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ONLINE COURSE WARE

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Module I 8L

Lecture 1:

Introduction

Some Factors that control the growth of microorganisms in food fermentation are as follows:

Intrinsic factors- Parameters that are an inherent part of the medium and can alter microbial growth.

- Nutrient content
- Substrate pH
- Antimicrobials
- Redox Potential
- Water activity

Extrinsic factors- Properties of the storage environment that affect the growth of microorganisms.

- Storage temperature
- RH of environment
- Atmosphere
- Presence of other microbes.

For a successful industrial fermentation, the following factors should be considered.

a)Selection of microorganism

The selected organism

1. Should be a potent strain
2. Should be able to ferment cheap raw materials
3. Should not produce any toxic material
4. Should be genetically stable
5. Should not undergo autolysis.

b)Selection of nutrient medium.

The nutrient medium should provide carbon, nitrogen, vitamin and minerals to microbes for their growth and production.

The medium should be

1. non toxic
2. cheap.
3. easily available
4. suitable for utilization for microbes..

c)Selection of optimum pH

d)Selection of optimum temperature

Lecture 2:

Basic idea about microbial growth and fermentation

Microbes(Bacteria, yeasts and molds) are involved in fermentation process.

Fermentation is a metabolic process which converts carbohydrates to alcohol, organic acids and gases by the activity of enzymes originated from microbial cells.

Fermentation may be of batch or continuous process.

Fermentation may be done by surface culture method or submerged culture method.

Surface and Submerged fermentation

Surface culture fermentation or Solid state fermentation is a manufacturing process used in the production of fuel, food, pharmaceutical and industrial products. It is used as an alternative to submerged fermentation. It is known in Japan as Koji fermentation and has existed for many years. It is the use of microorganisms in a controlled environment to produce enzymes, fuel and nutrients. Solid state fermentation occurs in the absence of free water. There are a number of advantages of the use of solid state fermentation over submerged fermentation. It is a much more simple process which requires a lot less energy. It produces a much higher volumetric productivity and it is similar to the natural environment of certain fungi. The volumetric productivity can be up to eight times greater than that of submerged fermentation. Solid state fermentation also has a more easy downstream process than submerged fermentation. The solid state fermentation process involves a solid matrix like rice bran and placing it on a medium to alongside microorganisms create a substrate. This is then stored in at a

specific temperature, between 5 and 95 degrees Celsius, for one to five days. It is also subject to agitation using constant or intermittent rotation. Solid state fermentation plays a key role in developing filamentous fungi. It allows air to come in contact with the mycelium by smearing the mycelium.

Submerged fermentation is a process involving the development of microorganisms in a liquid broth. This liquid broth contains nutrients and it results in the production of industrial enzymes, antibiotics or other products. The process involves taking a specific microorganism such, as fungi, and placing it in a small closed flask containing the rich nutrient broth. A high volume of oxygen is also required for the process. The production of enzymes then occurs when the fungi interact with the nutrients on the broth resulting in them being broken down. At industrial level this production of yeasts has become a major output of microbiological industries as a result of improved fermentation technologies.

Comparison between surface and submerged process

Surface fermentation is easy to control and to implement. It needs no aeration or agitation of the fermentation broth, so it needs no instrumentation for aeration and agitation. The separation of citric acid from the mycelium is easy because the microorganism is not dispersing into the medium. Only the temperature and humidity of the fermentation chamber need controlling. It can be used easily in small plants as well as in third world countries. With surface fermentation, the fermentation broth is concentrated due to a high evaporation rate during fermentation. Thus, expenses and losses during recovery and purification are low. However, surface fermentation has the following disadvantages: Building investment costs are high. Personnel expenses are high in developed industrial countries with extremely high wages. Fermentation time is long and therefore productivity is low.

Submerged fermentation is favored over surface fermentation for the following reasons: lower total investment costs; higher yields of citric acid; improved process control; reduced fermentation time; reduced floor space requirements; lower labor costs; simpler operations; and easier maintenance of aseptic conditions on an industrial scale. However, submerged fermentation has some disadvantages

compared to surface fermentation: expenses for equipment are higher; consumption of electrical energy is higher; and the process is very sensitive to short interruptions or breakdowns in aeration and vulnerable to infections, which result not only in losses of yield, but also in a total breakdown of respective batches.

