Discipline Courses-I

Semester-I

Paper: Phycology and Microbiology

Unit-IV

Lesson: Economic importance of- Bacteria

Lesson Developer: Pamela Singh

College/Department: Dept. of Biotechnology, Deen Bandhu Chhotu Ram University of Science and Technology

Table of contents

Chapter: Economic importance of Bacteria

- **Biodegradation and Biofuel Generation**
- **Bioremediation**
- **Biomining**
- **Biofertilizers**
- **Biopesticides**

- **Bacteria as causal organism of plant disease**

- **Summary**
- **Exercise/ Practice**
- **Glossary**
- **References/ Bibliography/ Further Reading**

Introduction

Microorganisms have a significant impact on human life by their beneficial and harmful activities. Since time immemorial, the pathogenic microbes have been a cause of concern for the society. Deadly infectious diseases like AIDS, Leprosy, Meningitis, etc. causes large scale morbidity and mortality. Besides, microorganisms also cause a number of plant diseases leading to massive economic setbacks. However, the useful activities of microbes far outnumber their harmful ones. Besides, with the latest advances in biological sciences and technology, it is now possible to manipulate microbes for the benefit of mankind.

Bacteria have been used for decades for generation of specific products with industrial and medical applications. Products like organic acids, solvents, enzymes, amino acids, etc. have immense economic value. They can be used as food supplements, additives and in pharmaceuticals. Besides, medical compounds like hormones, antibiotics, transformed steroids etc. will go a long way in alleviating human suffering. Bacteria and Viruses also have a significant role in environment management. They are important components of biogeochemical cycles with a major role in biodegradation.

In the forthcoming discussion, the economic importance of bacteria and viruses, one of the major groups of microbes is reviewed. The applications of microbes in environment, industry and medicine are listed to present a broader outline of their activities.

Role Of Bacteria In Industry (Fermentation And Medicine)

The microorganism of industrial significance is first identified in the environment. Thereafter, the productivity of the specific compound is enhanced either by mutation or biotechnological approaches. The modified test microorganism is then grown under controlled conditions (i.e., temperature, PH, aeration, etc.) and specific nutrient media, usually in a fermenter. The product is harvested after downstream processing. The microbial products are basically of two types: Primary metabolites and secondary metabolites. Primary metabolites are the compounds synthesized by microbes during their active growth phase i.e., log phase. Products like ethanol, organic acids, amino acids fall under this category. The secondary metabolites produced during nutrient limitation in stationary phase include antibiotics and mycotoxins. Most of the chemicals and medical

compounds are produced using industrial waste products like whey, molasses; corn steep liquor and potato starch containing fermentable sugars and protein compounds.

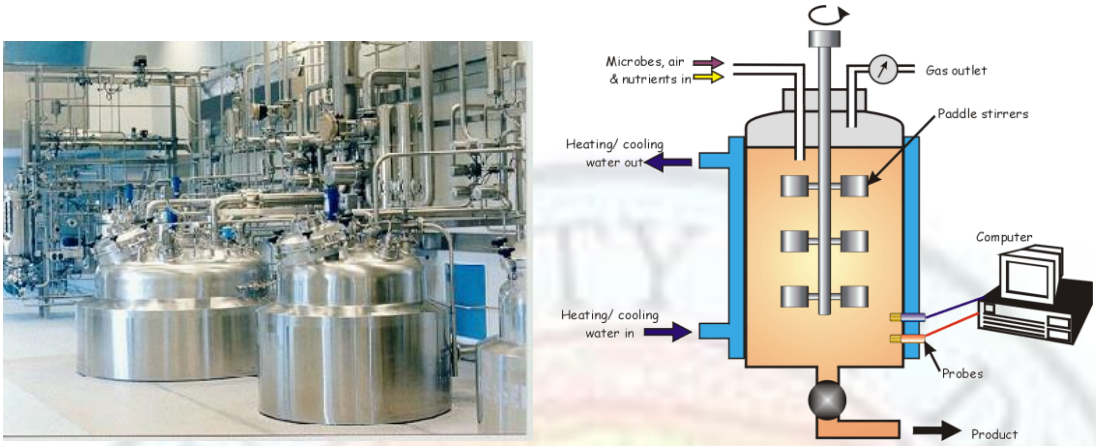


Figure: Fermenter unit

Source:
http://penicillin.wikispaces.com/file/view/Fermenters.jpg/111453935/Fermenters.jpghttp://www.woisd.net/moodle/file.php/6/assets/images/ks4/fermenter.gif

Major Industrial Products From Bacteria

Bacteria synthesize a number of compounds, which have immense commercial applications. These include fermentation products like organic acids, alcohol, amino acids, etc as well as medical products like antibiotics, therapeutic enzymes and hormones, etc.

Some of the important products generated by bacteria naturally or after genetic manipulation are discussed below:

Amino Acids

Amino acids are commonly used as additives in the food industry for enhancing the nutritional quality of food products. They can also be used as flavouring agents in specific foods.

Bacteria like *Enterobacter aerogenes*, *E.coli* and *Corynebacterium glutamicum* are used to produce lysine and glutamic acid. Regulatory mutants of this bacterium which show over production of certain intermediates are used for these fermentations. Lysine is an essential amino acid usually absent from most crop proteins. It is used in number of food material

and especially in bread. Glutamic acid on the other hand is used as a flavour enhancing agent in form of monosodium glutamate.

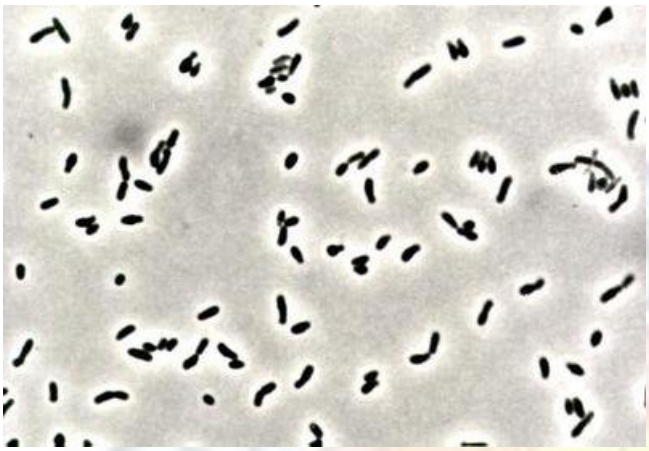


Figure: *Corynebacterium* –A microscopic view

Source:http://microbewiki.kenyon.edu/images/thumb/b/bc/Corynebacterium_glutamicum.jpg/400px-Corynebacterium_glutamicum.jpg

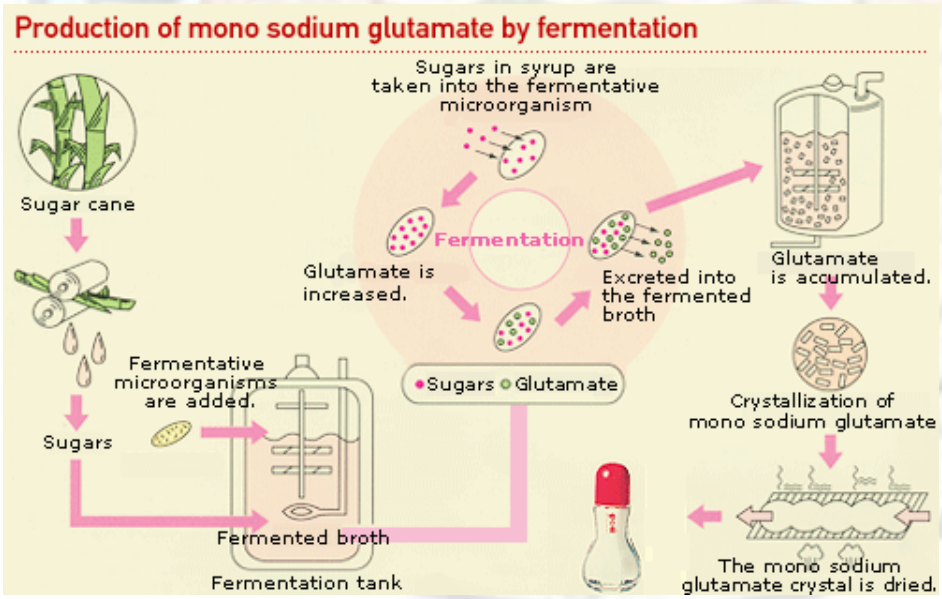


Figure: An overview of monosodium glutamate production

Source:http://glutamicacid.wikispaces.com/file/view/glutamate_production.gif/111376191/glutamate_production.gif

Organic Acids

Organic acids are also used in a variety of foods and pharmaceutical products, for example, lactic acid produced by the bacteria *Lactobacillus bulgaricus* and *L. delbrucckii* is used in food products and textile industry. Lactic acid derivatives like calcium lactate and iron lactate are used for treatment of calcium deficiency and anemia.

Another important organic acid – acetic acid is produced by oxidation of alcohol using the bacteria *Acetobacter* and *Gluconobacter*. Initially yeast is required for conversion of sugar into alcohol, which is the primary substrate. Acetic acid is used as vinegar which is an additive in number of food products.

