starch, such as maize and wheat. Sugar can be directly fermented to alcohol, while starch first needs to be converted to sugar. The fermentation process is similar to that used to make wine or beer, and pure ethanol is obtained by distillation. The main producers are Brazil and the USA. Ethanol can be blended with petrol or burned in nearly pure form in slightly modified spark-ignition engines. A liter of ethanol contains approximately two thirds of the energy provided by a liter of petrol. However, when mixed with petrol, it improves the combustion performance and lowers the emissions of carbon monoxide and sulfur oxide.

Biodiesel is produced, mainly in the European Union, by combining vegetable oil or animal fat with an alcohol. Biodiesel can be blended with traditional diesel fuel or burned in its pure form in compression ignition engines. Its energy content is somewhat less than that of diesel (85 to 95%). Biodiesel can be derived from a wide range of oils, including rapeseed, soybean, palm, coconut or jatropha oils and therefore the resulting fuel can display a greater variety of physical properties than ethanol.

Biogas is a fuel used as domestic purpose • Obtained from cow manure, fat and vegetable waste • Biogas is produced by the breakdown of organic waste by bacteria without oxygen anaerobic digestion.

Is Biomass Carbon Neutral ?

Biomass has long been investigated both as a (nearly) CO₂ neutral substitute for fossil fuel and as a means for sequestering carbon in terrestrial ecosystems. More recently, the potential to integrate carbon capture and storage technologies conceived to enable fossil fuel use without atmospheric CO₂ emissions with Bioenergy systems has emerged as a means to capture atmospheric carbon, fixed through photosynthesis, and sequester it from the atmosphere for geologic timescales. The ability of such integrated systems to produce energy products with negative net atmospheric carbon emissions could have important implications for mitigating anthropogenic climate change. The scale and timing of biomass-based mitigation is limited by

the availability and cost of conversion technologies, many of which are currently inefficient or technologically immature.

Biofuel and climate ethics

The term "biofuel" is a relatively recent neologism. Usage in earlier decades covered a wide variety of organism energy sources, such as "the biofuel cell" that was intended to power devices implanted in the body. It is today more generally used to indicate liquid fuels derived from plant-based sources that have been developed primarily for transportation. Plant-based liquid transportation fuels have been identified as a means to reduce harmful climate-related effects of fossil fuels. The emissions-remediation argument for biofuel is both elegant and relatively simple. When petroleum-based fuels are burned, they release carbon and other greenhouse gases into the atmosphere. The crude oil that is the source of most gasoline, diesel and kerosene liquid transportation fuels is mined from geologic sources, as is most natural gas. The carbon dioxide introduced into the atmosphere from the combustion of these fuels is now widely accepted to be altering the overall composition of atmospheric gases, precipitating the processes known as climate change. When crop transportation fuels derived from plants are burned, they also release carbon into the atmosphere. However, the process of plant growth removes carbon dioxide from the atmosphere through photosynthesis. Thus, the carbon released from burning of plant-based fuel is, in effect, being cycled through the atmosphere. It does not add to the proportion of carbon dioxide in the atmospheric composition and as such does not contribute to the processes that are thought to cause climate change. Shifting a proportion of liquid fuel usage to sources derived from plants, including algae, has been identified as one of the climate stabilization strategies by the Climate Mitigation Initiative (CMI).

The Ethics of Biofuels and the Nuffield Council report.

1. Biofuel development should not be at the expense of people's essential rights. 

2. Biofuels should be environmentally sustainable.

3. Biofuels should contribute to net reduction of total GHG emissions and not exacerbate global climate change.

4. Biofuels should recognize the rights of people to just reward.

5. Costs and benefits of biofuels should be distributed in an equitable way.

Mitigation is strongly coupled with a conception of sustainability that is reconciled to the idea that for-profit entities must more than recover their costs in order to be economically sustainable. And public sector entities cannot undercut the private sector without destroying the employment and tax base that is crucial to the economic sustainability of society as a whole. As such, pursuit of environmental sustainability here expressed in terms of mitigation must go hand in hand with economic sustainability. The idea of the triple-bottom line is to harness efforts for social betterment, human rights and the pursuit of justice to the economy and the environment in the search for economic and technological endeavors that are positive in all three dimensions (Rogers and Ryan 2001). Thus, the aim of the Nuffield Council and of the IAC can be accurately characterized as harnessing biofuels development to a triple-bottom line conception of sustainability in which private sector innovation is undertaken in partnership with government activities undertaken by voluntary non-profit organizations, as well as state-based regulatory agencies.

Technology

Biomass is uniquely positioned at the forefront of industrial energy systems and the natural carbon cycle. This enables four basic steps for carbon mitigation:

1. Carbon storage in terrestrial ecosystems by afforestation and conservation.

2. Substitution of bio-energy for coal such as bio-ethanol for diesel fuel.

3. Carbon storage in marine and oceanic biotic of terrestrial biomass in locations such as deep ocean sediments where carbon cycling is slow.

4. Substitution of bio-energy with remote carbon storage.

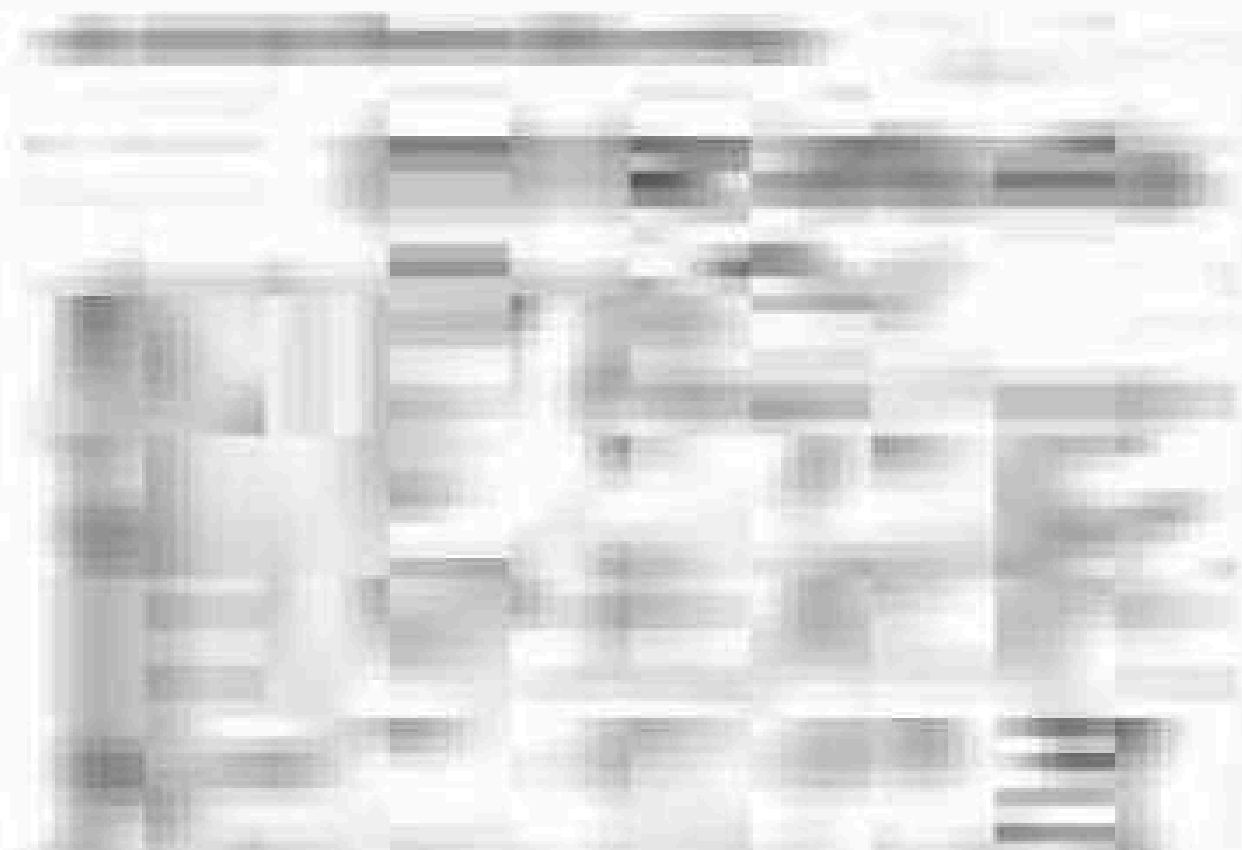
In addition to advancing carbon management objectives, biomass could be used to reduce petroleum consumption. With sufficient external inputs of hydrogen and electricity, essentially all of the carbon in biomass feedstock could be converted to liquid fuels (Agrawal et al., 2007). This differs from conventional biofuels production, in which at most a third of the carbon content of the feedstock ends up in the fuel, with the remainder being oxidized to provide process energy or left as waste. In such a system, biomass is used to capture and recycle carbon from the atmosphere, while primary energy inputs are provided externally. Assuming these inputs are

carbon-free, resulting liquid fuels can be (nearly) carbon neutral. Such systems could provide the largest displacement of petroleum-derived fuels per unit biomass, while biomass-CCS could provide the largest mitigation of CO₂ emissions per unit biomass—due to the double benefit of providing useful energy products and removing carbon from the active carbon cycle.

The technical performance of such pathways remains uncertain. Some, such as capture of CO₂ off-gases from fermentation, while others, such as those reliant on large-scale biomass gasification, may not be commercially viable for a decade or more, even in the presence of a strong carbon price. The mismatch between the scales of economically viable low-energy deployments and CO₂ capture, transport, and storage systems could be a challenge for biomass-CCS. However, supply chains supporting the current biofuel boom suggest that locally-available biomass supplies may not be fundamentally limiting, and biomass-to-liquid co-utilization could help resolve this issue.

Classification of biofuels

Classification According to Food and Agricultural Organization (FAO)



The term "by-products" includes the improperly called solid, liquid and gaseous residues and wastes derived from biomass processing activities.

Biofuels are generally classified as first, second and third generations:

First-generation biofuels are made from sugar, starch, vegetable oil, or animal fat using conventional technology. These are generally produced from grains high in sugar or starch fermented to methanol, or seeds that which are pressed into vegetable oil used in biodiesel. Common first-generation biofuels include vegetable oils, biodiesel, bioethanol, biogas, solid biofuels, and syngas.

Second-generation biofuels are produced from non-food crops, such as cellulosic biofuels and waste biomass (straw of wheat and corn, and wood). Common second-generation biofuels include vegetable oils, biodiesel, bioethanol, biogas, solid biofuels, and syngas. Research continues on second-generation biofuels including biohydrogen, biomethanol, DMF, Bio-DME, Fischer-Tropsch diesel, biohydrogen diesel, mixed alcohols and wood diesel.

Third-generation biofuels are produced from extracting oil of algae – sometimes referred to as “algae”. Its production is supposed to be low cost and high-yielding – giving up to nearly 30 times the energy per unit area as can be realized from current, conventional “first-generation” biofuel feedstocks.

Uses of Biofuels

Transportation

Nearly 30% of all energy consumed in the United States is used in transportation. To put this into perspective, residential and commercial uses combined only account for 10%. That means that humans in industrial nations use, on average, three times more energy to get around than they use to cook their food and heat their homes. This number does not include electricity generation, which accounts for 40% of all energy used.

Globally, transportation accounts for 25% of energy demand and nearly 62% of oil consumed. Most of this energy, two-thirds in fact, is burned to operate vehicles with the rest going to maintenance, manufacturing, infrastructure, and raw material harvesting. If we delve further into the numbers, we find that upwards of 70% of energy consumption in this segment is used to move people around and that most of this is used in private cars, the least efficient means of transportation. Only 12% of the energy burned by a car goes to moving it and only about 2% is actually used to move the occupants. The rest of the energy is lost to friction, heat, inefficient combustion, and moving about ever more heavy vehicles. Estimates are that we have hit peak oil or if we have not, it is very near. We won't actually know that we have peaked until we start down the slope toward the bottom again, but most experts agree that we are quite close. So, oil is running short and when this is combined with the tremendous environmental impact of petroleum recovery, refining, and eventual combustion, the drive for an alternative is clear. The problem with many alternatives, like wind, solar, etc. is that they simply aren't practical. Transporting enough stored electricity derived from these mechanisms to make an average journey is very difficult. Many experts believe that practical breakthroughs in these technologies are decades away at best. So, the challenge is to find a fuel that can replace the practical qualities of oil (like being easy to drive around), but which does not pollute the same way. The solution, at

Just for now, appears to be algal-based biofuels, which are still years if not decades away from commercialization. The idea is simple. Algae have lipid and lipid can be converted to a number of fuels including diesel, ethanol, butanol, and methanol. Because algae absorb CO₂ to make lipid, the net impact on the environment should be very small. Additionally, biofuels are biodegradable, so if they do spill, less harm is done compared to when fossil fuels spill. What is the hold-up you ask? At this point in time, developing fuel from algae requires huge investments of water and fertilizers because the algae must be killed in order to harvest the lipid and then a new stock is grown back up again. The energy needed to grow algae from a seed stock to "harvest-ready" is orders of magnitude larger than the energy obtained from harvesting them. In other words, more energy is put into the system than is taken out, so it leads to a net loss. Until the input of energy is lower than what the system produces (including energy from the sun of course), the system will not be viable.