Lecture 3 &4:

Methods for the microbiological examination of water and foods

Different methods of microbiological examinations are as follows:

1. Indicator organisms

Indicator organisms are microorganisms whose presence in water indicates probable presence of pathogens (disease-causing **organisms).**

A good indicator organism should always be present when the pathogen may be present, it should be present in relatively large numbers to facilitate its detection, it should not proliferate in the environment being monitored and its survival should be similar to that of the pathogen for which it is to be used as an indicator.

Escherichia coli is a natural component of the human gut flora and its presence in the environment, or in foods, generally implies some history of contamination of faecal origin. In water microbiology in temperate climates E. coli meets these criteria very well and has proved a useful indicator organism of faecal pollution of water which may be used for drinking or in the preparation of foods.

Traditionally the group chosen has been designated the coliforms – those organisms capable of fermenting lactose in the presence of bile at 37 °C.

The faecal coliforms, a more restricted group of organisms, are those coliforms which can grow at higher temperatures than normal, i.e. 44-44.5 °C and the methods developed for their detection were intended to provide rapid, reproducible methods for demonstrating the presence of E. coli without having to use time-consuming confirmatory tests for this species.

2. Direct organisms

When examining foods, the possibility of detecting the presence of micro-organisms by looking at a sample directly under the microscope should not be missed.

A small amount of material can be mounted and teased out in a drop of water on a slide, covered with a cover slip, and examined, first with a low magnification, and then with a x 45 objective. The condenser should be set to optimize contrast even though that may result in some loss of resolution.

Alternatively dark-field illumination or phase-contrast microscopy may be used. It is usually relatively easy to see yeasts and moulds and with care and patience it is possible to see bacteria in such a preparation.

The high refractive index of bacterial endospores makes them particularly easy to see with phase-contrast optics and, if the preparation is made as a hanging drop on the cover glass mounted over a cavity slide, it should also be possible to determine whether the bacteria are motile.

Since only a small sample of product is examined in this way, micro-organisms will not be seen unless present in quite large numbers, usually at least 10^6 ml^{-1}. In the case of some liquid commodities, such as milk, yoghurt, soups and fruit juices, it may be possible to prepare and stain a heat-fixed smear.
It may be necessary to dilute the sample with a little water, although that will reduce the concentration of micro-organisms further. The great advantage of such techniques is their rapidity, although in their simplest forms they do not distinguish between live and dead cells.

3. Cultural techniques

Agar is a polysaccharide with several remarkable properties which is produced by species of red algae. Although it is a complex and variable material, a major component of agar is agarose which is made of alternating units of 1,4-linked 3,6-anhydro-L-galactose (or L-galactose) and 1,3-linked D-galactose (or 6-0- methyl-D-galactose).

The properties of agar which make it so useful to microbiologists include the ability to form a gel at low concentrations (1.5-2%) which does not significantly influence the water potential of the medium. Such a gel is stable to quite high temperatures and requires a boiling water bath, or autoclave temperatures, to 'melt' it.

Once molten however, agar solutions remain liquid when cooled to relatively low temperatures (ca. 40 °C) making it possible to mix it with samples containing viable organisms before, or during, dispensing.

A further convenient property of agar is its stability to microbial hydrolysis, despite being a polysaccharide. Only a relatively small group of micro-organisms are able to degrade agar, presumably due to the presence of the unusual L-form of galactose in the polymer.

A very wide range of media are available to the microbiologist and details of their formulation, and how they are used, may be found in a number of readily available books and manuals.

Table *A selection of media commonly used in food microbiology*

Medium	*Use*
Plate Count Agar	Aerobic mesophilic count
MacConkey Broth	MPN of coliforms in water
Brilliant Green/Lactose/Bile Broth	MPN of coliforms in food
Violet Red/Bile/Glucose Agar	Enumeration of Enterobacteriaceae
Crystal Violet/Azide/Blood Agar	Enumeration of faecal streptococci
Baird–Parker Agar	Enumeration of *Staphylococcus aureus*
Rappaport–Vassiliadis Broth	Selective enrichment of *Salmonella*
Thiosulfate/Bile/Citrate/Sucrose Agar	Isolation of vibrios
Dichloran/18% Glycerol Agar	Enumeration of moulds
Rose Bengal/Chloramphenicol Agar	Enumeration of moulds and yeasts

Selective media contain one or more compounds which are inhibitory to the majority of organisms but significantly less so to the species, or group of species, which it is required to isolate.