Alcohol

Alcohol is the most widely used industrial chemical. It is used as major constituent component in the synthesis of pharmaceuticals, detergents, explosives etc. It is a commonly used solvent, extractant and disinfecting agent used in laboratories. Besides, alcohol is also used as a biofuel.

Alcohol is generally produced by fermentation using yeast *Saccharomyces cerevisiae*. However, few bacteria like *Zymomonas mobilis*, can catalyze glucose metabolism to form pyruvate which can then be converted into Ethanol. The bacterium *Sarcina ventriculi* is also known for its ability to produce ethanol in a manner similar to yeast. However, its use in alcoholic fermentation is still in infancy because of low yields. Besides certain members of Enterobactereaceae and Clostridia, also form small amounts of ethanol as a byproduct.

Enzymes

Few bacteria synthesize enzymes, which can be concentrated, purified and used for industrial applications like processing and refining. Further, newer techniques like immobilized enzyme technology have greatly increased the economic output of enzyme production units. Besides, the immobilized enzymes are reusable and the product can be easily recovered.

Various bacterial genera like *Bacillus, Pseudomonas, Clostridium, Proteus* and *Serratia* produce proteases – enzymes involved in the hydrolysis of protein molecules. Bacterial proteases are used in leather processing, tenderization of meat, digestion of fish liver to release fish oil etc. They are also used for clarification of alcoholic beverages.

Bacteria also synthesize cellulases, important in degradation of cellulosic component of plant material. Both aerobic and anaerobic bacteria are cellulolytic in nature for example

Cellulomonas, Thermomonospora (Aerobes) and *Bacterioides, Clostridium* (Anaerobic). This enzyme is used in bio degradation of lignocellulosic waste material and its conversion into useful products like fermentable sugars. In Industry, cellulases are used for clarification of fruit juices and beer.

Some bacteria like *Pseudomonas* sp. also produce lipases, responsible for the breakdown of triglycerides into fatty acids and glycerol. Bacterial lipases can be used in laundry, in food industry for flavour development of dairy products and cheese.

Vitamins

Vitamins are essential for healthy growth and development of living organisms and are primarily derived from food material. Vitamin deficiencies cause number of disorders like stunted growth, neurological malfunctioning, anemia, etc. Some bacteria synthesise vitamins naturally or after addition of certain basic components into the medium.

Vitamin B_{12} (cyanocobalamine) is produced by quite a few bacteria like *Bacillus megaterium, Streptomyces olivaceus, Propionibacterium shermanii, Pseudomonas denitrificans* etc.

Another vitamin, i.e, Vitamin B_2 (Riboflavin) is also produced by bacterial genera like *Clostridium acetobutylicum*, *Mycobacterium smegmatis*, etc. Vitamin B_{12} is important for reproduction in humans and animals. Its deficiency can cause dermatitis, eye damage, etc. Vitamins are being used as additives in animal feed and their use in human food is currently in advanced stages of research.

Bacteria in food industry

Since ages, bacteria have been used for generation of different fermented food and dairy products. These fermented products increase the shelf life of food besides imparting a variety of desirable flavours to it. However, it is very important to use the appropriate bacterial culture for such fermentations. The important fermented food products are discussed below:

Fermented milk

Yoghurt is the most commonly used form of fermented milk. Yoghurt formation involves a thermophilic fermentation (45°C). Cultures of lactic acid bacteria viz. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are used for acid and flavor production.

Butter milk production involves a mesophilic fermentation (20-30°C) by *Lactobacillus spp* and *Lactococcus lactis . L.lactis* subsp diacetilactis is responsible for formation of diacetyl, the compound imparting buttery flavour to the product.

Kefir and koumiss are fermented milk products and their production involves mixed fermentation by bacteria and yeast.

Milk (15-20% reduced water content) or Pasterized yoghurt milk (1-2% milk,fat)

Pasterurize at 95°C

Homogenize at 60 °C

Cool to 43 °C

Mixing starter culture

Package in containers

Incubate containers at 43 °C (pH 4.5)

Cool and store at 5 °C for upto 3-4 weeks

Figure: Steps in processing for fermented milk products

Source: Author

Cheese

Figure: A variety of cheese are available in market

Source: http://upload.wikimedia.org/wikipedia/commons/c/c6/Many_types_of_cheeses.jpg

A variety of cheeses are commercially available and formation of all of them involves lactic acid fermentation of milk. Milk is subjected to fermentation by using *Lactococcus lactis or Lactococcus cremoris*. The milk proteins on coagulation form curd. Enzymes like rennin can also be used for coagulation. The curd is then heated and pressed, separated from the watery part or whey and then salted. The product formed can be packaged as such or ripened for flavour production using other bacteria and even fungi. The common bacterial strains which are used for ripening include *Leuconostoc cremoris, Lactobacillus casei, Lactobacillus bulgaricus etc.*

Table: Major type of cheese and microorganism used in their production.

Source: Author

Country of Origin	**Earlier Stages of Production**	**Later Stages of Production**
Soft, unripened		
Cottage	*Lactococcus Lactis*	*Leuconostoc cremoris*
Cream	*L. cremoris, L. diacetylactis, S. thermophiles, L. bulgaricus*	
Mozzarella (Italy)		

Soft, ripened Brie (France) Camembert (France)	*Lactococcus Lactis, L.cremoris* *L. lactis, L.cremoris*	*Penicillium camemberti.* *P.candidum.* *Brevibacterium linens* *Peniclillium camemberti.* *Brevibacterium linens.*
Semisoft Blue, Roquefort (France) Brick, Muenster (U.S) Limburger (Belgium)	*Lactococcus lactis, L. cremoris* *L. lactis, L. cremoris* *L. lactis L. cremoris*	*Penicillium roqueforti* *Brevibacterium linens* *Brevibacterium lines*
Hard, ripened Cheddar, Colby (Britain) Swiss (Switzerland)	*Lactococcus lactis, L. Cremoris* *L. lactis, L. helveticus, S. thermophilus*	*Lactobacillus casei.* *L. plantarum* *Propionibacterium Shermanii.* *P. freudenreichii*
Very hard, ripened Parmesan (Italy)	*Lactococcus lactis, L. cremoris, S. thermophilus*	*Lactobacillus bulgaricus*