Power Generation

The generation of electricity is the single largest use of fuel in the world. In 2008, the world produced about 20,261 TWh of electricity. About 41% of that energy came from coal, another 21% came from natural gas, and the rest was covered by hydro, nuclear, and oil at 16%, 13%, and 9% respectively. Of the fuel burned, only 39% went into producing energy and rest was lost as heat. Only 3% of the heat was then used for co-generation. Of the 20,261 TWh produced, 16,430 TWh were delivered to consumers and the rest was used by the plants themselves. It is clear that a great deal of energy goes into producing electricity, which isn't surprising given that everything humans do in the industrialized world, from running water to surfing the internet, requires electricity. Most estimates suggest that about 40% of all GHG emissions come from the production of electricity, with transportation coming in a very close second. Coal, in particular, is highly problematic for its production of sulfur dioxide, which produces acid rain, amazingly, nuclear power is the least damaging in terms of pollutants produced, generating less carbon than any form of power generation other than hydro and including solar (PV panel production uses large amounts of water).

So, if humans are not going to switch to nuclear power, then a cleaner, more renewable form of energy is needed. Biofuels may provide at least a partial answer. Co-generation plants often use methane derived from landfills and there is vigorous interest in the use of syngas in many

agricultural areas. Like any biofuel, the balance of the equation lies in carbon generation. For syngas made from the agricultural waste, the net impact is lower than if the waste were allowed to decompose on its own. This is because natural decomposition in oxygen-rich environments produces nitrogen dioxide, which is over 300 times more potent of a greenhouse gas than carbon dioxide, as well as methane, which is over 20 times more potent. The same benefits exist for methane harvested from landfills.

Of course, these applications are not enough to meet our energy needs and so the conversion of crops grown specifically for energy is where most of the research and development is occurring at this stage. Algae and other plants that grown in harsh conditions and thus do not threaten the food supply are actively under investigation for potential sources of biofuel. At this point, only about 13% of all electricity in the United States is made from renewable sources (excluding hydro), but very little of this is biofuel. Most of the electricity from biofuels is produced as a byproduct of fuel production for transportation. The United Kingdom is the largest market for biofuel-to-electricity generation, generating enough power for 150,000 households from landfill gas alone.

Heat

The major use of natural gas from fossil fuels is heat, though a good deal of it also goes to energy. In the United States, a boom in hydraulic fracturing (called Fracking) has led to a huge surge in the production of natural gas from shale (a fossil fuel) and to the prediction that this will soon become the predominant form of energy, perhaps as soon as 2040. Of course, natural gas need not come from fossilized plant material; it can also be produced from recently grown plant material. Of course, the majority of biofuel used in heating is solid. Wood is both an aesthetic and a practical method of heating and many homes use wood burning stoves as supplements to other heating systems like natural gas or electricity. Renewed interest in solid biofuel, in part a response to rising energy prices, has led to a surge in innovation in the industry with research focusing on improved efficiency, reduced emissions, and enhanced convenience. Wood gasification boilers can reach efficiencies as high as 91%. To put the cost of biofuel into perspective, 1,000 BTUs of energy from wood cost about \$1.25. Natural gas, on the other hand, cost about \$2.60 per 1,000 BTUs. Wood pellets cost around \$2.10 per 1,000 BTUs, making them

less expensive than natural gas as well. The table below shows the cost of various fuels and provides a note on efficiency.

Charging Electronics

Just because you're off the grid doesn't mean you don't have friends to communicate with. If nothing else, you want to get on the Internet to let your computer pals know about the latest breakthrough in inserting the voltage on your Christmas lights. Plus, you want the convenience of charging your phone and laptop wherever you go. Recently, chemists from Saint Louis University developed a fuel cell that uses sugar and cooking oil byproducts to generate electricity. In the future, consumers may be able to use these types of cells in place of batteries to power anything from cell phones to computers. While the cells are still in development, they have the potential to become a very versatile power source.

Cooking

While kerosene is the most common ingredient to use for stoves and non-wick lanterns, biodiesel works just as great.

Create energy when fossil fuel runs out

As the oil supply is starting to run out, this has caused us to question how fuel can be extracted without destroying the environment. Biofuel will help the government create a stable method of producing energy that is cost-effective.

Reduce cost and need for imported oil

More than 84% of the world's petroleum is used in the United States. Despite the increase in fuel demands, the U.S. has recently started to decrease the need since 2006. This allows biofuel to become the best factor in energy reduction.

Analysts say that replacing imported oil with biofuel will help to stabilize the economy when oil is disrupted. It does not matter how much the United States spends on oil import, but how the overall economy must be stabilized.

Role of microbes in petroleum industry

Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. Microorganisms play an important role on nutritional chains that are an important part of the biological balance. With several abilities, microorganisms have become an important influence on the ecological systems, making them necessary for superior organisms and their life in this planet. Ability of microorganisms to transform and degrade many types of pollution in natural pollutant matrices (Soil, Water, Sediments and Air) has been widely recognized during the last decades. Microorganisms survive in contaminated habitat because they are metabolically capable of utilizing its resources and can occupy a suitable niche. Contaminates are their potential energy for microorganisms. Petroleum hydrocarbons are a major source of energy providing over 50% of the energy used all over the world. In addition, petroleum is the principal source of lubricants, solvents and a variety of large volume of chemical feedstock for synthesis of plastics, fibers, detergents, pharmaceuticals and cosmetics. A large scale of operations necessitated by the above demands renders the petroleum industry a potential source of air, water and soil pollution. Petroleum as crude oil while drilling, refinery effluents, sludge, emulsion solids and as water from ballast tanks may find its way into the adjoining air, soil surface or ground water through accidental or deliberate disposal. Petroleum exploration has been in operation for over 100 years.

Petroleum product spills during oil exploration, refining and transportation are very common. Many farmers have lost their agricultural land due to oil spills in their fields. Many fishermen have lost their livelihood due to oil slick in lakes and rivers. Petroleum industry by-products have a detrimental effects on the environment which is evident from their classification as new waste categories (Waste category number 10 and 12) under the recent Hazardous Waste act of 1988. Total petroleum hydrocarbons are composed of compounds varying from simple aliphatic and aromatic compounds to complex, multi-ring structure of high molecular weight hydrocarbons. It

also contains a wide range of substances that contain sulfur, nitrogen, oxygen and other elements. On average, crude oil contains about 84% C carbon, 14% hydrogen, 1 - 3% sulfur and less than 1% each of nitrogen, oxygen, metals, and salts. Petroleum hydrocarbons can be broadly divided into four fractions: the aliphatic fraction, the aromatic fraction, the asphaltene fraction and the resins. Among the aromatics, polycyclic aromatic hydrocarbons (PAHs) represent a unique class of petroleum hydrocarbons because of their pyrogenic nature and the complexity of the assemblages in which they occur. Polycyclic aromatic hydrocarbons (PAHs) are persistent pollutants, found to have toxic, mutagenic and carcinogenic properties and are listed among the priority pollutants by the U.S. Environmental Protection Agency (EPA). Petroleum hydrocarbon-metabolizing microorganisms are widely distributed in nature. Petroleum hydrocarbons can be degraded by microorganisms such as bacteria, fungi, cyanobacteria, and microalgae. However, bacteria play the important in hydrocarbon degradation. Over the last two decades the microbiological investigation of total petroleum hydrocarbon impacted environments have revealed the presence of a physiologically diverse microbial flora.

Due to the wide variety of complex reduced substrates constituting total petroleum hydrocarbons and different metabolic pathways, there are certain inherent difficulties in characterizing microbial communities impacted by total petroleum hydrocarbons. Despite these problems, there has been extensive research to analyze the microbial abundance in such contaminated sites and to assess the microbial communities with the ecosystem function. For the proper exploitation of the bioremediation potential of indigenous microflora, the study of microbial diversity of sites contaminated with total petroleum hydrocarbons with a polyphasic approach becomes imperative. The elucidation of diversity at the genomic level will help to understand the evolutionary and phylogenetic perspectives and will lead to conservation of indigenous microflora, which are of economic and environmental importance.

Whereas detection of the functional variability of the bacterial strains from the contaminated sites & investigating the degradative phenotype will allow the selection of specific bacterial strains for designing bioremediation strategies. Petroleum hydrocarbon waste is considered to be a degradation challenge to the microorganisms because of its persistence in the ecosystem. Further the low solubility in water (hydrophobic nature) and low volatility of the petroleum waste poses a significant threat to the environment. Thus there has been an increased interest in studying the microbial diversity of indigenous microorganisms capable of degrading pollutants.

such as crude oil, polycyclic aromatic hydrocarbons and polychlorinated biphenyl in different environments. Microorganisms adapt their catabolic activities to make use of toxic organic pollutants as food sources; thereby mineralizing complex organic compounds to simple compounds such as carbon dioxide and water. Identification of key organisms that play a role in degradation of the pollutants is relevant to development of optimal in situ bioremediation strategies. Extensive efforts have been made to characterize bacterial communities and their response to pollutants. Isolation of the potential degraders and identification of their functional genes involved in a particular degradation process have been elaborately studied. Many studies have also demonstrated the microbial diversity of different contaminated environments. With an ever increasing world's population, there is a concomitant increase in the demand for petroleum and petroleum products, which apparently constitutes a source of environmental pollution.

Oil pollution is a major environmental concern in many countries and this has led to a concerted effort in studying the feasibility of using oil-degrading bacteria for bioremediation. The discharge of used engine oil from vehicles is the main source of oil pollution in the environment. Engine oil is a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents that is used to lubricate the parts of an automobile's engine, in order

to keep everything running smoothly. The most important characteristic of the lubricating oil for automotive use is its viscosity. Large amounts of lubricating oils, composing long-chain saturated hydrocarbons (base oil) and additives are used in car engines. The main components of the base oil are cyclic alkanes or paraffins. Long-chain hydrocarbons and α -alkanes are known as recalcitrant to microbial degradation. The base oil contains C16-C36 hydrocarbons, and more than 75% α -alkanes. The ring number of α -alkanes in the base oil is from 1 to 3 and any ring contains 5 or 6 carbons. Most of the α -alkanes in the base oil have long alkyl side chains. It is mainly composed of base stock of tapered having alkyl benzene having carbon atom mainly n-C21 to C25 in the range of 80-90% by weight, antioxidant in the range of 0.06-0.05% by weight. Anticorrosion pressure additive in the range 0.01-0.05% by weight, antifoaming agent in the range of 0.01-1.0% by weight. Chemical analysis of 4 engine oil demonstrated a high contents of PAH's (naphthalene, anthracene, phenols and their derivatives) and a small fraction of aliphatic hydrocarbons (n-C15-n-C40). New motor oil contains a higher percentage of fresh and lighter (often more volatile and water soluble) hydrocarbons that would be more of a concern for acute toxicity to organisms. Used motor oil contains more metals and heavy polycyclic aromatic hydrocarbons (PAHs) that would contribute to chronic hazards including mutagenicity and

carcinogenicity. Prolonged exposure and high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer. Environmental pollution with petroleum and petrochemical products has attracted much attention in recent times. The illegal dumping of used motor oil is an environmental hazard with global ramifications. Thousand million gallons of waste engine oil is generated annually from mechanical workshops and discharged carelessly into the environment. According to USEPA (1996), only 1 liter of used engine oil is enough to contaminate one million gallons of freshwater. Used engine oil also renders the environment unsightly and constitutes a potential threat to humans, animals, and vegetation.

The presence of various kinds of automobiles and machinery vehicles has caused an increase in the use of motor oil. Spillages of used motor oils such as diesel or petrol, contaminate our natural environment with hydrocarbon compounds. Engine oil is the most widely used petroleum product. Research on biodegradation of engine oil is scarce in which screening of mixed bacteria is more important than of a single strain. Persistent screening and domestication are necessary to obtain a mixture of degrading bacteria through natural selection to produce a symbiosis system. Such a mixed bacterial flora is less vulnerable to environment and is more applicable than traditional treatment process.