Elective media on the other hand, are designed to encourage the more rapid growth of one species or group of micro-organisms so that they out-compete others even in the absence of inhibitory agents. Thus cooked-meat broth incubated at 43 to 45 °C allows rapid growth of Clostridium perfringens so that it may become the dominant organism after only 6-8 hours incubation.

The difference between selective and elective media must be seen from the viewpoint of the organism which it is desired to recover. By ensuring optimal growth in the elective medium for one organism, it is desirable that conditions are sub-optimal, or even inhibitory, to others.

4. Enumeration method

Plate Counts:
It has already been suggested that to count micro-organisms in a food sample by direct microscopy has a limited sensitivity because of the very small sample size in the field of view at the magnification needed to see micro-organisms, especially bacteria. In a normal routine laboratory the most sensitive method of detecting the presence of a viable bacterium is to allow it to amplify itself to form a visible colony.

This forms the basis of the traditional pour plate, spread plate or Miles and Misra drop plate still widely used in microbiology laboratories. In the pour plate method a sample (usually 1 ml) is pipetted directly into a sterile petri dish and mixed with an appropriate volume of molten agar.

Even if the molten agar is carefully, tempered at 40-45 °C, the thermal shock to psychrotrophs may result in them not producing a visible colony. The spread-plate count avoids this problem and also ensures an aerobic environment but the sample size is usually limited to 0.1 ml.

5. Alternative methods

A number of methods have been developed which aim to give answers more quickly and hence are often referred to as ‘Rapid Methods’.
Dye-Reduction Tests:

A group of tests which have been used for some time in the dairy industry depend on the response of a number of redox dyes to the presence of metabolically active micro-organisms. They are relatively simple and rapid to carry out at low cost. The redox dyes are able to take up electrons from an active biological system and this results in a change of colour.

Usually the oxidized form is coloured and the reduced form colourless but the triphenyltetrazolium salts are an important exception. Figure 10.1 shows the structures of the oxidized and reduced forms of the three most widely used redox dyes, methylene blue, resazurin and triphenyltetrazolium chloride.

Electrical Methods:

When micro-organisms grow, their activity changes the chemical composition of the growth medium and this may also lead to changes in its electrical properties. Measuring this effect has become the basis of one of the most widely used alternative techniques of microbiological analysis.

The electrical properties most frequently monitored are conductance (G), capacitance (C) and impedance (Z), the latter being influenced by both capacitance and resistance (R) as well as the frequency of the alternating current applied (f). The conductance is simply the reciprocal of resistance.

6. Rapid methods for rapid detection of specific organism and toxins

Immunological Methods:

Because of the potential specificity of immunoassays using polyclonal or monoclonal antibodies, there has been considerable effort devoted to developing their application in food microbiology. Commercial immunoassay kits are now available for detecting a variety of foodborne micro-organisms and their toxins, including mycotoxins.

Raising antibodies to specific surface antigens of micro-organisms, or to macromolecules such as staphylococcal or botulinum toxins, is relatively straightforward and can be achieved directly. Mycotoxins, however, belong to a class of molecules known as haptens which can bind to an appropriate antibody but are of relatively low molecular weight and are not themselves immunogenic.

Haptens can be made immunogenic by binding them chemically to a carrier protein molecule, and antibodies have now been raised using this technique to a wide range of mycotoxins including the aflatoxins, trichothecenes, ochratoxin and fumonisins.

Although a number of different formats are used in immunoassays, their essential feature is the binding of antibody to antigen. A commonly used protocol is that of the sandwich ELISA (enzyme-linked immunosorbent assay) in which a capture antibody is immobilized on a solid surface of say a microtitre plate well.

The sample containing antigen is then added to the well, mixed and removed leaving any antigens present attached to the antibodies.