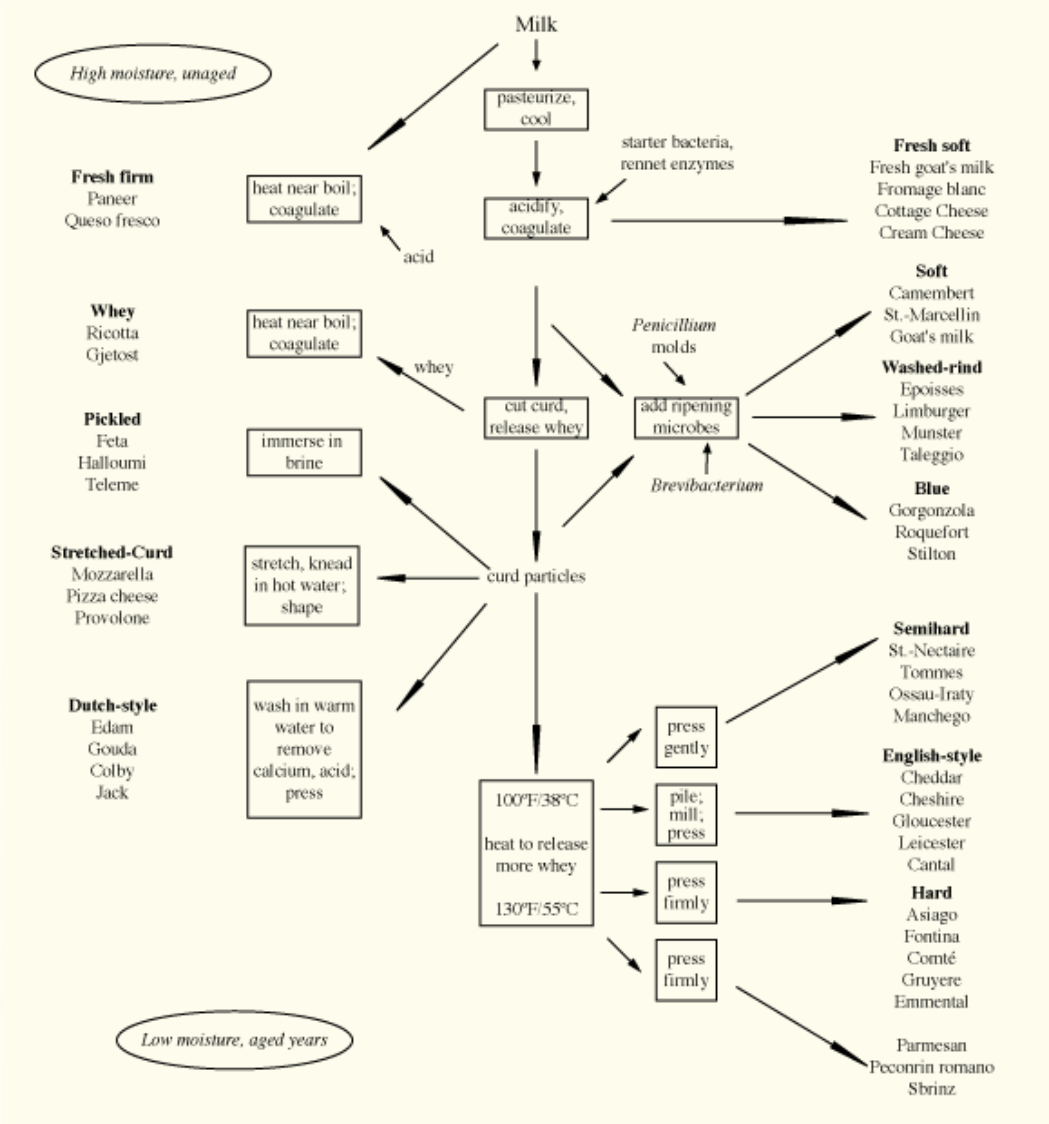


Figure: Steps involved in the production of different types of cheese

Source: http://curdnerds.com/files/cheese-varieties.png

Fermented food

In countries like Japan and China, a number of fermented meat and fish products are consumed. Sausages, hams, salami etc are fermented using *Pediococcus cerevisiae* and *Lactobacillus plantarum*. Fresh fish fermented using *Lactobacilus spp* forms Izushi, a fermented meal common in Japan. Fish sauces, commonly generated by halophilic Bacillus species are also popular.

Sauerkraut, a fermented product of cabbage is consumed worldwide. The fermenting bacteria include *Leuconostoc mesenteroides*, *Lactobacillus plantarum* and *Lactobacillus brevis*. Kimchii, a food made by cabbage and other vegetables is made by Lactic acid bacteria and is common in Korea.

Cabbage is shredded

Packed into bottles. Salt added to limit spoilage.

Fermentation for 20-30 days - lactic acid production
Final product ready for consumption

Figure: Processing of sauerkraut

Source:http://www.earthactionmentor.org/custom/gardeninggals/sauerkraut-cut.gif

Pickles are produced from a variety of vegetables by using liberal doses of salt, other spices and flavouring agents. The salt helps in extraction of water soluble components from vegetables directly as well as indirectly by preventing growth of undesirable microbes. Bacteria like *Pediococcus cerevisiae*, *Leuconostoc mesenteroids*, *Enterococcus faecalis* and *Lactobacillus plantarum* are important in fermentation reactions leading to generation of specific flavours in pickles.

Probiotics

Probiotics are substances, usually live microorganisms which are orally administered to promote good health. Probiotics are bacterial preparations which are used as health supplements. Constituent bacteria of Probiotics like *Lactobacillus, Streptococcus* and *Bifidobacterium* provide nutritional benefits and immunomodulatory activities like antigen processing, presentation and degradation. Other advantages of probiotics are that they are anti-inflammatory and anti cancerous. The probiotics have been found to have a profound effect on digestion, and can control diarrhea to a limited extend. Probiotic bacteria compete with pathogens for space, nutrients while producing certain antimicrobial compounds. The probiotics are being successfully used in animal feeds. A probiotic preparation for cattle containing *Lactobacillus acidophilus* has been found to decrease *E. coli* content of beef. Another probiotic *Bacillus* strain has been formulated as chicken feed additive. This has found to produce healthier chickens with limited pathogens like *Coliform* bacteria and *Campylobacter*.

Figure: Yakult, a probiotic drink for human consumption is currently available in the market and is showing encouraging results.

Source: http://upload.wikimedia.org/wikipedia/commons/1/1a/Yakult_65ml_Vietnam.JPG

Alcoholic Beverages

Alcoholic beverages are produced by fermentation of carbohydrate substrate (present either in grains or fruits) by yeast (*Saccharomyces cerevisiae*). However after fermentation the aging of wine (done to impart flavour) is carried out by bacteria. This involves a secondary fermentation called malolactic acid fermentation. This is done to convert malic acid (highly acidic and sour in taste) into lactic acid and carbon dioxide. Lactic acid is moderately acidic and imparts good flavour to the wine. The bacteria involved are *Leuconostoc oenos*, *L. plantarum* etc. Bacteria are also used for production of wine/ vinegar which uses bacteria like *Acetobacter* and *Gluconobactor* which oxidize ethanol to acetic acid.

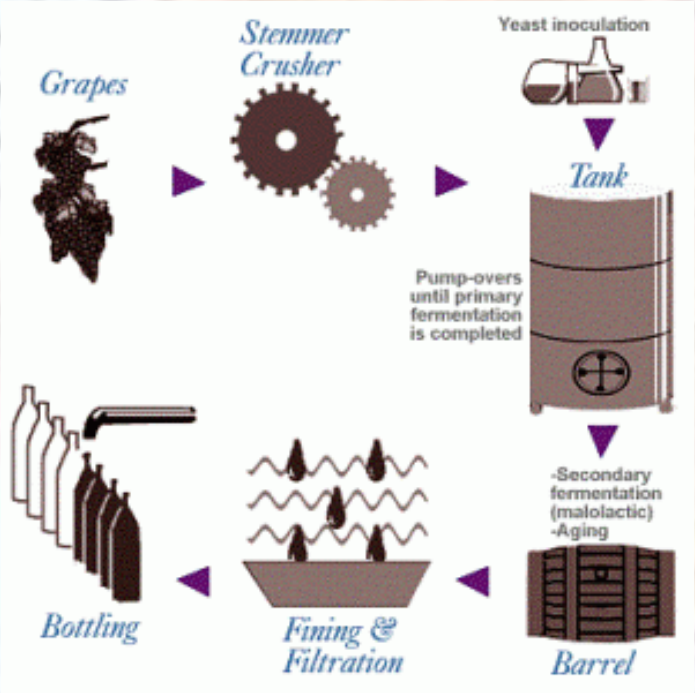


Figure: Wine making

Source: http://www.askwinespirits.com/wp-content/uploads/2011/09/wine-making-3651-300x300.gif

For production of Scotch whisky, the bacterium *Lactobacillus delbrueckii* is used after initial fermentation, i.e., after alcohol production. The production of lactic acid imparts flavor, lowers the pH of the product and prevents development of undesirable microbes as well.

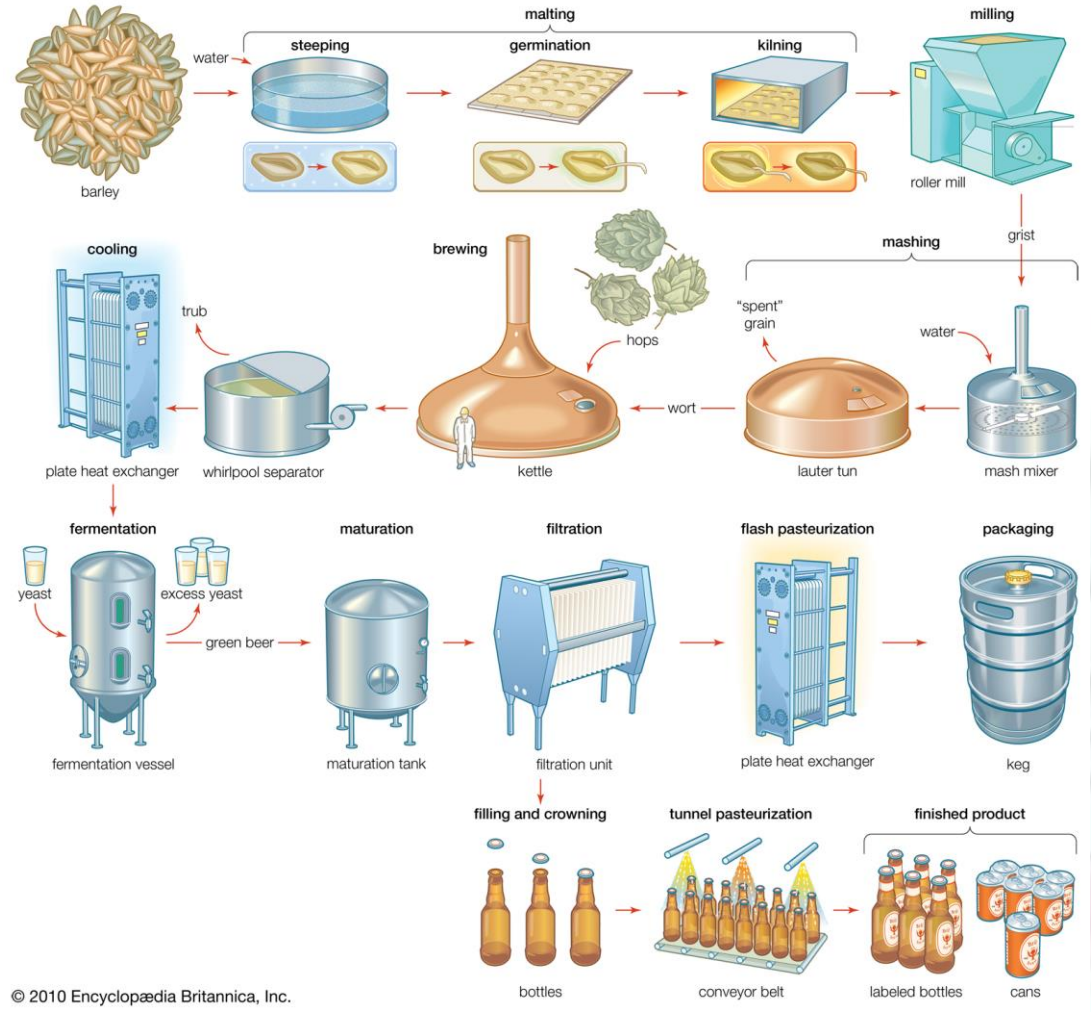


Figure: Beer production

Source: http://www.britannica.com/bps/media-view/70929/1/0/0

Bread

Breads are produced by using yeast fermentation of carbohydrate substrate. However certain special breads such as sour doughs are produced by mixed fermentation with *saccharomyces exiguus* and the bacterium *Lactobacillus* species. Such breads have specific flavour and aroma.