Plant-microbe interaction

Introduction

Interactions between plants and microorganisms occur in many different ways and on many different levels. Almost all organs of the plant interact with microorganisms at a certain stage of their life span, the interaction is not necessarily negative for the plant. Indeed, there are plenty of interactions where the plant benefits either through direct or through indirect effects of the associated microbes. In these interactions, plants serve as sheltered habitats for the microorganisms that may colonize apoplastic spaces, plant surface areas or areas adjacent to the plant surface, e.g., the rhizosphere, the soil in the vicinity of roots. In addition to a sheltered habitat and a future source of nutrients that are liberated upon plant death, many plants release compounds that attract and feed the associated microbes. The associated microbes may in turn secrete compounds that lower plant growth, they may make the plant more resistant to abiotic or biotic stress, or they may defend the plant against more malignant microbes. With the

development of techniques to identify and quantify the microbial diversity associated with plants, we begin to grasp the intensity of the interactions to which plants are exposed. Complete microbiomes can be evaluated that are associated with different parts of the plant, and microbes can be found wherever they are looked for. Since functional effects of these multidimensional interactions are difficult to disentangle, most researchers stick to easier tractable and less complex interactions that can be experimentally tackled. This holds also true for researchers studying plant-microbe interactions that are clearly negative for the plant and result in the development of plant disease symptoms. Research on these negative interactions focuses on identifying microbial and plant factors necessary for establishment of the disease and elucidating their molecular function. The sum of all these research endeavours will lead to an increased, detailed and more and more complex understanding of the multidimensional interactions that plants keep with microbes. In this issue, three aspects of plant-microbe interaction are highlighted: the good, the bad and the diverse.

The Good: Soil Microbes Positively Affecting Plant Growth Of the microorganisms

Colonizing the rhizosol, *Streptomyces* species are special. They grow filamentously and can colonize not only soil but also roots and aerial parts of the plants; they are active producers of antibiotics and can save the plant from attack by more dangerous bacteria; and they produce volatile organic compounds that give rise to the typical fragrance of fresh forest soil. As such, they qualify as biocontrol agents in several cropping systems, and strains serving as antagonists of various plant pathogens can be identified. The versatile *Streptomyces* species also have plant growth-promoting abilities and can be used as biofertilizers. Because of their ability to form spores and survive adverse conditions in the soil, they are also more competitive than other microbes. In addition, they produce various lytic enzymes that can break down insoluble organic polymers and generate nutrients that can be used by plants.

Plant growth-promoting bacteria can also be used in phytoremediation of metal-contaminated soils. Exposing *Helianthus tuberosus*, a high biomass crop used for bio-ethanol production, to particulate plant growth-promoting bacteria that were isolated from plants growing on a metal-contaminated soil increased the ability of the plant to sustain elevated concentrations of cadmium and zinc. The bacteria were shown to grow endophytically in the root and resulted in a

significantly increased cadmium uptake into the plant. In presence of the bacteria, the plant showed a decrease of metal-induced stress and an improved growth. Thus, these plant growth-promoting bacteria can help both in phytoremediation and in sustainable biomass production.

Plant growth-promoting bacteria can induce drought and salt tolerance. The beneficial soil bacterium *Bacillus amyloliquefaciens* G803 together with a water-retaining agent consisting of super absorbent hydrogels used for soil erosion control can significantly improve the drought resistance of perennial ryegrass; this was true even compared to application of single components that already significantly improve drought resistance of the plant relative to control. A novel bacterium isolated from a C4 perennial succulent semihalophyte, Arab with excellent drought and salt tolerance, to significantly increase both the growth and salt tolerance of perennial ryegrass. The positive role of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* CDI on plant growth promotion and the *Verticillium* wilt resistance of cotton. They determined the symbiotic efficiency of 17 cotton varieties to *R. irregularis*. The best one, Lurlian 1, was used for a two-year field trial. Presence of the arbuscular fungus significantly increased plant growth and plant disease resistance against *Verticillium dahliae* wilt. While the negative effect on *V. dahliae* colonization could be due to mycorrhiza-induced resistance, the authors show that growth of *R. irregularis* may directly inhibit growth of *V. dahliae* by releasing as yet unknown volatiles. Thus, microorganisms can be used to positively change growth capacities of plants and to make them more resistant against biotic and abiotic stresses like drought and salt stresses that will likely occur much more often with progressing climate change.

The Host Elucidative Molecular Strategies of Plant Pathogens Of

The plant-pathogenic microorganisms, fungi are an enormous threat to plant health. While a lot of plant-pathogenic fungi are highly host-specific, host switching events are often at the base of emerging fungal diseases. Therefore, the elucidation of host-specificity factors that allow fungal proliferation and disease formation on particular host plants is one of the hot research topics in plant pathology. It turns out that the elucidation of host-specificity factors requires several successive steps, for each of which several comparative methods exist. In most cases, comparison of molecular characteristics of different host-specific strains or species resulted at best in a list of target genes potentially involved in host-specific virulence that await verification and functional validation. The authors indicate that intelligent combination of classical genetics, genomics, and transcriptomics covering both the pathogen and the host may lead to host-

specificity factor identification and a mechanistic understanding of host specificity. Biotrophic plant-pathogenic fungi live in close intimacy with the plant because they feed on living plant tissue and have to subvert the defense systems of the plant. One of their strategies for survival in the hostile plant tissue environment is the secretion of effector proteins that interact with plant proteins to the advantage of the pathogen.

Types of plant microbe interaction

Symbiosis is any relationship between two or more biological species. Such relationships are usually long term and have a strong impact on the fitness of one or both organisms. Symbiotic relationships are categorized by the benefits and physical relationships experienced by each species.

Common types of symbiosis are categorized by the degree to which each species benefits from the interaction:

- **Mutualism** In mutualistic interactions, both species benefit from the interaction. A classic example of mutualism is the relationship between insects that pollinate plants and the plants that provide those insects with nectar or pollen. Another classic example is the behavior of mutualistic bacteria in ecology and human health. Gut bacteria in particular are very important for digesting our food and other species. In humans, gut bacteria assist in breaking down additional carbohydrates, out-competing harmful bacteria, and producing hormones to direct fat storage. Humans lacking healthy mutualistic gut flora can suffer a variety of diseases such as irritable bowel syndrome. Some ruminant animals, like cows or deer, rely on mutualistic bacteria to help them break down the tough cellulose in the plants they eat. In return, the bacteria get a steady supply of food.
- **Commensalism** In commensalism, one organism benefits while the other organism neither benefits nor suffers from the interaction. For example, a spider may build a web on a plant and benefit substantially, while the plant remains unaffected. Similarly, a clown fish might live inside a sea anemone and receive protection from predators, while the anemone neither benefits nor suffers.
- **Parasitism** Parasites are organisms that harm their symbiotic partners. Parasitism is extremely common in nature, depending on the definition, more than half of all species

may go through at least one parasitic stage in their life cycle. There are many well-documented examples of parasitic bacteria and microorganisms throughout this text.

Symbiosis can also be characterized by an organism's physical relationship with its partner.

- **Endosymbiosis:** a relationship in which one of the symbiotic species lives inside the tissue of the other. For example, Coral polyps have special algae called zooxanthellae that live inside their cells. Zooxanthellae provide sugars to the coral through photosynthesis. Similarly, nitrogen-fixing fungi often live inside the roots of plants, providing nitrogen in exchange for the sugars of photosynthesis.
- **Ectosymbiosis:** a relationship in which one species lives on the outer surface of the other. Barnacles that live on whales and bromeliads that live on tropical trees are examples of ectosymbiosis.

These categories can be paired with the above terms to better describe the species' interactions. For example, you might say that a gut bacteria is an "endosymbiotic mutualist," or that a flea is an ectosymbiotic parasite.

Associative Interaction

Associative microorganisms mostly live in the phyllosphere, rhizosphere and rhizoplane or sometimes reside inside the plant tissue and organ. Rhizosphere and rhizoplane associated bacteria are termed as rhizobacteria, whereas those strictly found inside the plant are known as endophytic bacteria. The term rhizoplane refers to the root surface, whereas, the rhizosphere is the immediate thin zone of soil surrounding the root. Legume root nodules also accommodate various non-nodulating plant growth promoting endophytic bacteria, which influence the survival of rhizobia and impact the nodulation and grain yield of crop. The influence associative bacteria may be positive, but most often they promote plant growth. Phyllosphere refers to the aerial part of the plant that also carries microorganisms. Bacteria are the most extensively studied microorganisms in this part of plant. Rhizosphere and rhizoplane associating bacteria generally resides in the soil and move towards the plant as it produces root exudates. Microorganisms attach themselves with to the root surface via cell surface polysaccharides or cell structures.

including fibrinase and flagella. Bacteria, cyanobacteria, fungi and algae reside in and around the root zone but bacteria is the most abundant. The associative microorganisms establish a relationship with plant for nutrient and shelter and in return promote plant growth mainly by fixing nitrogen, indole acetic acid production, and phosphate solubilization, biocontrol of plant diseases and by many other mechanisms, which are being explored.

Pathogenic Interaction

1. Pathogenic Relationship •

The contact between plant and pathogenic microorganism lead to a particular series of events in plant organism. • The extracellular space between cell wall and plasma membrane acts as a first battle field between plants and pathogen. • Bacteria, fungi, viruses and protozoetes that colonize the living plant tissues are encased in this narrow region in the initial step of infection. • Therefore, the apoplastic region is believed to be an interface which mediates the first crosstalk between host and pathogen. • The secreted proteins and other metabolites, derived from both host and pathogen, interact in this apoplastic region and govern the final relationship between them. • Types of pathogen based on effects.

1. Necrotrophy: plant cells are killed.

2. Biotrophy: plant cells remain alive.

3. Hemibiotrophy: plant cells remain alive later killed.

Applications of plant-microbe interactions

Although the rational use of plant-microbe interactions has been recommended as a means to maximize the economic returns of agribusiness, still there are widespread concerns regarding the use of plant-microbe chemical signaling and their interactive co-ordination.

Bioremediation of soil pollutants

Recent advancements in several meta and omics-technologies provide the moment to exploit proteomic, metabolomic, genomic, metagenomic, and transcriptomic approaches to identify traits that maximize the benefits of modern agricultural practices and manipulate the plant-microbe relations toward the sustainable agriculture. Microbes are the causes of both beneficial

(bioremediation, biofertilization, and biocontrol) and pathogenic effects. But the pathogenesis is just a functional phase of the microorganism occurred at the propagating phase of their life cycle which happens at the expense of the host. Hence, modern agriculture practices and increasing awareness about plant-microbe interactions tend to focus more on the development of breeding or crop-protection schemes than making the attempt to eliminate the potential pathogens as making it more effective, or sustainable.

Treatment of roots of young plants with several PGPR strains as biofertilization process, inoculating plants with a number of bacterial and fungal species as biocontrol methods, plant-microbe interactive consumption of organic pollutants as bioremediating phenomenon, are some of the recent progressions made in the way of sustainable agriculture and environment restoration having a low-input biotechnology prospects.

Utilization of whole organisms, that is, bacteria and fungi, for the improvement of crop yield, became the method of old days, while the recent technology lends molecular and sub-molecular units of the microbes. Along with the betterment of crop yields and help in sustainable agriculture, mankind also gets served via Genome's variety of medical and economic benefits that's coming from the overexpression of the secondary metabolites such as alkaloids, volatile oils, and antibiotics by the use of the molecular pharming techniques. Hence, molecular insights of plant-microbe interactions bring the advancement in agriculture practices along with the ecological gains. Moreover, more manipulation of desired plant-microbe interactive conquest with genetic engineering approaches should facilitate more advance and organic agro-practices ensuring complete utilization of beneficial microbial ecological interactions for additional benefits.

BIOCONTROL OF PESTS AND PATHOGENS:

The control of insects and agents responsible for disease production in plants has been achieved through the use of chemicals. However, an increased concern for the addition of chemicals into the environment has prompted scientists to explore the use of biological agents to control or prevent the growth of pathogens. Plant-growth-promoting rhizobacteria (PGPR) produce a variety of antibiotic compounds that inhibit a variety of Gram-negative and Gram-positive soil bacteria. Nematodes found in the soil are roundworms of a few millimeters in length, and some of these are parasitic for plants.

- As a result of nematodes attacking roots and underground parts of the plant, death of the plant may be due to direct damage by the nematode or to secondary infection by bacteria, fungi, or viruses.
- Certain strains of *Bacillus thuringiensis* produce a protein that has been used to control insect populations and some of the insects controlled. *Bacillus thuringiensis* is an aerobic, Gram-positive bacterium found broadly distributed in soil.