These are then detected lay adding a second antibody which is coupled to an enzyme such as horseradish peroxidase or alkaline phosphatase. This antibody will also bind to the antigen producing an antibody sandwich. Binding is detected by addition of a chromogenic substrate for the enzyme attached to the second antibody and measuring the colour developed (Figure).

Alternative detection systems are used, such as attachment of antibodies to latex and looking for agglutination in the presence of the antigen and fluorescence-labelled antibodies which can be used to detect target organisms using a fluorescence microscope or flow cytometry.

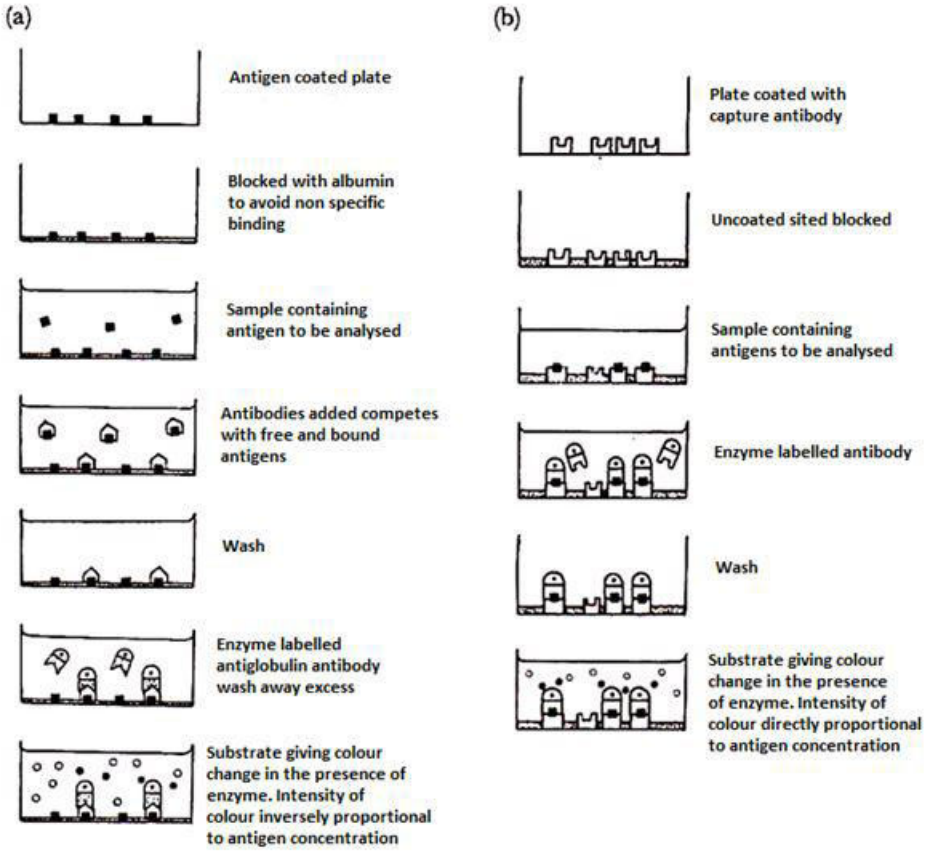


Figure *Sandwich ELISAs. (a) Competitive sandwich ELISA, (b) direct sandwich ELISA*

Commercial ELISAs are available for such organisms as Salmonella and Listeria monocytogenes but they still require the presence of at least 10^5— 10^6 organisms. Detection of smaller numbers therefore depends on some form of enrichment or concentration by one of the separation methods briefly mentioned above, so that although the immunoassay itself may be rapid the whole analytical protocol may take almost as long as conventional procedures.

Some advantage can be gained from the automation of the assay and a number of instruments are commercially available. There may also be some concern over the specificity of immunoassays.

While striving for antibodies that are sufficiently broad in their specificity to recognize all strains of the desired target organism, it is difficult to avoid the problem of cross reactivity with organisms other than those under investigation.

DNA/RNA Methodology:

All biochemical, immunological and other characteristics used in the detection of micro-organisms are governed directly or indirectly by the base sequences encoded in the organism's genome. The specificity of this information can now be mobilized to provide methods capable of identifying genera, species or even strains within a species.