Single cell protein

Microorganisms are rich in protein and can grow rapidly on cheap industrial wastes. Proteins extracted from dried cells of microbes can be used as a source of nutrient in both

human and animal feed. Such protein preparations are referred to as single cell protein (SCP). SCP has high protein content (43-85%), low fat content and is a good source of vitamins. Moreover, microbes have faster growth rate and can serve as perennial sources of protein feed.

Bacteria are commonly used as sources of single cell proteins because they can readily utilize a number of substrates as energy source, simultaneously generating other useful byproducts. Bacteria like *Methylophilus, Methylococcus*, *Pseudomonas, Brevibacterium ketoglutamicum* etc are used to produce bacterial SCP using hydrocarbons like methane, ethane etc as a substrate . Cellulose degrading bacteria like *Cellulomonas* are also used to generate SCP using cellulosic wastes.

Bacteriocins

A number of methods like refrigeration, Pasteurization, canning, chemical treatment, radiations, etc. are commonly used for food preservation. However, each of these methods has some or the other disadvantage. Thus, the scientists are always exploring other methods of food preservation. Recently, the use of Bacteriocins to prevent food spoilage has been proposed. Bacteriocins are antibacterial proteins produced by bacteria, which inhibit closely related bacterial groups. They either act by binding to cell membrane and affecting its function or inhibition of protein, DNA or RNA synthesis.

Additional Information

Bacteriocin as food preservatives: over the last 2 years, a number of bacteriocins produced by bacteria have been characterized biochemically and genetically. Currently, Nisin is the only purified bacterium approved for food use in the US and has been successfully used for several decades as food preservatives in various countries. Lactic acid bacteria produce class 1 and class 2 bacteriocin that have playing role as food preservatives. Nisin like substances were found to be commonplace among cheese culture (Hurst 1967) and now it is understood that Lactic acid Bacillus can produce other bacteriocins.

Streptococcus lactis produces Nisin, a bacteriocin active against gram-positive bacteria, mainly, *Enterococcus faecalis* and *Clostridium botulinum*. Nisin is a small sized protein,

hydrophobic in nature and nontoxic to humans. Bacteriocins are produced by other bacteria also and are quite effective in preservation of variety of foods.

Table: Classification of Bacteriocins of Lactic Acid Bacteria

Source: Author

Producer Strain	**Bacteriocin Example**
Lac. Lactis ATCC11454	Nisin A
Lac lactis CNRZ481	Lacticin 481
Lab sake L45	Lactocin S
Ped.acidilactici H	Pediocin AcH
Leu. gelidum UAL 187	Leucocin A
Lab plantarum LCP 010	Plantaricin S
Car.piscicola LV17A	Carnobacteriocin A
Ent.faecium L51	Enterocin L50
Ent.faecium P13	Enterocin P31
Lab.helveticus J	Helveticin J

Role of bacteria in medicine

Antibiotics

Antibiotics are compounds produced by microorganisms which either kill the target pathogen or inhibit its growth. The first antibiotic was discovered by Alexander Fleming in 1929. He accidentally discovered that *Pencillium notatum* inhibited the growth of gram positive bacterium staphylococcus. Antibiotics are secondary metabolites, mostly produced by streptomyces, a bacterial genus belonging to actinomycetes.

One of the most common antibiotics produced by bacteria is streptomycin. Streptomycin is a broad spectrum antibiotic active against different types of pathogens esp. Gram-negative bacteria and *Mycobacterium*. It is produced by *Streptomyces griseus*.

This bacterium also produces antibiotics like kanamycin, neomycin and tobramycin. These drugs inhibit protein synthesis in pathogen by binding to the small subunit of ribosome. This group of antibiotics are bactericidal and on prolonged usage cause a number of side effects like allergy, deafness and other neurotoxicities.

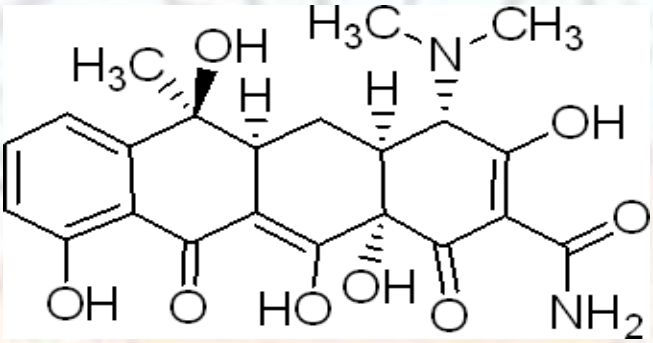


Figure: Tetracycline

Source:Author

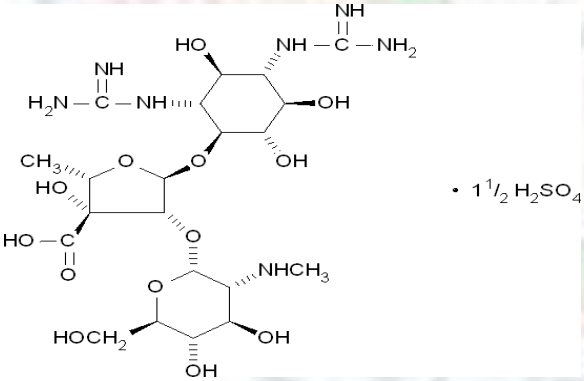


Figure: Streptomycin

Source: Author

Erythromycin, an antibiotic synthesized by *Streptomyces erythraeus* is bacteriostatic and acts by interfering with protein synthesis. This antibiotic is also broad spectrum and used in treatment of diphtheria, mycoplasmal infections, whooping cough etc.

Tetracycline, another antibiotic produced by *Streptomyces* inhibits protein synthesis of pathogens. It is active against bacteria, mycoplasma, rickettsia and Chlamydia.

In addition to antibacterial antibiotics, bacteria also produce antifungal drugs like Nystatin and amphotericin B. Nystatin, an antibiotic produced by *Streptomyces* is used in treatment of superficial mycoses caused by fungi. Nystatin is active against infections of skin, vagina or alimentary tract caused by *Candida*. Nystatin binds to membrane e sterols thereby affecting its permeability. Amphotericin B, in contrast is used to control systemic mycoses, which is resistant to treatment. This drug is also produced by *Streptomyces* spp. and alters membrane permeability by binding to sterols. However, it's judicious use is advised because it is very toxic and has serious side effects.

Vaccines

A vaccine is a preparation of either the altered whole microorganism or its specific components, that is used to induce immunity in host. Bacteria can be thus used for preparation of vaccines which evoke hormonal and cell mediated responses to ward off pathogens during active infection. Vaccines are of different types with their corresponding advantages and disadvantages.

Attenuated bacterial vaccines include vaccine against tuberculosis (*Mycobacterium tuberculosis*) and typhoid (*Salmonella* spp) bacteria. Inactivated whole agent bacterial vaccines have been formulated for *Vibrio cholera, Pneumococcus, Bordetella and Salmonella.* Bacterial vaccines prepared from bacterium *Clostridium tetani* and *Corynebacterium diptheriae* are the toxoid vaccines that is they contain the inactivated toxins of these bacteria. For diphtheria, conjugated vaccines have also been developed containing both the proteins as well as the polysaccharide component of bacterium. A number of recombinant bacterial vaccines are now being produced.