SUMMARY

A diverse group of microorganisms are found in the root zone rhizosphere, and bacteria present in the rhizosphere have considerable influence on the plant. Free-living bacteria may enhance plant growth, due to the development of mycorrhizae, or prevent growth of phytopathogenic fungi. Fungi may be found in symbiotic association with plant roots, where the plant provides sugars and organic acids while the fungal partner enhances mineral uptake by plants. The two principal types of fungal association are endomycorrhizae found commonly on herbaceous plants and ectomycorrhizae, which are generally associated with woody plants.

- Symbiotic nitrogen-fixing plants have specific bacteria as partners for the conversion of atmospheric N_2 to ammonia. Establishing the plant-bacteria activity results from signal responses on the part of both partners in the symbiosis. A few of the fungi and bacteria are plant pathogens producing highly destructive plant pathologies. An important bacterial disease in plants is crown rot, caused by *Agrobacterium tumefaciens*.

Bio-fertilizers

Bio-fertilizers

Bio-fertilizers include the chemical which in turn make full use of bacteria for you to fertilize your land. These kinds of fertilizers are certainly not unhealthy for plant life as well as various other plant life such like the compound fertilizers. These are truly removed from the dog waste items with the microbial recipes. Bacteria are widely-used to take how much nutrients inside plant life. That they let the plant life expand in a very balanced natural environment. Fortunately they are environment-friendly and bring about your urea involving just about any form. Using bio-fertilizers inside land creates your plant life balanced and also shields these people via receiving just about any ailments.

Advantages of bio-fertilizers

(i) Bio-fertilizers are usually eco-friendly and also guard the particular ecosystem in opposition to pollution.

2) Bio fertilizers: Farmers people dumping parts from your earth which usually result in conditions inside the crops. Crops can be safeguarded in opposition to drought as well as other stringent ailments through the use of resource fertilizers.

3) Bio fertilizers usually are not expensive and also weak farmers can easily utilize these. They help acquire large produce regarding vegetation simply by creating the particular earth abundant together with vitamins and minerals and also microbes required for the particular progress with the crops.5) Bio fertilizer increases the actual and also substance attributes regarding earth.

Types of bio fertilizers

Nitrogen bio fertilizers

The sort of bio fertilizers can help any farmers to determine the nitrogen point during the soil ground. Nitrogen may be a crucial factor which happens to be put to use in any growing for put.

Including, Acetobacteria must be used to your non-legume bounty. Rhizobium it takes in your legume bounty. Purple earth-friendly algae are needed to progress hemp despite the fact that Acetobacter must be used to progress sugarcane.

Phosphorus bio fertilizers

Phosphorus bio fertilizers widely used to determine the phosphorus point during the soil ground. The demand for phosphorus in your put growing is small. Phosphorus bio fertilizers come up with all soil ground which demanded use of phosphorus. But I think that your particular selected phosphorus bio fertilizers must be used for that selected types of head. They are put to use in all different kinds of any head including, Acetobacter, Rhizobium can implement.

Compost bio fertilizers

Compost bio fertilizers really are those that use the pet animal dung to make sure you greatly enhance all the dirt by means of advantageous microorganisms and additionally vitamin supplements. To make sure you replace the dog waste matter right into bio fertilizers, all the microorganisms want initially bacteria proceed through organic technique and additionally help in breaking down all the waste matter. Cellulytic fungal civilization and additionally Acetobacter civilization can be installed for these compost bio fertilizers.

Application/ methods of bio fertilizers

Seedling root dip

This method is applied to the rice crop. A bed of water is spread on the land where the crop has to grow. The seedlings of rice are planted in the water and kept there for eight to ten hours.

Seed treatment

Within this process your nitrogen along with phosphorus fertilizers are generally put together jointly throughout normal water. And then vegetables are generally dropped within this mix. Following purposes on this substance on the vegetables, vegetables are generally dried up. As soon as they normally dry out, they must always be sown immediately ahead of that they had harmed by simply small bacteria.

Soil treatment

All the bio fertilizers along with the compost fertilizers are mixed together. They are kept for one night. Then the next day the mixture is spread on the soil where seeds have to be sown.

Symbiotic nitrogen fixers: Most important symbiotic Nitrogen fixing bacteria is **Rhizobium** and **Azospirillum**.

Rhizobium: Rhizobium lives in the root hairs of the legumes by forming nodules. Plant root supply essential growth and root synthesized substance to the bacteria. The name Rhizobium was established by Frank in 1889. This genus has seven distinct species based on 'Cross Inoculation Group' concept. More than twenty cross-inoculation groups have been established. A new classification has been established for Rhizobium. That is 'slow growing rhizobia' known as Bradyrhizobium and the other group is 'fast growing rhizobia' called Rhizobium. Rhizobium can fix 30-100 kg/ha Rhizobium.

Azospirillum: It mainly present in cereal plants. Inhabits both root cells as well as surrounding of roots forming symbiotic relation and increasing nitrogen fixing potential of the cereal plant. Azospirillum is recognized as a dominant soil microbe | nitrogen in the range of 20- 40 kg/ha in the rhizosphere in non-leguminous plants such as cereals, millets, Oilseeds, cotton etc. Considerable quantity of nitrogen fertilizer up to 25-30 % can be saved by the use of

Azospirillum inoculant. These species have been commercially exploited for the use as nitrogen supplying Bio-Fertilizers.

Free living bacteria

Large number of free living or non -symbiotic bacteria (does not form nodules but makes association by living in the rhizosphere) present in soil. Commonly used free living bacteria are *Azotobacter* *Klebsiella* it will not associated with plant. *Azotobacter* is a heterotroph which provides the required amount of nitrogen to the plant from the soil.

Azotobacter

Azotobacter is a heterotrophic free living nitrogen fixing bacteria present in alkaline and neutral soils. *Azotobacter* is the most commonly occurring species in arable soils of India. Apart from its ability to fix atmospheric nitrogen in soils, it can also synthesize growth promoting substances such as auxins and gibberellins and also to some extent the vitamins. Many strains of *Azotobacter* also exhibit fungicidal properties against certain species of fungi. Response of *Azotobacter* has been seen in rice, maize, cotton, sugarcane, pearl millet, vegetable and some plantation crops. It improves seed germination and plant growth. *Azotobacter* is aerobic breathing organism and requires a large amount of organic carbon for its growth.

Biopesticides

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. For example, camels oil and baking soda have pesticidal applications and are considered biopesticides.

Biopesticides fall into three major classes:

Biobiochemical pesticides

Biobiochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biobiochemical pesticides include substances that interfere with mating, such as insect sex pheromones; as well as various scented plant extracts that attract insect pests to traps. Because it is sometimes difficult to determine whether a substance meets the criteria for

classification as a bioherbicide pesticide. EPA has established a special committee to make such decisions.

Microbial pesticides:

Microbial pesticide consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest(s). For example, there are fungi that control certain weeds and other fungi that kill specific insects. The most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis*, or Bt. Each strain of this bacterium produces a different mix of proteins and specifically kills one or a few related species of insect larvae. While some Bt ingredients control moth larvae found on plants, other Bt ingredients are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular Bt produces a protein that fits bind to a larval gut receptor, thereby causing the insect larvae to starve.

Plant-Incorporated-Pesticides (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Bt pesticidal protein and introduce it into the plant's own genetic material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest. The protein and its genetic material, but not the plant itself, are regulated by EPA.

Advantages of Biopesticides:

- Biopesticides are usually inherently less toxic than conventional pesticides.
- Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum conventional pesticides that may affect organisms as different as birds, insects and mammals.
- Biopesticides often are effective in very small quantities and often decompose quickly, resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides.

- When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly reduce the use of conventional pesticides, while crop yields remain high.
- To use biopesticides effectively (and safely), however, users need to know a great deal about managing pests and must carefully follow all label directions.

In 1994, we established the Biopesticides and Pollution Prevention Division within the Office of Pesticide Programs to facilitate the registration of biopesticides. This division promotes the use of safer pesticides, including biopesticides, as components of IPM programs. The division also coordinates the Pesticide Environmental Stewardship Program (PESP). Since biopesticides tend to pose fewer risks than conventional pesticides, EPA generally requires much less data to register a biopesticide than to register a conventional pesticide. In fact, new biopesticides are often registered in less than a year, compared with an average of more than three years for conventional pesticides. While biopesticides require less data and are registered in less time than conventional pesticides, EPA always conducts rigorous reviews to ensure that registered pesticides will not harm people or the environment. For EPA to be sure that a pesticide is safe, the Agency requires that registrants submit the results of a variety of studies and other information about the composition, toxicity, degradation, and other

Composting

Composting is a great way to reduce your food waste and contribution to greenhouse gas emissions. Composting is nature's process of recycling decomposed organic materials into a rich soil known as compost. Anything that was once living will decompose. Basically, backyard composting is an acceleration of the same process nature uses. By composting your organic waste you are returning nutrients back into the soil in order for the cycle of life to continue. Finished compost looks like soil-dark brown, crumbly and smells like a forest floor.

Compost is simply decayed organic matter — and “organic matter” is a pretty wide-ranging label. A twig can be organic matter, but so can a banana peel. When you mix a bunch of these items together in a compost pile, they break down naturally into a nutrient-rich fertilizer that helps gardens grow.

Composting Benefits

Soil Conditioner

With compost, you are creating rich humus for your lawn and garden. This adds nutrients to your plants and helps retain soil moisture. They don't call it “black gold” for nothing.

Recycles Kitchen and Yard Waste

Composting can divert as much as 30% of household waste away from the garbage can. That's important because when organic matter hits the landfill, it rots, the air it needs to decompose quickly. Instead, it creates harmful methane gas as it breaks down, increasing the rate of global warming and climate change.

Introduces Beneficial Organisms to the Soil

Microscopic organisms in compost help aerate the soil, break down organic materials for plant use, and ward off plant disease.

Good for the Environment

Composting offers a natural alternative to chemical fertilizers when applied to lawns and garden beds.

Reduces Landfill Volume

Most landfills in North America are quickly filling up; many have already closed down. One-third of all waste is made up of compostable materials. Diverting this waste from the landfill **means that our landfills will last longer (and so will our soil spaces).**

Types of composting:

- Backyard composting If you have a yard and a balance of browns (fallen leaves or straw) and greens (grass clippings and food scraps), you have all you need to make compost.

- Worm composting (vermicomposting) If you have a tiny yard or live in an apartment or have an abundance of food scraps, this type of composting is for you.
- Grass cycling If you have grass clippings and don't want to use them in a compost pile, you can leave them on the lawn to decompose. Read about grass cycling for tips, techniques and benefits.

10 good reasons why you should compost:

1. Yard and food waste make up 30% of the waste stream. Composting yard wastes and yard trimmings helps divert that waste from the landfill, waterways and water treatment facilities.
2. You will significantly reduce pest problems—and your use of pesticides.
3. Healthy plants from healthy soil look better, produce better and have a much greater ability to fight off pests and diseases.
4. Adding organic materials to the soil improves moisture retention.
5. Adding decomposed organic material to the soil feeds beneficial organisms.
6. Compost amends both sandy and clay soils.
7. Compost provides a balanced, slow-release source of nutrients that helps the soil hold nutrients long enough for plants to use them.
8. Composting saves money—you avoid the cost of buying soil conditioners, bagged manure, etc.
9. For less, eat plants well: soil will improve your own diet. Plants grown in depleted soils have a reduced nutrient content.
10. Home composting is a valuable tool in educating children about nature and the cycle of life.

How to Compost

1. Start your compost pile on bare earth. This allows worms and other beneficial organisms to aerate the compost and be transported to your garden beds.

2. Lay bays or straw first, a few inches deep. This aids drainage and helps aerate the pile.
3. Add compost materials in layers, alternating moist and dry. Moist ingredients are food scraps, tea bags, seaweed, etc. Dry materials are straw, leaves, woodchip pellets and wood ashes. If you have wood ashes, sprinkle in thin layers, or they will clump together and be slow to break down.
4. Add manure, green manure (clover, buckwheat, wheatgrass, grass clippings) or any nitrogen source. This activates the compost pile and speeds the process along.
5. Keep compost moist. Water occasionally, or let rain do the job.
6. Cover with anything you have - wood, plastic sheeting, carpet scraps. Covering helps retain moisture and heat, two essentials for compost. Covering also prevents the compost from being over-watered by rain. The compost should be moist, but not soaked and sodden.
7. Turn. Every few weeks give the pile a quick turn with a pitchfork or shovel. This aerates the pile. Oxygen is required for the process to work and turning "adds" oxygen. You can skip this step if you have a ready supply of coarse material, like straw.

Once your compost pile is established, add new materials by mixing them in, rather than by adding them in layers. Mixing, or turning the compost pile is key to aerating the composting materials and speeding the process to completion.