Nucleic acid probes can be designed which recognize and bind (hybridize) to specified regions of either chromosomal or plasmid DNA or to RNA, and the region chosen to give the desired level of specificity. Thus, for example, ribosomal RNA contains both conserved and variable regions, the former being suitable for recognition at the genus level whereas the latter may be considerably more specific.

Although RNA is a more labile molecule than DNA, there are many more copies of ribosomal RNA in a cell than genomic DNA which should make methods based on this molecule more sensitive.

The nucleic acids have to be released from the cells by some form of lysis and, in the case of double-stranded DNA, it has also to be denatured, usually by heat treatment, to the single-stranded form. The denatured nucleic acid is then adsorbed onto a membrane, fixed to it by heat or alkali treatment, and the membrane is treated with some form of blocking agent to prevent nonspecific binding of the probe.

After incubating with the labelled probe and washing off un-adsorbed probe, the presence of the hybridization product is measured using the label attached to the probe. In the earliest stages of the development of this methodology probes were directly labelled with radioactive isotopes such as ^{32}P or ^{35}S and hybridization was detected by autoradiography.
This is a very sensitive method but the routine use of radioactive compounds in a food-associated environment is not usually acceptable. Probes can be labelled with an enzyme and detected with a chromogenic substrate.

Lecture 5:

Coliform bacteria

Coliform bacteria are a natural part of the microbiology of the intestinal tract of warm blooded mammals, including man. Coliform bacteria can also be found in soil, other animals, insects, etc. The total coliform group is relatively easy to culture in the lab, and therefore, has been selected as the primary indicator bacteria for the presence of disease causing organisms.

Coliform bacteria are defined as rod-shaped Gram-negative non-spore forming and motile or non-motile bacteria which can fermentlactose with the production of acid and gas when incubated at 35–37°C. They are a commonly used indicator of sanitary quality of foods and water. Coliforms can be found in the aquatic environment, in soil and on vegetation; they are universally present in large numbers in the feces of warm-blooded animals. While coliforms themselves are not normally causes of serious illness, they are easy to culture, and their presence is used to indicate that other pathogenic organisms of fecal origin may be present. Such pathogens include disease-causing bacteria, viruses, or protozoa and many multicellular parasites. Coliform procedures are performed in aerobic or anaerobic conditions.

Typical genera include:[1]

- *Citrobacter*
- *Enterobacter*
- *Hafnia*
- *Klebsiella*
- *Escherichia*

Escherichia coli (*E. coli*), a rod-shaped member of the coliforms group, can be distinguished from most other coliforms by its ability to ferment lactose at 44°C in the fecal coliformtest, and by its growth and color reaction on certain types of culture media. When cultured on an eosin methylene blue (EMB) plate, a positive result for *E. coli* is metallic green colonies on a dark purple media. Escherichia coli have an incubation period of 12–72 hours with the optimal growth temperature being 30–37°C. Unlike the general coliform group, *E. coli* are almost exclusively of fecal origin and their presence is thus an effective confirmation of fecal contamination. Most strains of *E. coli* are harmless, but some can cause serious illness in humans. Infection symptoms and signs include bloody diarrhea, stomach cramps, vomiting and occasionally, fever. The bacteria can also cause pneumonia, other respiratory illnesses and urinary tract infections.

An easy way to differentiate between different types of coliform bacteria is by using an eosin methylene blue agar plate. This plate is partially inhibitory to Gram (+) bacteria, and will produce a color change in the Gram (-) bacterial colonies

based on lactose fermentation abilities. Strong lactose fermenters will appear as dark blue/purple/black, and *E.coli* (which also ferments lactose) colonies will be dark colored, but will also appear to have a metallic green sheen. Other coliform bacteria will appear as thick, slimy colonies, with non-fermenters being colorless, and weak fermenters being pink.

A **fecal coliform** (British: **faecal coliform**) is a facultatively anaerobic, rod-shaped, gram-negative, non-sporulating bacterium. Coliform bacteria generally originate in the intestines of warm-blooded animals. Fecal coliforms are capable of growth in the presence of bile salts or similar surface agents, are oxidase negative, and produce acid and gas from lactosewithin 48 hours at 44 ± 0.5°C The term "thermotolerant coliform" is more correct and is gaining acceptance over "fecal coliform".