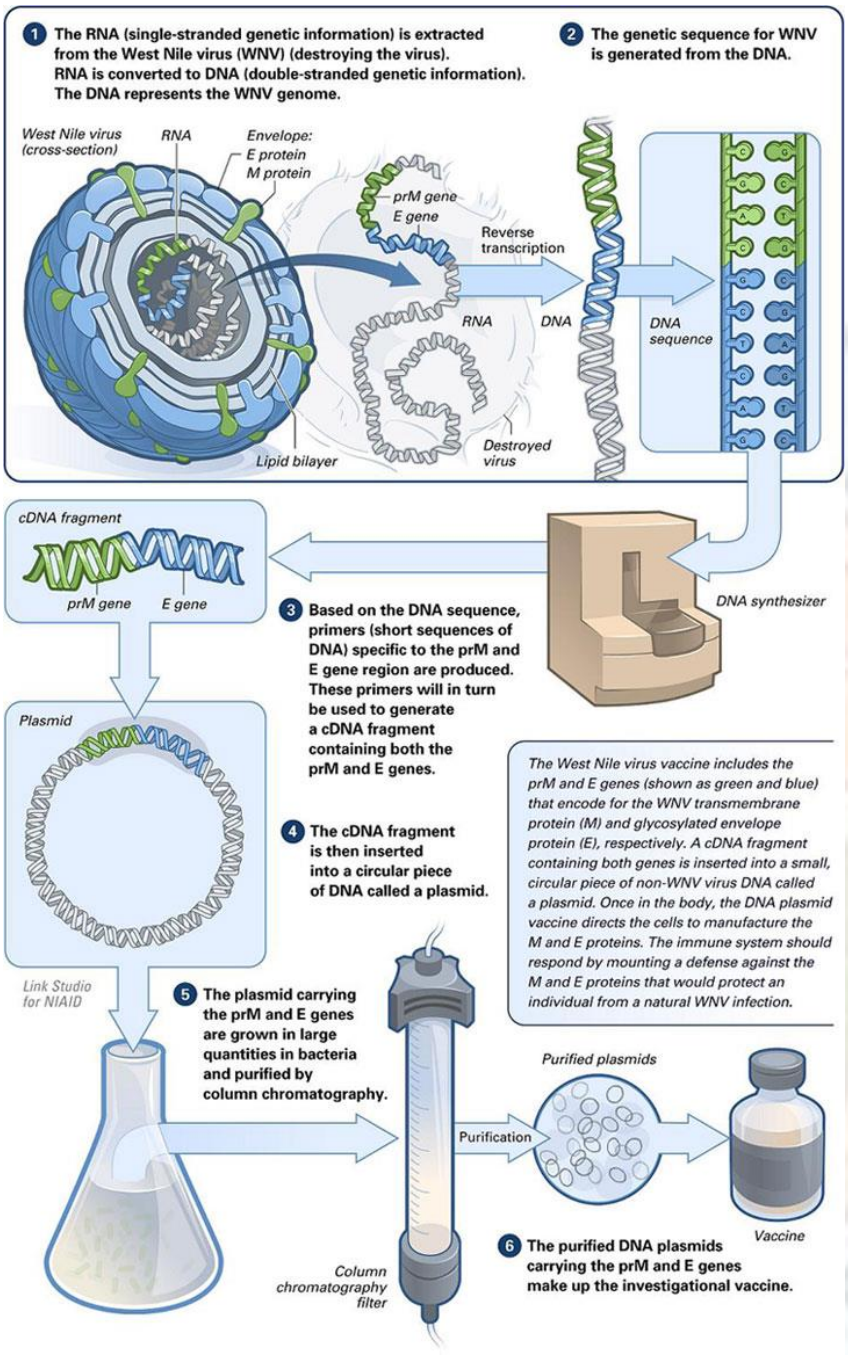


Figure: Recombinant vaccines production

Source:http://upload.wikimedia.org/wikipedia/commons/d/da/Making_of_a_DNA_vaccine.jpg

Steroid Biotransformation

Steroids are used as drugs in the treatment of number of diseases like asthma, rheumatoid arthritis, reproductive and other hormonal disorders. Animal steroids are produced in limited quantity and cannot possibly be extracted from human sources. The usual process of steroid hormone production is by chemical synthesis. However, this process is complicated, low yielding and requires high economic inputs. Another feasible option for production of steroid hormones is bio-conversion of plant sterols into animal steroids. Plant sterols like stigma sterol, Sapogenins are abundant in nature and can be converted into animal steroids using microorganisms. Few bacterial can alter the plant sterols, for example, *Corynebacterium simplex* is used for production of prednisolone.

$$\text{Hydrocortisone} \xrightarrow{\text{Hydroxylation of C-1 \& C-2}} \text{Prednisolone}$$

This bacterium can introduce a hydroxyl group at first and second position at carbon^{-11} of the steroid molecule, hydrocortisone which is otherwise difficult by chemical synthesis. This step is very important and required for biological activity of steroid molecule.

In such biotransformations, steroid precursors are also added besides the steroid to be transformed, microbe and the nutrient medium. Nowadays, research is underway on the usage of purified enzymes for biotransformations rather than the microorganism.

Role of genetically engineered bacteria in medicine

The naturally occurring strains of bacteria produce a variety of compounds with industrial, environmental and medical significance. However, with the advent of genetic engineering technology, the bacterial genes can be manipulated to produce a much larger number of useful molecules. Genes coding for the synthesis of useful products can be transferred through a vector into a bacterial cell using the procedures of recombinant DNA technology.

Hormones

Therapeutic hormones like insulin and growth hormone have been synthesized in the genetically engineered bacterium *E. coli.* Insulin is a glucose catabolizing hormone secreted by islets of Langerhans cells in Pancreas. It is very commonly used for treatment of diabetes. The gene coding for insulin production can be isolated from pancreatic cells or can be chemically synthesized. This gene is then inserted into a vector and cloned in *E. coli* cells. Human insulin was the first genetically engineered product which was approved for use. Similarly, few human

growth hormones viz somatotropin, somatostatin and beta-endorphin have also been synthesized using genetically manipulated *E. coli* cells.

Interferon

Interferons are antiviral proteins produced by virally infected cells. Besides eliminating viruses, interferons have also been found to be anticancerous. Thus interferons form a major group of therapeutic proteins. There are three types of interferons with varied functions viz. interferon alpha, interferon beta and interferon gamma.

Genes for INF-alpha and INF-gamma have been successfully expressed in genetically engineered *E. coli* cells. However, research to increase their yields is still underway.

DNA probes

DNA probes, used for the diagnosis of disease like tuberculosis, leprosy, malaria etc can be synthesized by genetically engineered bacteria eg. *E. coli*. The specific DNA sequences of pathogenic microbes are identified, isolated and inserted into a vector. The host *E. coli* cells are transformed by this vector, followed by cloning. As a result of cloning, a number of copies of foreign DNA fragments are synthesized. For use in disease diagnosis these specific DNA fragments can be radiolabeled and incubated with DNA extracted from patients sample. The hybridization if any can be detected by Autoradiography.

Currently such probes are available for detection of quite a few disease like tuberculosis, leprosy etc.

Bacteria in environment management and agriculture

Industrialization and population explosion have brought with them the menace of pollution. The increased accumulations of industrial, municipal and agricultural waste not only occupy space, but are breeding grounds of pathogens. However microorganisms can be effectively used to degrade this bio-waste, simultaneously generating a number of useful products. The significance of bacteria in environment management and agriculture will be discussed under various headings in the following write-up:

Biodegradation and biofuel generation

The conversion of organic waste into usable forms of energy, fertilizers and other products by microorganisms is known as Biodegradation. The major components of waste are the lignocellulosic agricultural residues. Bacteria which produce *cellulases*, *hemicellulase* and *ligninase* are efficient in bioconversion of such wastes. *Cellulolytic* bacteria include Bacillus, *Cellulomonas*, *Clostridium* etc. while *ligninolytic* bacteria are *Pseudomonas*, *Micrococcus*, *Arthrobacter*, etc. These bacteria either acting singly or in combination with other microorganisms are primarily responsible for biodegradation of lignocellulolytic wastes. Bacteria involved in breakdown of recalcitrant pollutants include *Desulfitobacterium*, *Dehalococcoides* etc.

Biofuels are alternative sources of energy generated by using either plants or micro organisms using readily available cheap substrates like plant residues, municipal wastes etc. Different types of Biofuels are currently either in use or in research stages. Ethanol is generated by fermentation of sugar /cellulose /starch in agricultural crops residues. The waste is first hydrolysed by ligninolytic and cellulolytic bacteria. This leads to production of sugars which are converted to pyruvate using the bacterium *Zymomonas mobilis* followed by decarboxylation to form alcohol.

Additional Information

Biofuels: A group of scientists from university of Exeter have developed a new technique which makes use of bacteria to produce diesel fuel. Ex- *E. coli* bacteria naturally turn sugars into fat or oil that is almost identical to conventional diesel. In addition to *E. coli*, many bacteria as well as number of algae and other organism produce various types of oil.

Biogas is another biofuel commonly generated and used in India for cooking and lighting purposes. It is a mixture of gases primarily composed of methane and carbon dioxide. Bacteria are responsible for generation of Biogas. *Methanogens*, obligate anaerobic bacteria convert organic matter into methane. Hydrogen, a potential biofuel is produced by *Halobacterium*, a halophilic bacteria, which requires high concentration of salt for growth.

Bioremediation

Bioremediation is the process of modification of environment to enhance the degradation or transformation of environmental pollutants by microorganisms. Microorganisms have a natural potential to degrade organic matter, however, this capability could be enhanced by provision of

additional readily utilizable growth substrates like sugar, other nutrients (N_2/P sources) and oxygen. Thus bioremediation is normally achieved by stimulating indigenous microorganisms. However, microbial cultures grown in laboratories could also be inoculated at these polluted sites. Bacterial population used in bioremediation include bacteria with degradative capabilities like cellulolytic, ligninolytic bacteria as discussed before.