Antimicrobial

Antimicrobial the word **antimicrobial** was derived from the Greek words *anti* (against), *mikros* (little) and *bios* (life) and refers to all agents that act against microbial organisms. This is not synonymous with antibiotics, a similar term derived from the Greek word *anti* (against) and *bios* (concerning life). By strict definition, the word "antibiotic" refers to substances produced by microorganisms that act against another microorganism. Thus, antibiotics do not include antimicrobial substances that are synthetic (sulfonamides and quinolones), or semi-synthetic (methicillin and amoxicillin), or those which come from plants (quercetin and alkaloids) or animals (lysozyme). In contrast, the term "antimicrobial" include all agents that act against all types of microorganisms — bacteria (antibacterial), viruses (antiviral), fungi

(antifungal) and protozoa (antiprotozoal). Notice that the term “antibacterial”, being the largest and most widely known and studied class of antimicrobials, is often used interchangeably with the term “antimicrobials” and will be the major focus of this website.

An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicides, while those that merely inhibit their growth are called biostats. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.

Antimicrobial products have a wide range of uses

One recent study indicated that personal care products such as soaps, body washes and hand sanitizers that contain antimicrobial chemicals are more effective at limiting bacteria than soaps without antimicrobial agents – this 2011 study found that washing hands with an antimicrobial soap reduced bacteria on skin at a greater rate than non-antimicrobial soap.

- Triclosan, an antimicrobial compound, has been found to promote oral health and aid in the fight against gingivitis when added to toothpaste.
- Kitchen counters, office desks, bathroom sinks, and other high-traffic areas in homes and offices may accumulate germs that can make people sick. Cleaning products without antibacterial/antimicrobial ingredients will clean the surface, but will not kill the germs on it.
- Antimicrobial pesticides help to slow the growth of human pathogenic microorganisms and are used in food processing plants, dairies, breweries, poultry houses and other animal feeding operations, hospitals and medical and dental clinics and offices, municipal drinking water and water treatment facilities, swimming pools and spas, schools, day-care centers, public access facilities and homes.

Types of antimicrobials

Antibacterials

Antibacterials are used to treat bacterial infections. The drug toxicity to humans and other animals from antibacterials is generally considered low. Prolonged use of certain antibacterials can decrease the number of gut flora, which may have a negative impact on health. Consumption of probiotics and reasonable eating can help to replace destroyed gut flora. Stool transplants may be considered for patients who are having difficulty recovering from prolonged antibiotic treatment, as for recurrent *Clostridium difficile* infections. The discovery, development and use of antibacterials during the 20th century has reduced mortality from bacterial infection. The antibiotic era began with the prescient application of nitroglycerine drugs, followed by a "golden" period of discovery from about 1945 to 1970, when a number of structurally diverse and highly effective agents were discovered and developed. Since 1980 the introduction of new antimicrobial agents for clinical use has declined, in part because of the enormous expense of developing and testing new drugs. In parallel there has been an alarming increase in antimicrobial resistance of bacteria, fungi, parasites and some viruses to multiple existing agents. Antibacterials are among the most commonly used drugs and among the drugs commonly misused by physicians, for example, in viral respiratory tract infections. As a consequence of widespread and injudicious use of antibacterials, there has been an accelerated emergence of antibiotic-resistant pathogens, posing a serious threat to global public health. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibacterials. Possible strategies towards this objective include increased sampling from diverse environments and application of metagenomics to identify bioactive compounds produced by currently unknown and uncultured microorganisms as well as the development of small-molecule libraries customized for bacterial targets.

Two types of antibiotics are commonly available. These are as follows:

1. **Bactericidal antibiotics** - These antibiotics are fast-killing effects on bacteria. Example: Penicillin, Aminoglycosides, Ofloxacin.
2. **Bacteriostatic antibiotics** - These antibiotics have an inhibitory effect on bacteria. Example: Erythromycin, Tetracycline, Chloramphenicol.

Depends on the spectrum of action, antibiotics are further classified into three types. These are as follows:

1. **Broad-spectrum antibiotics:** These antibiotics are widely used to kill or inhibit the Gram-positive and Gram-negative bacteria. Example: Chloramphenicol
2. **Narrow spectrum antibiotics:** These antibiotics are widely effective against specific groups of bacteria. Example: Penicillin G
3. **Limited spectrum antibiotics:** These antibiotics are effective against a single organism or a single disease. Example: Dysalazine.



Antifungals

Antifungals are used to kill or prevent further growth of fungi. In medicine, they are used as a treatment for infections such as athlete's foot, ringworm and thrush and work by exploiting differences between mammalian and fungal cells. They kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus, fungal and human cells are similar at the molecular level, making it more difficult to find a target for an antifungal drug to attack that does not also exist in the infected organism. Consequently, there are often side effects to some of these drugs. Some of these side effects can be life-threatening if the drug is not used properly. As well as their use in medicine, antifungals are frequently sought after to control mold growth in damp or wet home materials. Sodium bicarbonate (baking soda) blasted on to surfaces acts as an antifungal. Another antifungal serum applied after or without blasting by soda is a mix of hydrogen peroxide and a thin surface coating that neutralises mold and encapsulates the surface to prevent spore release. Some paints are also manufactured with an added antifungal agent for use in high humidity areas such as bathrooms or kitchens. Other antifungal surface treatments typically contain variants of metals known to suppress mold growth e.g. pigments or solutions containing copper, silver or zinc. These solutions are not usually available to the general public because of their toxicity.

Antivirals

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics, specific antivirals are used for specific viruses. They are relatively harmless to the host and therefore can be used to treat infections. They should be distinguished from virucides, which actively deactivate virus particles outside the body. Many antiviral drugs are designed to treat infections by retroviruses, mostly HIV. Important antiretroviral drugs include the class of protease inhibitors. Herpes virus, best known for causing cold sores and genital herpes, are

usually treated with the nucleoside analogue acyclovir. Viral hepatitis is caused by five unrelated hepatotropic viruses (A-E) and can be treated with antiviral drugs depending on the type of infection. Influenza A and B viruses have become resistant to neuraminidase inhibitors such as oseltamivir, and the search for new substances is on.

Antiparasitics

Antiparasitics are a class of medications indicated for the treatment of infection by parasites, such as nematodes, cestodes, trematodes, infectious protozoa, and amoebae. Like all antimicrobials against intracellular microbes, they must kill the infecting pest without serious damage to the host.

Non-pharmaceutical

A wide range of chemical and natural compounds are used as antimicrobials. Organic acids and their salts are used widely in food products, e.g. lactic acid, citric acid, acetic acid, either as ingredients or as disinfectants. For example, beef carcasses often are sprayed with acids, and then rinsed or steamed, to reduce the prevalence of *Escherichia coli*. Copper-alloy surfaces have natural intrinsic antimicrobial properties and can kill microorganisms such as *E. coli* and *Staphylococcus*. The United States Environmental Protection Agency has approved the registration of 355 such antibacterial copper alloys. In addition to regular cleaning, antimicrobial copper alloys are being installed in some healthcare facilities and subway transit systems as a public hygiene measure. Other heavy metal cations such as Hg^{2+} and Pb^{2+} have antimicrobial activities, but can be toxic. Traditional herbals use plants to treat infectious disease. Many of these plants have been investigated scientifically for antimicrobial activity, and some plant products have been shown to inhibit the growth of pathogenic microorganisms.

Antimicrobial pesticides

According to the U.S. Environmental Protection Agency (EPA), and defined by the Federal Insecticide, Fungicide, and Rodenticide Act, antimicrobial pesticides are used in order to control growth of microbes through disinfection, sanitation, or reduction of development and to protect inanimate objects, industrial processes or systems, surfaces, water, or other chemical substances from contamination, fouling, or deterioration caused by bacteria, viruses, fungi, protozoa, algae, or slime.

Antimicrobial pesticide products

The EPA monitors products, such as disinfectants/sanitizers for use in hospitals or homes, in order to ascertain efficacy. Products that are meant for public health are therefore under this monitoring system, including products used for drinking water, swimming pools, food sanitation, and other environmental surfaces. These pesticide products are registered under the premise that, when used properly, they do not demonstrate unreasonable side effects to humans or the environment. Even once certain products are on the market, the EPA continues to monitor and evaluate them to make sure they maintain efficacy in protecting public health.

Public health products regulated by the EPA are divided into three categories:

- **Sterilizers (Sporicides):** Eliminate all bacteria, fungi, spores, and viruses.
- **Disinfectants:** Destroy or inactivate microorganisms (bacteria, fungi, viruses,) but may not act as sporicides (as those are the most difficult form to destroy). According to efficacy data, the EPA will classify a disinfectant as limited, generalbroad spectrum, or as a hospital disinfectant.
- **Sanitizers:** Reduce the number of microorganisms, but may not kill or eliminate all of them.

Antiseptics and Disinfectants

Antiseptics and disinfectants are the chemical components which are used as antimicrobial agents.

Antiseptics are applied to the injured tissues, cuts, and infected skin surfaces. Antiseptics are not prescribed to be taken orally. A few examples are given below:

1. **Dettol** – It is a mixture of chloroxylenol and soap. It is used to apply on the wounds.
2. **Iodine tincture and Iodofors** – It has very good antiseptic properties.
3. **Boric acid** – It is used as an antiseptic agent for eyes.

Disinfectants are used to destroy the pathogenic microorganisms in the nonliving objects such as floors and drainage systems.

Example: Chlorine and sulphur dioxide at low concentrations.

Significance of microbial biotechnology in the economic development of Pakistan

Biotechnology is generally defined as application of living system based technologies to develop commercial processes and products. Over the last few decades, several fundamental discoveries in life sciences have given rise to Modern Biotechnology which is now one of the fastest growing areas of science; hence this century has rightly been termed as 'Century of Biology', hoping that such advances in life sciences will yield changes more momentous than those of electricity and computers. In view of these developments, Biotechnology was included among the six priority areas of Science & Technology by the National Commission of Science & Technology. Biology in this century has become an information science. Many programs and initiatives underway at major research institutions and leading companies are already giving shape to this assertion.

Biotechnology and Agriculture

Agricultural biotechnology offers efficient and cost-effective means to produce a diverse array of novel, value-added products and tools. It has the potential to increase food production, reduce the dependency of agriculture on chemicals, and lower the cost of raw materials, all in an environmentally friendly manner. The initial phase of a revolution in agriculture has already occurred. Large areas of genetically modified (GM) crops of soybeans, maize, cotton, and canola have been successfully grown in the Western Hemisphere. In the United States in 1999, of the total of 72 million acres planted with soybeans, half were planted with GM herbicide-resistant seeds. When herbicide-resistant seeds were used, weeds were easily controlled, less tillage was needed, and soil erosion was minimized. The total global area under cultivation with transgenic crops as of 1999 was 98 million acres, while by year 2001, it have been cultivated on 125 million acres (ISAAA). The commercialisation of other Bt crops such as canola, cotton, and maize is in progress in several countries including Asian countries such as India and China. The main objectives of creating transgenic plants are attempts to engineer metabolic pathways for the production of tailor-made plant polymers or low molecular weight compounds, increased resistance towards pathogens and pesticides, improved food quality, and the production of polypeptides for pharmaceutical or technical use. Plant-made vaccines or antibodies are

especially attractive as plants are free of human diseases, reducing screening costs for viruses and bacterial toxins.

Bt Cotton

Pakistan is the world's fourth largest producer of cotton after China, the USA and India, according to statistics from the All Pakistan Textile Mills Association. Cotton and textiles make up over 60 per cent of Pakistan's Rs. 488.00 billion annual export. Cotton or white gold as it is aptly called is grown for its lint and seed, which yield cotton fiber and seed oil, respectively. This crop occupies 70-75 million acre of world area with a production of 10-15 metric tons. In Pakistan, its area spans over 12-14 million acre with an average yield of 485kg/acre or 210kg/hectare of lint and 500kg/acre of seed cotton. To meet the challenges of the century with a population of more than 140 million, a total production of 12 million bales is required as against the 7-8 million bales of today. This can be achieved by the use of improved crop production practices coupled with appropriate pest management tactics. In addition, generation of novel Bio-technology can help to achieve the near impossible. Genes that have been identified as potentially profitable, if engineered into acceptable cultivator methodology can be used to generate such transgenic. Among these are genes imparting resistance to herbicides, insects, pathogens and biotic stresses. It is also widely accepted now that a number of other qualitative characters can be improved, such as fiber strength, fineness, color and thermal adaptability of the fiber. Pakistan offers a rapidly expanding market for insecticides and pesticides. The total value has expanded from Rs. 7.20 billion (\$120m) in 1990 to Rs11.00 billion (\$180m) in 2000. By the introduction of the Bt cotton in Pakistan could result in a 45-55 per cent reduction in insecticide use on cotton (Which is 85per cent of Rs11 billion). This would mean a benefit of about of about Rs 4.2 to Rs 5.40 billion apart from the favorable impact on the environment and increase in cotton yield. In Pakistan, average yield of conventional cotton per acre is around 25-28 maund or 933 kg-1,044 kg. Bt Cotton in Pakistan can increase per acre yield from 14 to 30 per cent. Which means that, on the one hand, it will bring prosperity for Pakistani farmers, on the other, it will bring a boom to all industries and business activities which are directly or indirectly associated with agriculture sector.