Lecture 6:

Coliform Test

Approved tests for total coliform bacteria include the membrane filter, multiple tube fermentation, MPN and MMO-MUG ("Colilert") methods. The membrane filter method uses a fine porosity filter which can retain bacteria. The filter is placed in a petri (culture) dish on a pad with growth enrichment media (mEndo) and is incubated for 24 hrs at 35 degrees C. Individual bacteria cells which collect on the filter grow into dome-shaped colonies. The coliform bacteria have a gold-green sheen, and are counted directly from the dish. Since some other bacteria may develop a similar color, a confirmation test using more specific media is required. The confirmation procedure requires an additional 24 to 48 hrs to complete the test for suspected positive total coliform tests.

Most Probable Number (MPN)Test

Principle

Water to be tested is diluted serially and inoculated in lactose broth, coliforms if present in water utilize the lactose present in the medium to produce acid and gas.The presence of acid is indicated by color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes giving positive reaction *(i.e both color change and gas production)* and comparing the pattern of positive results *(the number of tubes showing growth at each dilution)* with standard statistical tables.

MPN test is performed in 3 steps

1. Presumptive test
2. Confirmatory test
3. Completed test

1. Presumptive test:

The presumptive test, is a screening test to sample water for the presence of coliform organisms.

If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe.

If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe. If, however, any tube in the series shows acid and gas, the water is considered unsafe and the confirmed test is performed on the tube displaying a positive reaction.

The method of presumptive test varies for treated and untreated water.

Requirements :

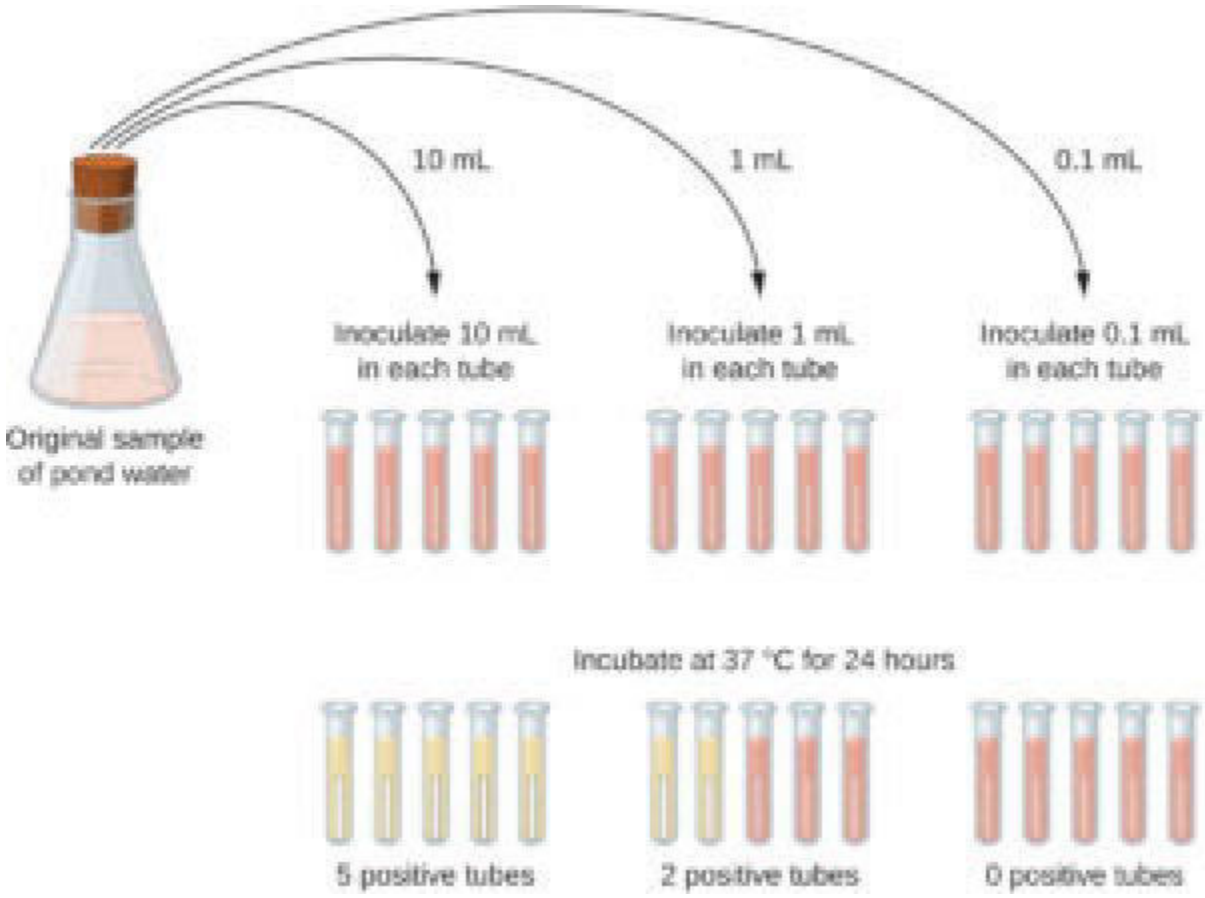
- Medium: Lactose broth or Mac Conkey Broth or Lauryl tryptose (lactose) broth
- Glasswares: Test tubes of various capacities (20ml, 10ml, 5ml), Durham tube
- Others: Sterile pipettes