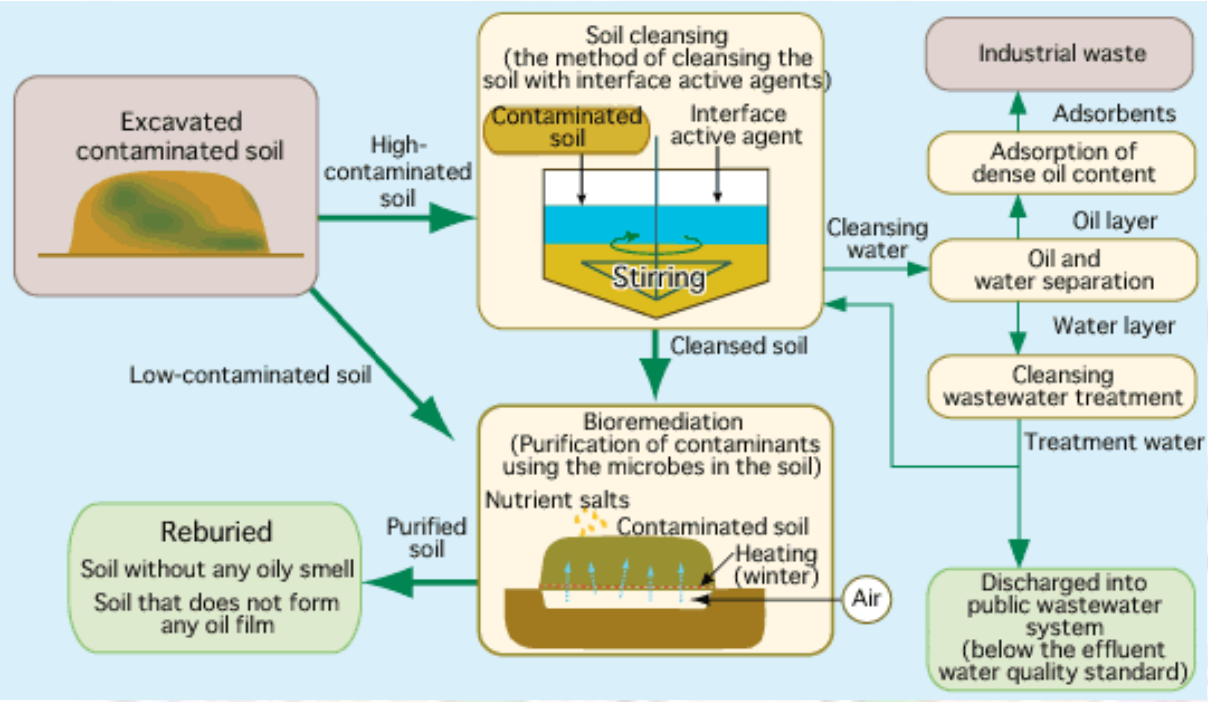


Figure: Bioremediation of contaminated soils

Source: http://www.gses-uae.com/images/gallery_clip_image001.gif

Marine oil spills generate a lot of hydrocarbon pollutants which cannot be easily dispersed and degraded. Addition of oleophillic (hydro-carbon soluble) preparations, nutrients and hydrocarbon degrading bacteria like *Pseudomonas sp.*, *Corynebacteria*, *Microbacteria*, *Bacillus* etc. enhance the bioremediation of oil spills.

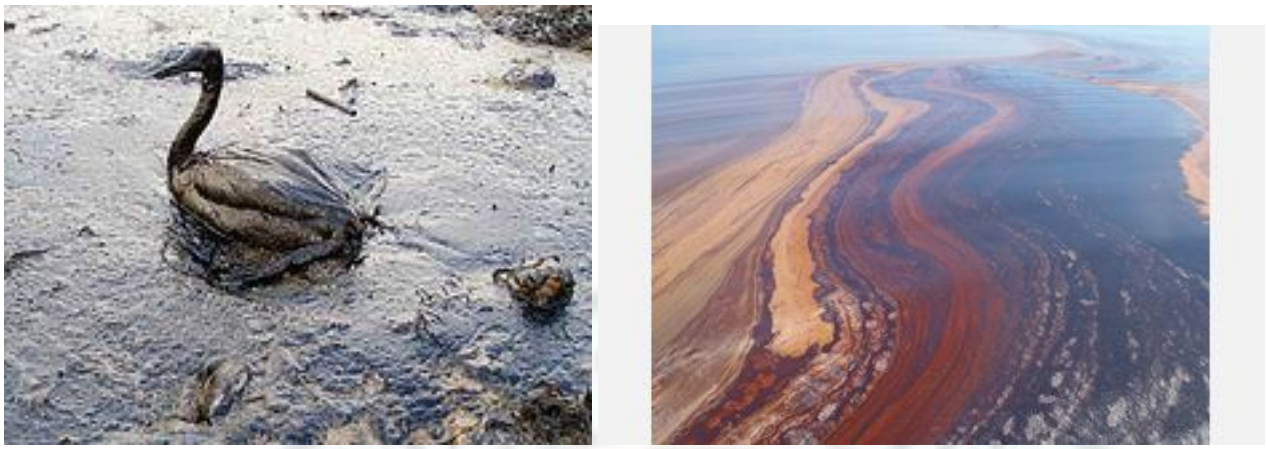


Figure: Oil spills are disastrous for the flora and fauna

Source: http://upload.wikimedia.org/wikipedia/commons/thumb/f/fc/Oiled_Bird_-_Black_Sea_Oil_Spill_111207.jpg/220px-Oiled_Bird_-_Black_Sea_Oil_Spill_111207.jpg, http://www.eoearth.org/files/152101_152200/152141/calc_img03.jpg

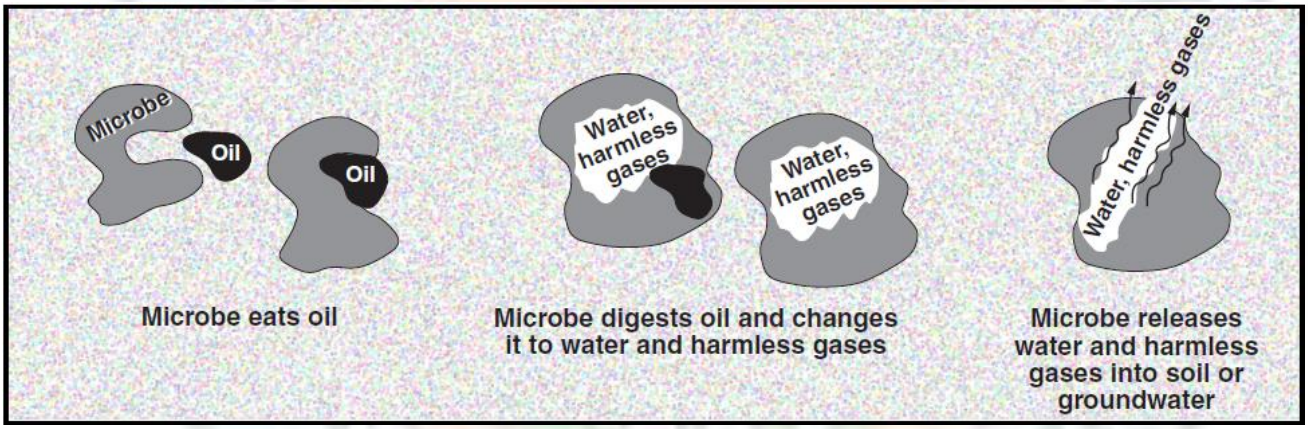


Figure: An illustration depicting the role of microbes in controlling oil spills

Source: http://www.eoearth.org/files/152101_152200/152139/biorem_img01.png

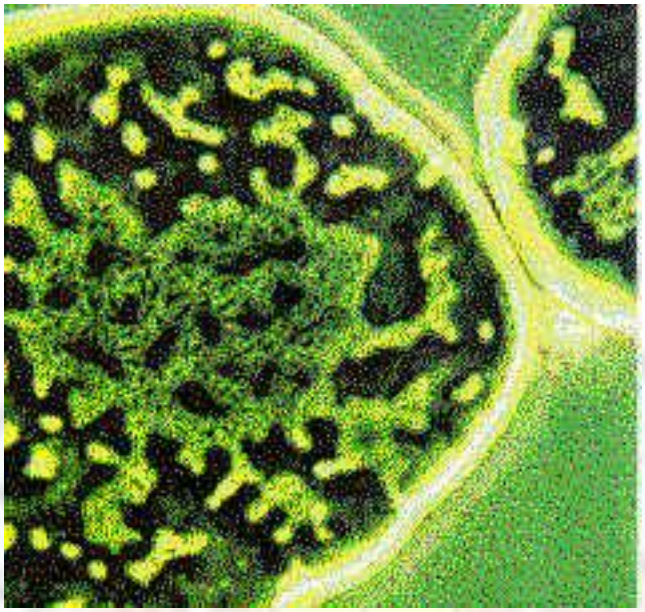


Figure: Magnified image of an oil droplet showing the microbes.