Bt Rice:

Bt Rice will reduce yield losses caused by caterpillar pests, the most important of which are the yellow stem borer, in Pakistan and other parts of Asia and the striped stem borer, in temperate areas. Average yield losses to stem borers in Asia are often estimated at 5%, and vary from region to region. In some areas, stem borers are among the major constraints on yield, while in others they occur at levels too low to cause yield loss. Similarly in Pakistan a fungal disease "Blight" reduce substantial yield in Basmati rice which causes economical loss about Rs. 1.5 billions annually.

Significance of microbial biotechnology in the economic development of Pakistan II

The Green Revolution ushered in the late 1960s has transformed some countries from a food-grain importer to a self-sufficient one. However, with our burgeoning population having already crossed the 150 millions mark, the achievements of the Green Revolution are unlikely to be sufficient. Injurious but common farm practices have damaged the cultivated land through water and wind erosion, compaction, salinization, and water logging. Our forest cover is a sad mismatch of desirable levels and actual ground realities. Over-tilage rapidly depletes prime agricultural lands and today little quality land is available to increase the area under farm production. It is therefore not surprising to learn that Pakistan will need to import an estimated 10-12 million tons of food-grains to meet its basic requirement of food by the year 2050. There is little doubt that agricultural research will have to be rejuvenated to meet the increasing demands of farm production. To achieve this, there will have to be a paradigm shift from the methodologies held in the 1970s and 1980s. Radical changes will have to be made even in the thought processes that plan the needs of the new century. Conservative and obsolete policies drawn for an older era will have to give way to realistic measures reflecting the urgency of the demand for technological breakthroughs. The success of the Green Revolution of the earlier decades will now have to be repeated through a Gene Revolution. Biotechnological interventions

can play a vital role by not only increasing crop productivity but also taking care of the increasing threat of biotic and abiotic stresses on crop plants.

Biofuel:

Another growth area in this century will be the development of alternatives to non-renewable resources especially fossil fuels. Biotechnology will provide answers through modified enzymes and microorganisms that can turn abundant biomass into feedstocks for the production of synthetics, plastics, polymers and bio-fuels like Ethanol and

Biodiesel:

Growth in the ethanol industry offers enormous potential for overall economic development and additional employment in these smaller communities. In Pakistan it is estimated that 400 million litres ethanol production facility can create 5000 - 6000 local jobs. The processing of grains for ethanol production can provide an important value-added market for Pakistani farmers, helping to raise the value of commodities they produce. Pakistan's economy faces some of the toughest times in recent history. Therefore, production of ethanol can spark new capital investment and economic development in rural communities across Pakistan as well. There are a number of benefits to producing and using bio-fuel aside from the transportation fuel supply issue. First, bio-fuels are produced domestically, and the feed-stocks for them are grown domestically. This helps reduce our country's trade deficit and creates jobs in our country, both of which are good for our economy. Our agricultural community especially stands to benefit, since bio-fuels are made from crops and agricultural residues, providing options for new valuable crops and new uses for existing crops and residues. Producing our fuel domestically also improves our energy security; we become less dependent on the strategic, political and economic whims of other countries.

Our country's heavy reliance on imported oil is becoming a serious energy security issue, and it is clear that our vulnerability will get worse with time. Key among the reasons for rising oil imports is the limited domestic resource base of crude oil. Producing and using bio-fuels is much better for the environment than burning fossil fuels. Bio-fuels produce fewer harmful emissions during production and combustion and they contribute virtually no carbon dioxide to the atmosphere, which is very important for reducing the build-up of greenhouse gases.

Biotechnology has tended to favour the industrialized world, where most of the research is concentrated. In Pakistan number of "Mujahads" like - Dr Ansar Numan, Chairman National Commission on Bio- Technology, Dr Kausar A Malik Bioscience Pakistan Atomic Energy Commission, Dr. Sheikh Rasuddin Director of Centre of Excellence in Molecular Biology, Dr. Zahoor Ahmad CEMR, and Dr Yusuf Zaka of National Institute of Bio Technology and Genetic Engineering (NIbge) are participating in this jihad since 1990's and working hard to put biotech tracks in the country. Unfortunately progress in Pakistan is hindered by number of reasons like inadequate funding/ lack of human resources, restricted information, poor higher education, weak links between universities and research institutions, lack of appropriate legal regimes, little private sector involvement, while the major hamper which is prohibiting biotechnology to flourish in our country is "The Ministry of Environment" which is taking hell of Biosafety guidelines from more than one year. Such obstruct will not attract foreign investment and development in our country while our country would be remain far behind in this emerging technology.

Genomics

Genome sequencing of viruses began in the late 1970s. The basic technique involved, the random sequencing of fragments by the Sanger dideoxy termination method, was proposed and applied successfully by Fred Sanger and associates to the complete sequencing of bacteriophage DNAs, notably that of phage λ in 1980 (for the principle of the random shotgun method). Historically, the first attempt to obtain a complete genome sequence of a cellular organism was geared toward *Escherichia coli*, the best studied organism outside of humans. This project started in 1980 and used a "directed" approach. Because a fairly detailed genetic map of *E. coli* was available thanks to the efforts of bacterial geneticists, it was possible to first produce a set of λ -based clones, each containing up to 20 kb DNA, with overlapping ends. The sequencing from here on represented the shotgun phase: The inserted segments in the λ vector were then randomly cut into much smaller fragments based clones, each containing up to 20 kb DNA, with overlapping ends. The ends of larger inserts in a plasmid vector, when sequenced, were useful also in positioning some raw sequences that overlap with them, where the "source DNA" corresponds to the 20-kb inserts in the λ vector). This two-stage approach (also called "clone-by-

clone shotgun") was taken because it was thought that the shotgun approach could not be used for larger segments of DNA or for an entire genome.

It was a surprise, therefore, that J. Craig Venter and his associates at The Institute of Genomic Research successfully used the random shotgun approach for the entire 1.8-megabase (Mb) genome of *Haemophilus influenzae* without first directly cloning and ordering large inserts (Figure 4.1, where the "source DNA" corresponds to the entire bacterial genome in this case) and published the results in 1995, two years before the publication of the *E. coli* genome sequence. The determination of *H. influenzae* genome sequence involved the use of 24,000 "reads," each with the average length of about 400 bases or slightly longer. Thus, the total sequences used for assembly were almost 12 Mb long, meaning that each segment of the genome was covered more than six times by the sequencing reads; this is called "depth of coverage" and will be mentioned in the "Metagenomics" section later in this chapter. The assembly required a newly developed computer program. For many of us, the crowning glory of the genome sequencing projects was that of the human genome—at about 3 Gb, over 1600 times larger than that of *H. influenzae*. The International Human Genome Sequencing Consortium used the clone-by-clone shotgun approach, and the "first draft" was published in 2001. This approach was taken because the presence of very large numbers of repeated sequences in the mammalian genome was predicted to make the assembly of random sequences difficult.

Prokaryotic Genomes

There is a large variation in the size of bacterial genomes. Compared with the *H. influenzae* genome (1.8 Mb), other obligate parasites, such as *Chlamydia trachomatis* (1.0 Mb) and *Mycoplasma genitalium* (0.6 Mb), have smaller genomes. On the other hand, the genome of *E. coli* (4.6 Mb) is much larger, and even larger genomes are found in *Pseudomonas aeruginosa* (6.3 Mb), *Streptomyces coelicolor* (8.7 Mb), and *Bradyrhizobium japonicum* (19.1 Mb). (With rare exceptions, most archaeal genomes have a rather small size, between 1.5 and 3 Mb). In the prokaryotic genome, most of the DNA represents coding sequences or genes, unlike in higher animals and plants. So this large difference leads us to two questions. The first is, what is the minimal set of genes that allow simple cellular organism to grow and replicate? Here we have to classify genes, and the protein coded by these genes, on the basis of homology. Homology means descent from a common ancestor, and this is usually predicted by comparing sequences.

using computer programs such as BLAST. We distinguish two kinds of homologs. Homologous proteins in two different organisms, where they developed independently of each other, are called *orthologs*. In contrast, homologous proteins that exist in the same species, presumably created originally by gene duplication and often differentiated later in terms of functions, are called *paralogs*. Comparison of the genomes of *M. genitalium* (coding for 468 proteins) and *H. influenzae* in terms of orthologs led to the definition of a "minimal gene set" of about 300 genes.

(The list was expanded to 382 genes in 2006 by experimental gene disruption using transposons. This set includes genes needed for DNA replication and repair, transcription, translation (including chaperones) and for biosynthesis of nucleotides, coenzymes, and lipids, as well as the glycolytic pathway and the F1F0 ATPase. However, because *Mycoplasma*, which does not have peptidoglycan, was used as the basis, the list does not contain genes needed for peptidoglycan synthesis, nor does it contain genes involved in the biosynthesis of amino acids or purines and pyrimidines. The set of genes needed for an independent survival of a bacterium in an environment is usually thought to contain about 1500 genes.)

The second question regarding different genome sizes is: what do larger genomes contain in addition to the minimal set just mentioned? Comparison of the genome of *H. influenzae*, which basically resides in only one environment – the upper respiratory tract of animals – with the much larger genome of *E. coli* already indicates that the latter organism, which must face "trans-or-larvae" existence in the intestines, and must survive (at least for a short period) in natural waters, contains many more genes (often paralogs) needed for adaptation in different types of environment. *E. coli* also needs genes for complex regulatory responses for this adaptation.

The same theme is repeated over and over. Thus, *P. aeruginosa*, which is basically a resident of soil and water but can also cause severe infection in humans, has a larger genome filled with a more complex array of genes. This tendency reaches its zenith in *S. cerevisiae*, a soil bacterium that produces several antibiotics and undergoes differentiation into aerial spores. This genome is equipped for very complex regulatory responses, as seen, for example, from the record number of sigma factors (that control transcription specificity at the most basic level) – 55 – in comparison with *E. coli*, which produces only seven. Genes for many enzymes occur as a set of multiple paralogs, each gene known or suspected to be expressed only under certain conditions. For example, five paralogs of *fabH*, coding for the first enzyme of fatty acid biosynthesis, are found in *S. aureus*. One is the main fatty acid synthesis enzyme and is essential. There are in gene clusters involved in the biosynthesis of antibiotics and probably a polyunsaturated fatty

acid and are predicted to function for these specialized pathways without interfering with the major "housekeeping" metabolism of the cell.

Another major mechanism that increases the prokaryotic genome size is the addition, presumably through horizontal transfer from other organisms, of large genomic islands. In *Salmonella*, "pathogenicity islands" 1 through 5 are located at the 63-, 31-, 82-, 92-, and 20-min positions on the circular map (with a circumference of 100 min). They range in size from several kbp bases to 40 kb, and they code for proteins involved in pathogenesis, such as type III secretion systems that inject toxic proteins directly into mammalian cells. These islands are genetically different from the codogenous sequence of the genome – for example, in GC content – a fact that suggests their "foreign" origin. The function of islands is not limited to pathogenesis. For example, the chromosome of *R. japonicum*, an N₂-fixing symbiont of the soybean root, contains a very large (610-kb) "symbiosis island" of much lower GC content that apparently codes for many functions needed for symbiosis. The analysis of prokaryotic genomes thus shows that the genome often has a mosaic origin, and horizontal transfer has occurred many times during evolution.

Proteomics

Proteomic analysis requires the separation of thousands of proteins or their fragments and then their identification. It is impossible to identify this many proteins and their fragments by using the traditional approaches, such as reactivity with specific antibodies or functional properties. One of the advances in mass spectrometry, now almost the universal approach in identification, were the major factor that made proteomics possible. Previously, mass spectrometry, which measures the mass-to-charge ratio of ions in a vacuum required the "hard" ionization methods that fragmented the sample molecule. Mass spectrometry became extremely useful in the study of proteins and peptides when the two "soft ionization" methods – matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) were developed. These methods made possible the analysis of (unfragmented) peptides and proteins at high sensitivity and great precision. The significance of these advances may also be seen from the fact that two of the Nobel prizes in chemistry in 2002 were awarded to scientists who contributed to the development of these methods.