Source: http://www.eoearth.org/files/152101_152200/152140/biorem_img03.jpg

Agrobacterium tumefaciens, a bacteria is used to transfer microbial genes into plants to enhance their capability for biodegradation. Microbial genes like merA and merB, coding for enzymes involved in the degradation of mercury, have been transferred to the plant *Arabidopis thaliana*. In addition, genes coding for tetranitrate reductase, an enzyme which can transform nitrate esters and nitroaromatic compounds present in explosives into less toxic molecules have been transferred from bacteria into tobacco plants. Bacteria can also degrade pesticides like nitrophenols, organophoshates etc. *Pseudomonas diminuta*, *Flavobacterium* are the most potent bacteria involved in pesticide degradation.

Bioremediation can be used for treatment of wide variety of wastes like agricultural sewage and wastes from food industries. Bioremediation can be conducted by using genetically engineered microbes capable of novel degradative abilities. Dr A.M. Chakrabarty designed a super bug, a genetically altered strain of the bacterium *Pseudomonas putida* which could efficiently degrade a number of environmental pollutants like toluene, xylene, octane, naphthalene etc. This superbug was created by introducing plasmid carrying genes coding for pollutant degrading enzymes from different bacterial strains into *Pseudomonas putida*.

Biomining

Microorganism can be used for the recovery of metals from their ores especially the low grade ores. The microbes involved in biomining produce acid, which solublizes the metal from their ore bearing rocks. The bacteria identified for such bioleaching processes are *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*. These bacteria are used to economically extract copper from its low grade ores which is not fit for smelting. Beside there are reduced problems of pollution. Thus use of bacteria for metallurgy is very promising, especially in India which has vast reservoirs of minerals.

Biofertilizers

Biofertilizers are preparations containing live beneficial microorganisms and certain plant nutrients (amino acids, growth hormones, trace elements, carbohydrates etc) which promote plant growth. These formulations help the plant to assimilate environmental nutrients by increasing their availability. Biofertilizers can be usually applied either by seed or soil treatment. They are ecofriendly, cost effective and renewable in contrast to the chemical fertilizers commonly used for soil enrichment.

Nitrogen is a vital nutrient required for growth of living organisms. The atmospheric nitrogen is fixed by microbes and converted into usable form. Nitrogen fixing bacteria such as *Rhizobium, Azotobacter, Azospirillum, Bacillus, Enterobacter, Klebsiella* etc. are used for production of nitrogeneous fertilizers. These fertilizers have found to be quite effective in increasing yields of crop plants when applied in appropriate concentration on seed or in soil.

Figure: Blue green algae are used as bio fertilizers

Source: http://upload.wikimedia.org/wikipedia/commons/thumb/1/18/Blue-green_algae_cultured_in_specific_media.jpg/220px-Blue-green_algae_cultured_in_specific_media.jpg, http://1.bp.blogspot.com/_WoXqOmz71vk/TIEyDa6oZPI/AAAAAAAAABU/HCcVKVERCqI/s320/Shootup.3.jpg

Phosphorus, another essential nutrient is also required for growth of plants and microbes. However, most of the phosphorus is present in insoluble forms and is not readily available. As a consequence, phosphate fertilizers like superphosphates are applied to the crop plants. However, due to agronomic constraints and pollution problems, alternative sources of phosphorus are preferred. The use of phosphate solubilizing bacteria for producing soluble phosphorus is a safe and economic alternative *Pseudomonas, Bacillus, Micrococcus, Flavobacterium* are commonly used phosphate solubilizing bacteria. These bacteria can utilize rock phosphate, aluminum and ferrous phosphate compounds to produce usable forms of phosphorus.

Biopesticides

Plant diseases cause huge economic losses and chemicals have been used for decades to control plant pathogens. However, the synthetic pesticides are toxic and hazardous. The chemical insecticides are gradually becoming ineffective and many species of pathogens have developed resistance to them. More over their widespread use cause pollution. Thus, biological control measures using microorganisims are a feasible alternative. Biopesticides are easy to produce and store, especially the ones from sporulating bacteria. However biopesticides from gram negative bacteria have limited shelf life. In addition, some of these formulations might not be very effective on sturdy pathogens.

Quite a few sporulating species of bacterial genera *Bacillus* are used as bioinsectides viz *B.thuringenesis, B.popilliae, B.lentimorbus, B.sphaericus* etc. Among these *B.thuringiensis* is the most widely used bacterial biopesticides. The mode of action of *B.thuringensis* toxin is significant and peculiar Toxins of *B.thuringensis* are crystalline proteins formed after the bacterium differentiates to form an endospore. The larvae of insects ingest the spore, which reaches its gut. On reaching the gut, the toxins become active and disrupt the permeability of intestinal cell of insect. The intestinal cells swell and burst, as a result of which feeding stops and insect dies within 2-3 days. This toxins produced by the bacterium is known as Bt toxin. Bt toxin is commercially produced by fermentation and has been successfully used against 140 insect species of *Lepidoptera, Diptera* and *Coleoptera*.

The Bt toxin genes have been cloned into fast growing bacteria like *Pseudomonas* and *E.coli* and also into plants like cotton, tobacco, tomato etc for effective results.

Bacteria as causal organisms of plant disease

Bacteria are not only important pathogens of humans and animals diseases, they also cause significant economic losses by infecting plants. The plant pathogenic bacteria are transmitted by wide variety of mechanisms like soil movements, wind, water, infected seeds and infected agricultural tools. The plant bacteria affect plants causing a number of symptoms like wilts, rots, galls dwarfing, cankers, leaf spots and discolorization of plants parts etc. There are a number of bacteria infecting plants, however, the bacterial genera acting as significant plant pathogens include the following :

***Pseudomonas*:** Various species of *Pseudomonas* cause plant disease like wilts, dwarfing, leaf spots, discoloration etc. *P. syringae* is an important plant pathogen causing widespread infections of plants.

Figure: *Phaseolus vulgaris* (common bean) infected by *Pseudomonas syringae*

Source: http://www.invasive.org/images/384x256/5363920.jpg

P.syringae produces cankers and gummosis in various trees even during winter. Another variety of *P.syringae* causes blight of oats producing yellowish patches, due to production of a toxic compound. A genetically engineered strain of *P.syringae* is now being used to prevent ice–crystal damage.

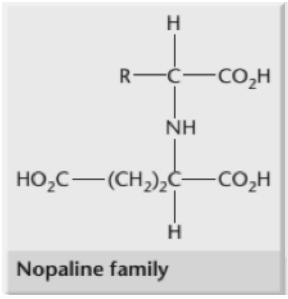
Xanthomonas: *X. campestries* is a common pathogen causing necrosis and canker of citrus fruits. Another variety causes spotting and discoloration in peaches. These species form pectolytic enzymes that hydrolyze pectin between individual plant cells. This causes cells to get separated leading to bacterial soft rot disease.

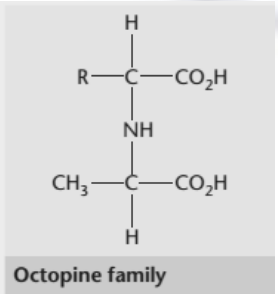
***Erwinia*:** Number of species of *Erwinia* cause plant disease. The common symptoms they induce are rots, wilts, galls and necrosis. Slime producing *Erwinia* species like *E. stewarti* block the passage of water causing wilts in sweet corn.

***Corynebacterium*:** *Corynebacterium* spp cause pathogenesis in varied plant species eg. rots of potato, grasses and tomato. *C. insidiosum* an important plant pathogenic species of *Corynebacterium,* causes vascular infection in alfalfa. *C. michiganense* causes canker (lesion formed after necrosis) formation in tomatoes.

***Streptomyces*:** This bacterial genera usually affects potato and sweet potato causing scabs. *Streptomyces scabies* is a *Streptomycete* bacterium species mainly found in soil. This plant pathogens form lesions on tuber and root crops. This bacterial genera usually affects potato and sweet potato causing scabs. *Streptomyces scabies* mainly cause disease on tuber and tap root crops. It cause scab on potato (*Solanum tuberosum)*, beet (*Beta vulgaris*), carrot (*Daucus carota*), parsnip (*Pastinaca sativa*) and radish (*Raphanus sativus*).

***Agrobacterium*:** These bacteria are very important plant pathogens and commonly inhabit the soil, roots or stems of plants producing galls. Galls are tumors of plant containing undifferentiated cells .When these galls start growing, they tend to cut off the supply of water and nutrients to plants causing plant death. *Agrobacterium tumefaciens* is the most important species of *Agrobacterium* producing marked galls in plant. This bacterium has a plasmid known as Ti plasmid which is mainly responsible for tumor induction and propagation in plant.





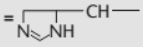
Octopine	$R = NH_2—C(=NH)—NH—(CH_2)_3—$
Octopinic acid	$R = NH_2(CH_2)_3—$
Lysopine	$R = NH_2(CH_2)_4—$
Histopine	
Nopaline	$R = NH_2—C(=NH)—NH—(CH_2)_3—$
Nopalinic acid	$R = NH_2(CH_2)_3—$

Figure : Structure of some opines

Source: Author

Ti plasmid of the bacterium is transferred to the plant cell during course of infection and it gets integrated into the plant .The infected plant gets transformed and starts producing opines which in turn act as a carbon and nitrogen source for bacteria. The bacteria flourish after utilization of opines producing severe infections.