For the separation of proteins and peptides, two major approaches are used. In one, proteins are separated by two-dimensional polyacrylamide gel electrophoresis, usually by isoelectric points in the first dimension, and by molecular weight in the second dimension using sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis. The protein spots are stained, and in this way they can be roughly quantitated. Each of the protein spots can then be digested with trypsin, and the fragments are analyzed by mass spectrometry. Commonly, MALDI is the ionization method used, and time-of-flight (TOF) detection is used. The molecular weights of various fragments are compared with their predicted sizes, obtained from the protein database, and this results in the identification of the protein. The TOF analyzer is sufficient in this setup, because the identification relies on the comparison of sizes of multiple fragments from a single protein. The weakness of this method is that only the highly expressed proteins can be analyzed in this way because the resolution of the two-dimensional gel is limited and poorly expressed proteins may be obscured by the spots of strongly expressed ones.

In the second, "gel-free" approach, the mixture of proteins is digested first with trypsin, and the resultant, continuously complex mixture of peptides is separated by liquid column chromatography. Because a single column is unlikely to resolve this mixture, multidimensional approaches are used. For example, a column may contain an ion exchange resin in its upper part, and a reverse phase matrix in its lower part, and it can be eluted by pulses of salt solutions followed each time by gradients of a water-organic solvent mixture, such as acetonitrile. The advantage of this method is that the effluent from the column can be directly introduced into a mass spectrometer, thus avoiding the cutting out of a gel spot, subsequent digestion, and preparation of the small sample for MALDI. It is thus a method suitable for very large-scale analysis. The preferred detector is a tandem mass spectrometer (MS/MS), in which the isolated ion can then be analyzed further after collision-induced fragmentation. Such a setup is necessary because the size of a peptide alone is not sufficient for identification as the initial mixture may contain many different peptides of approximately the same size; thus, the fragmentation pattern of each peptide, determined in the second step, becomes important. Another advantage of this approach is the sensitivity that is achieved because the entire sample can be introduced into the mass spectrometer. A sample containing about 50 proteins was successfully analyzed by using a total of 0.2 approximately this method.

Following are a few examples. Samuel Miller and associates wanted to examine the expression levels of proteins in *P. aeruginosa*, a human pathogen that plays a major role in cystic fibrosis airways. They examined the effect of low Mg^{2+} concentration in the medium and also strains isolated from cystic fibrosis patients. They took the second approach mentioned above, liquid chromatography of trypsin digests followed by ESI ionization and MS/MS analysis. Mass spectrometry is inherently poor in terms of quantitation. Thus, they labeled the two sets of proteins by using either a light or heavy version of the isotope-coded affinity tag (ICAT) that reacts covalently with the sulfhydryl group of cysteine residues. Because ICAT increases 100-fold in mass, only the labeled peptides can be pulled out by using an avidin column, thus simplifying the analysis. They found that, among 1337 proteins thus examined, the expression of 145 proteins was affected by low Mg^{2+} , and the strains from cystic fibrosis patients had similar changes.

The second example is the analysis of protein expression in the malarial parasite *Plasmodium falciparum*. This parasite undergoes extensive morphological and biochemical changes in its development from the sporozoite stage (present in the salivary gland of mosquitoes), through the merozoite stage (invasive to red blood cells) to trophozoite (the form multiplying in erythrocytes), and finally gametocytes (the sexual stage). Samples of the parasites that could be collected were very small for some stages and were heavily contaminated by other human or mosquito proteins.

This precluded gene expression analysis with the DNA arrays as it requires a few micrograms of mRNA; proteins, by contrast, are much more abundant by a couple of orders of magnitude. Two-dimensional capillary electrophoresis coupled with MS/MS was used, and the study resulted in showing that many proteins are expressed in a stage-specific manner, a finding that will be most useful in the development of vaccines and therapeutic agents.

PROTEIN INTERACTIONS

Many proteins function by interacting with other proteins. For example, the RNA polymerase II preinitiation complex in yeast is thought to contain at least 68 different proteins. Myriads of signaling pathways in eukaryotic (and also prokaryotic) cells involve cascades of protein-to-protein interactions. If a protein of unknown function can be shown to interact with proteins of

known function, this will be a major step toward the understanding of the function of the former. Besides, some scientists argue that the complexity in higher animals is not the result of a large number of genes, but is caused by the increased complexity in protein-to-protein interactions.

For all these reasons, the study of protein-to-protein interactions is extremely important. A

classical strategy for such a study is the use of a yeast two-hybrid system. Here the two proteins to be studied are produced as fusions to two domains of an activator of gene transcription in yeast. If the two proteins associate with each other, the two domains of the activator come together and the transcription of the indicator gene ensues. Although examining a few million combinations of the 6000 genes in yeast in this manner may sound like an impossible task, scientist's overcome the difficulty by creating pools of domains of genes. However, there are potential problems with this approach. First, because we are dealing with a transcriptional activator, the protein-to-protein interaction must occur in the nucleus, and we cannot use the method for cytoplasmic membrane proteins, for example.

Second, it is difficult to deal with higher-level interactions involving more than two proteins. Finally, because we are dealing with fusion proteins, there is always a possibility that we may have interfered with the proper folding of the protein by making fusions. Mass spectrometric protein identification provides an ideal approach to the study of protein interaction. An early example is the identification of about 100 novel proteins that are found in spliceosomes, which splice out introns from the primary transcript in eukaryotes. On a larger scale, tags were added to the C-terminus of more than 1500 proteins in yeast, out of which close to 1200 were expressed at a reasonable level. Pulling out tagged proteins showed that at least 230 protein complexes exist in yeast. When the unknown proteins in these complexes were identified by mass spectrometry, most of them were found to contain one or more proteins of unknown function. As described already, the first major step toward of the proteins might be contaminants, not the intrinsic components of the complexes. However, an approach that uses the differential labeling with light and heavy ICAT was proposed, and this should work when the formation of complexes can be controlled experimentally.

PROTEIN ARRAYS

Can we make protein arrays, as we can make gene arrays? Indeed this is possible. In one study, 5000 open reading frames in the yeast genome were cloned and expressed as a fusion with GST

and a beta histidine tag. The proteins were purified on glutathione-agarose and attached to glass slides coated with nickel, using the affinity of the beta histidine tag. The utility of such a “chip” was demonstrated when it was treated with a solution of histidine-labeled calmodulin. When the binding of the histidine tag was detected with a fluorescence-labeled streptavidin, 39 proteins were shown to bind calmodulin, out of which only six were the already known calmodulin binding proteins (Figure 4.6). Similar approaches should be possible with many reagents, including drug candidates, and protein arrays may one day become the standard approach for screening for proteins with specific functions on a global scale.

Metagenomics

A fundamental operation in biotechnology is the expression of a known gene coding for a useful production in a suitable host organism, such as *E. coli* or yeast. However, because what is cloned is a piece of DNA, it may come directly from environmental sources. This approach of direct cloning is extremely valuable because most of the microorganisms in the environment have not yet been cultured, and the use of samples (for example soil samples) from a diverse range of environments is likely to supply genes for genes or proteins with widely different properties. Thus, it has now become a standard procedure in the industry to maintain such samples for the direct cloning of useful genes. We can extend this approach, and instead of cloning just a gene or a gene cluster, we may hereafter construct the entire genomes of yet-to-be-cultured organisms from DNA samples in the environment. We will then be dealing not with the genome of a single cultured organism, but with genomes of the entire community of microorganisms, many of which may be currently uncultured. This is what is often called *metagenomics*.

Metabolomics

Once we learn the expression levels of proteins at the global level, it becomes important to know the levels of many metabolites, which represent the integrated results of the activities of these proteins, including enzymes and regulators. Such an analysis at the global scale – *metabolomics* – became possible in recent years, thanks to technical developments in mass spectroscopy and nuclear magnetic resonance (NMR). Because a very large amount of data is generated, its mathematical analysis becomes very important. Metabolomics on its own holds much promise in

the area of clinical diagnosis; for example, an inexpensive, sensitive NMR analysis of ingredients of patient sera was reported to diagnose coronary artery disease, although a later study found problems with the statistical treatment of data. In this case also, the visual inspection of NMR spectra showed no difference between patients and healthy people, and mathematical analysis was obligatory. However, from the perspective of biotechnology, metabolomics becomes significant only when it is combined with Transcriptomics and proteomics.

In this way, we are trying to integrate all the "omics" information together in the direction of *systems biology*, where we are trying to consider all components of cells, tissues, and so on, from the level of genes up to the level of metabolites, as an integrated whole. Metabolomics, for example, supplies an exceptionally sensitive indicator of phenotypes, showing that metabolism becomes altered in mutant strains of yeast, in which conventional tests showed no alteration of phenotype. Metabolomics was also embraced eagerly by scientists working on plants, as plants produce a very large number of compounds. Here we give two examples in which a metabolomic approach was used in microorganisms, a topic relevant to biotechnology. In *Corynebacterium glutamicum*, which produces large amounts of the amino acid lysine (Chapter 9), lysine begins to be secreted abruptly when the growth rate begins to decrease at the end of the exponential phase of culture. Metabolomic analysis showed that there is a rapid change in the flux of metabolites at this time, yet hardly any change is noted in the gene transcription pattern, except in the down-regulation of glucose-6-phosphate dehydrogenase. Although the data did not show us how the switching of metabolism occurs, they serve as a starting point for future studies. In *Aspergillus terreus*, the producer of the cholesterol-lowering drug lovastatin about a dozen genetically defined strains were constructed and lovastatin production and the gene expression profile were determined. Genes whose expression levels were correlated with lovastatin production were then modified to increase their expression levels to produce a strain improved in terms of drug production. Although the number of measured metabolites was very small (and thus the study hardly qualifies as a metabolomic study), this approach suggests that the combination of metabolomics with transcriptomics may be fruitful in the future.

Transcriptomics

The presence of a gene in a genome does not obviously mean that it is expressed to produce mRNA and subsequently a protein. Furthermore, regulation of metabolism often involves alteration of expression of various genes, and therefore it is important to know their expression level in order to understand the physiology of the cell. Before the genome sequences became available, gene expression had to be examined on a one-by-one basis. The techniques used included Northern blot, which involves annealing of fractionated mRNA with the relevant pieces of DNA, and reverse transcriptase-PCR, in which mRNA is used as a template to produce cDNA, which is then amplified by PCR. These methods are still used when we already know the mechanism of regulation in precise detail and when we want to know the expression level of the genes involved. However, when the regulation mechanism is not known entirely, examining the expression levels of only a few selected genes out of many thousands becomes pure guesswork, and has led to many wrong conclusions.

When genome sequences became available, methods were developed in 1995 to "print" fragments of many genes onto a glass or plastic slide and to examine the binding of mRNA to these *microarrays*. The invention of the microarray or "chip" technology allowed us, for the first time, to examine the gene expression in an entire cell, for example, on an unbiased, "global" scale; then RNA to a certain piece of DNA fragment is obviously affected by the length, base composition, and other characteristics of the probe, and the binding itself cannot be used as the measured marker. However, we are usually interested in *changes* in transcription patterns, and comparison can be made by labeling the mRNA populations from two samples (query and reference) by different fluorescent dyes. In 1997, this methodology was successfully used to identify changes in pattern of gene expression in yeast cells undergoing physiological adaptation, and has produced a revolutionary change in the way we approach the molecular basis of the biology of the cell. Its impact may be seen by the fact that more than 10,000 papers using this technology were published in less than 10 years since its introduction.

Two methods are used to construct the microarrays. In one approach, usually 100- to 300-bp-long fragments of genes are amplified by PCR and deposited on glass slides. In another, slides

containing much shorter (about 20- to 25-nucleotide) pieces of DNA are fabricated by Affymetrix by synthesizing each chain on the chip. In both cases, efforts are made to decrease the false-positive signals. Important features of the microarray method are that it generates data of an enormous size that biologists have not encountered earlier and that the data contain significant amounts of statistical variation as well as "noise." Thus, it is extremely important for the data to be treated in a manner that is statistically correct.

What types of results were obtained by studies of these "transcriptomes" or gene expression profiles? The list will be too large because it covers almost every branch of biology. One of the areas investigated most intensely is the gene transcription pattern in human diseases such as cancer. In a study of human breast cancer in 2000, many clusters of genes were found to be overexpressed in different patients. In 2002, a group of scientists tried to define the "signature" genes, whose expression could serve as the prognostic marker in breast cancer. RNA was extracted from biopsy samples from primary breast cancer patients with no known metastasis to lymph nodes at the time of diagnosis. Because the purpose was to examine variations in individual tumors rather than to find variations in cancer in relation to normal tissues, a mixture of all samples was used as the reference. One of a microarray containing 25,000 human genes showed that there were significant differences in expression in about 5000 genes in the 78 tumor tissues studied. For each of these genes, the correlation coefficient was calculated between the expression level of that gene and the prognosis (whether there were distant metastases within five years). Most of the genes showed only a significant correlation and were discarded from further analysis. However, 311 genes showed significant correlation. These potential marker genes were used in regression numbers to find out if a group of them could successfully predict the outcome of the disease, and it was found that the use of 70 genes with the highest correlation coefficient was enough for this purpose. This method could classify about 90% of the poor-prognosis group accurately so that they could have been directed to "adjuvant" therapy, such as chemotherapy or radiation therapy; whereas the patients in the good prognosis group could have escaped such therapy, which puts a heavy burden on the patient's body. The prediction procedure was validated by using a series of 203 patients and, more recently, in an international multicenter study in 2006.

Another focus of transcription pattern studies has been the effort, mostly by pharmaceutical companies, to find a new target for a drug. Although transcriptomes can show only that the gene is transcribed, by using a bioinformatics approach, the functions of many genes may be predicted. If certain organs or pathological tissues, such as cancer cells, overexpress any of the genes, they may be attractive candidates for selective therapy. In fact, gene expression array analysis of multiple sclerosis lesions showed that hitherto unsuspected genes were up-regulated, potential targets of intervention. Pharmaceutical companies were thus the first to adopt transcriptomics, and the expectations were that a great many new targets would be discovered in a record short time, followed by hundreds of new drugs. In spite of a very large amount of work invested in this effort, however, it appears that a flood of new drugs has not yet been developed in this manner, with the approval of drugs for entirely new targets still at a steady rate of a few per year. Possibly this is because most of the newly discovered targets are not really "druggable" targets – such as G-protein-coupled receptors, serine/threonine kinases, tyrosine kinases, transcription factors, nuclear receptors, some ion channels, or ion channels, for which pharmaceutical companies already know how to develop low molecular weight drugs. Some scientists blame the poor correlation between the steady-state expression levels of mRNA, measured by transcriptomics, and the level of proteins that are produced. In any case, gene profiling is a very useful tool to predict the types of drug action and its efficacy, as the drugs of one type, say, opioids, generate a very own characteristic pattern of up- and down-regulation of various genes. Furthermore, a similar technique is used in predicting the toxicity of the drugs in development. In the future, it may even be possible to predict the specific response of an individual to various drugs, so that to tailor the use of drugs to each individual, the ultimate goal of pharmacogenomics.

In the past, basic research, expression profiling is now essential in the effort to understand the

molecular mechanisms of regulatory processes, as mentioned already. In addition, because genes will be simultaneously induced or repressed under a given condition if their products work together, the global gene expression profile may give hints to the "unknown" function of some proteins. In an extensive study of 228 disease mutants and 11 mutants of essential genes under the regulatable promoter in 2000, many expression patterns were defined, and in fact the functions

of eight hitherto uncharacterized open reading frames were predicted. Recent studies also showed that in higher animals, alternative splicing of pre-mRNA, use of alternative promoters, and use of alternative polyadenylation sites generate many mRNA species from one single gene; such a mechanism for the generation of multiple mRNA species may be used quite extensively and often appears to occur in an organ- or tissue-specific manner.

One important development in biology, aided in many cases by transcriptomic studies, is our realization that the untranslated portion of the genome plays major roles in cellular control. Even in bacteria, small untranslated RNAs (more than 50 are now known to exist in *E. coli*) play important roles in regulating the translation of many messages. In higher animals and plants, small interfering RNA (siRNA) is known to be generated from double-stranded RNA by a complex called Dicer and to inhibit gene expression through degradation of mRNA, most likely as a defense mechanism against double-stranded RNA viruses. In higher organisms, it is now known that hundreds of microRNA species, which are small in size (18 to 24 nucleotides long), play major roles by inhibiting mRNA translation and by inducing the degradation of mRNA. MicroRNA species are usually transcribed as a larger primary transcript, which folds upon itself to produce a double-stranded RNA structure that in turn is processed by nucleases including Dicer to produce the final short single-stranded form. Expression of some species of microRNA was known to be altered in cancer. MicroRNA levels are not detected in the conventional DNA arrays. However, recent examination of the levels of more than 200 microRNA species, using specially designed arrays and beads, showed very widespread changes in their levels in tumor cancer, suggesting that they play important roles in the development of cancer. The importance of the noncoding DNA region is not limited to the production of microRNA. A recent study of the transcription of 10 selected human chromosomes, using tiling arrays spaced at five nucleotide intervals, showed that about 9% of the probes hybridized with some transcripts. The surprising finding was that even when polyadenylated cytosolic RNA fractions were used, more than half of them did not come from known genes (exons) but came from elsewhere. Some came from introns, but more came from intervening regions of chromosome, hitherto considered as "junk DNA." The fraction of such transcripts coming from an intervening region is even higher among cytosolic, nonpolyadenylated RNA, reaching 48% of the total. It is not known what function these novel transcripts are performing, but this analysis

shows both the power of array analysis and the extent of our ignorance on how the genome of higher organisms really functions.

POLYSACCHARIDES

Microorganisms and plants produce polysaccharides of widely varying composition and structure. Cellulose, hemicelluloses, and starch from plants are the most abundant, important, and familiar of these biopolymers, but many other naturally occurring polysaccharides have interesting and useful properties. Agar, a mixture of polysaccharides isolated from marine red algae, has been manufactured in Japan since about 1760 and is one of the most effective gelling agents known. Gel formation occurs at agar concentrations as low as 0.04%. Dried agar suspended in water melts in the temperature range of 85°C to 90°C (the precise temperature varies with the algal source) and, at about 1% to 1.5% by weight, sets between 32°C and 39°C to form a firm gel. This large temperature hysteresis is a particularly valuable property of agar that it shares with few other polysaccharides. Among polysaccharides produced by bacteria and fungi, some have properties resembling those of agar whereas others have distinctive rheological (flow) properties valuable in certain pharmaceutical or industrial applications. Only one microbial polysaccharide, xanthan gum, among the 10 industrial polysaccharides utilized in the largest amount. In terms of market value, it ranks high among fermentation products. Much of this chapter is devoted to this biopolymer. Other microbial exopolysaccharides have found only modest commercial use, and only a few are produced on a large scale. However, the enormous range of polysaccharides synthesized by microorganisms has yet to be adequately explored.

BACTERIAL POLYSACCHARIDES

The majority of bacterial species, under the proper culture conditions, secrete mucoid substances of high molecular weight. When these viscous materials remain associated with the cell, they are called variously *capsules*, *sheaths*, or *slime layers* (Figure 8.1). Whereas capsules and sheaths are well defined layers external to the cell wall, slime accumulates in large quantities outside the cell wall and diffuses into the medium. The slime formers may produce such copious amounts of

viscous slone when they are grown in a liquid medium so that the culture flask may be inverted and the culture will remain in place. Mucoid colonies grown on solid media usually have a moist, glistening surface. Capsules have long been a subject of great interest because of their roles as important virulence factors in bacteria that cause invasive infections. Capsules protect bacteria from phagocytosis and impart resistance against bactericidal effects of serum. Frequently, pathogens cultured in the laboratory will spontaneously produce unencapsulated mutants; uniformly, these mutants are no longer pathogenic. Most of the extracellular polymers produced by bacteria are polysaccharides, although a few bacteria produce capsules made up of polypeptides of d-amino acid residues. The precise structure of capsular polysaccharides of many pathogenic organisms is strain specific. Such individual "coding" units' outermost layer protects the bacterial cell from being attacked by an immune system that may have developed antibodies in response to an earlier, perhaps closely related, invader. In contrast, the structures of the extracellular polysaccharides of many slime-forming organisms are simpler in composition and less homogeneous in size. The molecular weight and even the composition of these extracellular polysaccharides may vary depending on the culture conditions.

ASPECTS OF POLYSACCHARIDE STRUCTURE

The glycosidic bond between two monosaccharides is formed between the hydroxyl group on the anomeric carbon of one monosaccharide and any hydroxyl group on the other monosaccharide. Hence, the formation of a disaccharide from two identical hexopyranose ring structures of the d-series may result in 11 different isomers. In eight of the isomers, the glycosidic linkage is between C-1, in either the α or β anomeric configuration, and C-2, C-3, C-4, or C-6 of the other pyranose residue. These are designated α -D-(1 $\rightarrow$ 2) linkages, α -D-(1 $\rightarrow$ 3), linkages, and so on, where α and β refer to the anomeric configuration at C-1. The other three isomers are created by acetal formation between both C-1 atoms through the glycosidic oxygen atom in the α,α , α,β , or β,β configuration. A similar series of 11 isomers will result if the two identical hexopyranose residues are of the l-series. The number of possible isomers is even higher if furanose forms (five-membered rings) are included. When the two monosaccharides are not identical, the number of possible structures increases, because either carbohydrate residue can occupy the first or the second position — that is, can be the reducing or non-reducing residue. Each additional

carbohydrate residue brings a large increase in the number of possible isomers. For complex polysaccharides, the number of theoretically possible structures is astronomical.

ROLES OF MICROBIAL POLYSACCHARIDES IN NATURE

Organisms that produce extracellular polysaccharides have been isolated from a wide range of environments. The functions of these polysaccharides are equally varied. The capsular polysaccharides that protect pathogenic microorganisms from immune system defenses were mentioned above. Capsules may also serve as physical barriers to infection by bacteriophages. Moreover, capsules and sheaths retain water and in some settings play an important role in preventing dehydration of the cells. A common function of extracellular polysaccharides of water and soil microorganisms is to bind cells to surfaces, such as soil particles or rocks, as well as to each other. Likewise, it has been proposed that the extracellular polysaccharides of some plant pathogens play a role in attaching the bacteria to the surfaces of host plants.

XANTHAN GUM

Xanthan gum was discovered in the mid-1950s, during a systematic search for useful biopolymers. In a screen of a large microbial culture collection, the Northern Utilization Research and Development Division of the U.S. Department of Agriculture discovered that the bacterium *Xanthomonas campestris* (originally isolated from the rutabaga plant) produced a polysaccharide with potentially valuable physical properties. Substantial commercial production of xanthan gum began in 1964, and in 1969 the U.S. FDA authorized its use in food. Today, xanthan gum has numerous uses in the food industry and elsewhere. The total U.S. consumption of xanthan in 2005 is estimated at more than 80 million pounds.

XANTHOMONAS A PLANT PATHOGEN

The genus *Xanthomonas* belongs to the γ subdivision of the Proteobacteria and consists exclusively of plant pathogens. It is a relative of the genus *Pseudomonas*, a group of bacteria discussed elsewhere in this book. The bacteria of the genus *Xanthomonas* are yellow-

pigmented, motile, aerobic, Gram-negative rods. *X. campestris pathovar* (abbreviated "pv.") *campestris*, the producer of xanthan gum, causes black rot, one of the most serious diseases of plants in the genus *Brassica*, which includes vegetables such as cabbage, cauliflower, brussels sprouts, broccolo, rutabaga, and turnips. Seeds contaminated with bacteria spread the infection. As the seedling emerges and grows, the bacteria colonize the surface of the plant.

STRUCTURE OF XANTHAN GUM

The primary structure of xanthan gum is common with cellulose; the xanthan backbone consists of β -(1 $\rightarrow$ 4)-linked d-glucose; however, the 3-position of alternate glucose monomer units carries a triaccharide side chain containing one glucuronic acid and two mannose residues. The nonterminal d-mannose unit carries an acetyl group at the 6-position, and a pyruvate is attached to the terminal d-mannose residue by a ketal linkage to the 4-position. Occasionally, the terminal-mannose carries a 6-O-acetyl group rather than pyruvate. The degree of acetylation and the pyruvate ketal content vary with culture conditions and from one strain of the microorganism to another. In solution, xanthan forms a left-handed double helix with fivefold symmetry and a pitch of 4.7 nm, with the two chains of the double helix running antiparallel to each other as shown in Figure 8.5. The triaccharide branches are closely aligned with the polymer backbone. The double-helical molecules interact side-by-side to form body-centered lattices, which in turn form microcrystalline fibrils. Estimates of the apparent molecular weight of xanthan in solution range from 2 to 20 million. The wide spread in estimated values reflects the varying extent of intermolecular association between xanthan chains. In solution, xanthan chains transform from single- to double-chain structures in the presence of salts. As noted below, this transition is dependent on the shielding of the negative charges on xanthan by cations.

VU Medical Zone Effort By Amaan Khan