Figure: *Agrobacterium tumefaciens* induces plant tumours.

Source: http://www.bioimages.org.uk/html/p5/p56559.php

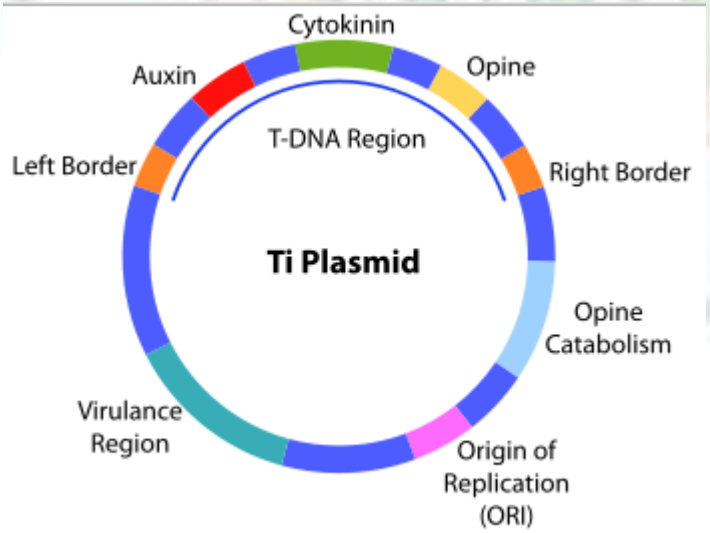


Figure: Ti plasmid gene map

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/d/d1/Ti_plasmid.svg/793px-Ti_plasmid.svg.png

Summary

Microorganisms have a significant impact on human life by their beneficial and harmful activities. With the latest advances in biological sciences and technology, it is now possible to manipulate microbes for benefit of mankind. The applications of microbes in environment, industry and medicine are listed to present a broader outline of their activities. Bacteria plays major role in food industry, medicine, environmental management and agriculture.

Bacteria synthesize a number of compounds which have number commercial applications. These include fermentation products like organic acids alcohol amino acids, etc. as well as medical products like antibiotics, therapeutic enzymes and hormones, etc.

Amino acids are commonly used as additives in the food industry for enhancing the nutritional quality and flavour of food products. Bacteria like *Enterobacter aerogenes*, *E. coli* and *Corynebacterium glutamicum* are used to produce lysine and glutamic acid. Organic acids are also used in variety of foods and pharmaceutical products. Few Bacteria synthesize enzymes which can be concentrated, purified and used for industrial applications like processing and refining. Various Bacterial genera like *Bacillus, Pseudomonas, Clostridium, Proteus* and *Serratia* produce proteases – Enzymes involved in the hydrolysis of protein molecules. Vitamins are essential for healthy growth and development of living organisms and are primarily derived from food material. Vitamin B_{12} (cyanocobalamine) is produced by quite a few bacterial like *Bacillus megaterium, Streptomyces olivaceus, Propionibacterium shermanii, Pseudomonas denitrificans* etc.

Recently, the use of Bacteriocins to prevent food spoilage has been proposed. Bacteriocins are antibacterial proteins, which inhibit closely related bacterial groups. They either act by burning to cell membrane and affecting its function, inhibition of protein or RNA synthesis.

Since ages bacteria have been used for generation of different fermented food and dairy production. These fermented products increase the shelf life of food besides imparting a variety of desirable flavours to it. The important food fermentation by bacteria includes- fermented milk (yoghurt, buttermilk, kefir, koumiss), Cheese, Sauerkraut, Probiotics, various alcoholic beverage, bread and single cell proteins.

Bacteria plays major role in medicine. Bacteria like *Pencillinum notatum*, *streptomyces griseus* etc produce number of antibiotics e.g. Streptomycin, Kanamycin, Neomycin and Tetracycline etc.

Vaccines: A vaccine is a preparation of either the altered whole microorganism or its specific components that is used to induce immunity in host**.** Genetically engineered bacteria with the recombinant DNA technology produce products such as **Hormones, Interferon, DNA probes** etc.

Microorganisms can be effectively used to degrade the bio-waste, simultaneously generating a number of useful products. The significance of bacteria in environment management and agriculture is discussed under various headings –

Biodegradation and biofuel generation- The conversion of organic waste into usable forms of energy, fertilizers and other products by micro organisms is known as Biodegradation.

Bioremediation: Bioremediation is the process of modification of environment to enhance the degradation or transformation of environmental pollutants by micro organism. *Agrobacterium tumefaciens*, a bacteria is used to transfer microbial gene into plants to enhance their capability for biodegradation. Bacteria can also degrade pesticides like nitrophenols, organophoshates etc. *Pseudomonas diminuta*, *flavobacterium* are the most potent bacteria involved in pesticide degradation.

Biomining: Process by which micro organism can be used for the recovery of metals from their ores especially the low grade ores is Biomining.

Bacteria are not only important pathogens of humans and animals disease, they also cause significant economic losses by infecting plants**.** The bacterial genera acting as significant plant pathogens include *Pseudomonas, Xanthomonas, Erwinia, Cornybacterium, Streptomyces, Agrobacterium* etc.

Exercises

Subjective Questions

Q1. What is industrial microbiology? Explain economic importance of bacteria in industry.

Q2. Describe how the *Bacillus thuringiensis* toxin kills insects?

Q3. Elaborate the role of genetically engineered bacteria in medicine.

Q4. Discuss the significance of bacteria in environment management and agriculture .

Q5. Explain in detail giving suitable examples, role of bacteria as causal organisms of plant diseases.

Objective Questions

a) Name the bacteria used for acetic acid production by oxidation of alcohol.

1. *Acetobacter aceti* 2. *Acetobacter xylinum*

3. *Lactobacillus* 4. *Lactococcus*

b) Which temperature is required for yoghurt formation?

1. 38 2. 45

3. 20 4. 25

c) Name the first discovered antibiotic.

1. Ampicillin 2. Penicillin

3. Streptomycin 4.Tetracycline

d) Name the disease caused by *Agrobacterium tumefaciens*.

1. Crown gall disease 2. Rot disease

3. Eyespot disease 4. Crown rust disease

Glossary

Tuberculosis: An infectious disease caused by *Mycobacterium tuberculosis*. Primarily infects the respiratory system but can also spread to other parts of body.

Leprosy : A highly contagious disease caused by *Mycobacterium leprae.* There is massive disfigurement of skin in this disease.

Meningitis: Meningitis could be infectious [caused by microbial agent] or non infectious. This involves inflammation of meninges of brain and spinal cord.

Fermentor : An equipment in which any type of fermentation reaction is carried out. Commonly used in industries for many mass production of various useful compounds like enzymes , amino acids,etc

DNA vaccine : A type of newly discovered vaccine. It contains DNA, encoding for specific proteins. This can be directly injected into the muscle cells and the specific proteins synthesized. DNA vaccines evoke both humoral and cell mediated immune response

Mycotoxins: Toxins produced by fungi affecting various organisms.

Regulatory mutants: There are mutant's which lack regulation of any specific step/steps in a bio chemical pathway.

Lignocellulosic waste: Any waste composed of lignin and cellulose components, usually agricultural wastes

Vector: An autonomously DNA molecule like plasmid or bacteriophage , carrying marker genes for easy identification, which is used to deliver foreign gene of intrest into a host cell.

Clone: A group of genetically identical cells or organisms which are produced either by asexual reproduction or recombinant DNA technology.

Ti Plasmid :A plasmid isolated from Agrobacterium tumefaciens which is used as an efficient plant vector.

Biofuels: The fuels like biodiesel , alcohol, hydrogen etc produced by living organisms, like plants [jatroplia] and microbes [algae]

Rennin: A proteolytic enzyme [which breaks down the proteins]

References:

1. Pelczar M. J., Chan E.C.S., Krieg N. R. 2008. Microbiology (5th Ed.). Tata Mcgraw Hill,New Delhi.

2. Prescott L.M., Harley J.P. and Klein D.A., 2005. Microbiology (6th Edition). Mcgraw Hill Higher Education, New York.

3. Tortora G.J, Funke B.R, Case C.L. 2008. Microbiology: An introduction (9th Ed.). Pearson Education New Delhi.

4. Panicker, Ananthanarayanan: Textbook of Microbiology and Immunology.

5. Primrose S. B., Twyman , R.M. and Old,R.W. 2002. Principles of Gene manipulation:Sixth Edition. Blackwell Publishing. Lodish et. al. 2013.Molecular Cell Biology- (Fifth Edition) W.H. Freeman and Company.

6. Bibek R. 1996. Fundamental of Food Microbiology, Third Edition. CRC Press.

7. Brown T.A.2010. Gene Cloning and DNA analysis: An Introduction, (Fifth Edition). Wiley- Blackwell Publications.

8. Sambrook J., Fritzsch E.F. and Maniatis T.1989. Molecular Cloning .A Laboratory Manual, second edition ,vol 1-3, Cold Spring Harbour Laboratory ,New York