Preparation of the Medium

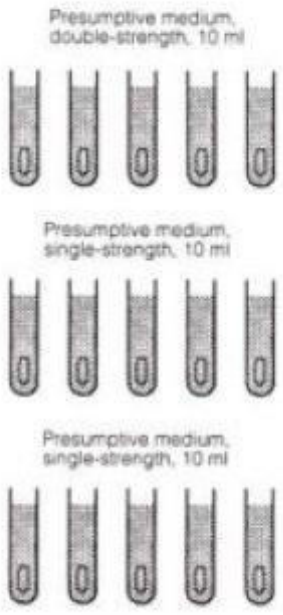
- Prepare medium (either mac conkey broth or Lactose broth)in single and double strength concentration.
- **For untreated or polluted water :**
    - Dispense the double strength medium in 10 tubes (10ml in each tube) and single strength medium in 5 tubes (10 ml in each tube)and add an durham tube in inverted position.
- **For treated water:**
    - Dispense the double strength medium in 5 tubes (10ml in each tube) and 50 ml single strength medium in 1 bottle and add an durham tube in inverted position.

- Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.
- Sterilize by **autoclaving at 15 lbs pressure (121°C) for 15 minutes**.

Procedure of the test



A. For untreated water



MPN Water Testing

1. Take 5 tubes of double strength and 10 tubes of single strength for each water sample to be tested.
2. Using a sterile pipette add 10 ml of water to 5 tubes containing 10 ml double strength medium.
3. Similarly add 1 ml of water to 5 tubes containing 10 ml double strength strength medium and 0.1 ml water to remaining 5 tubes containing 10 ml double strength medium.
4. Incubate all the tubes at 37°C for 24 hrs. If no tubes appear positive re-incubate up to 48 hrs.
5. Compare the number of tubes giving positive reaction to a standard chart and record the number of bacteria present in it.
   For example: a water sample tested shows a result of 3–2–1 (3 × 10 ml positive, 2 × 1 ml positive, 1 × 0.1 ml positive) gives an MPN value of 17, i.e. the water sample contains an estimated 17 coliforms per 100 ml

MPN values per 100 ml of sample and 95% confidence limits for various combinations of positive and negative results (when five 10-ml, five 1-ml and five 0.1 ml test portions are used)

No. of tubes giving a positive reaction :			MPN (per 100 ml)	95% confidence limits	
5 of 10 ml	5 of 1 ml	5 of 0.1 ml		Lower	Upper
0	0	0	<2	<1	7
0	1	0	2	<1	7
0	2	0	4	<1	11
1	0	0	2	<1	7
1	0	1	4	<1	11
1	1	0	4	<1	11
1	1	1	6	<1	15
2	0	0	5	<1	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	40

B. For untreated water

1. Take 1 tube of single strength (50ml) and 5 tubes of double strength (10ml) for each water sample to be tested.
2. Using a sterile pipette add 50 ml of water to the tubes containing 50 ml single strength medium.
3. Similarly add 10 ml of water to 5 tubes containing 10 ml double strength medium Incubate the tubes at 37°C for 24 hrs. If no tubes appear positive re-incubate up to 48 hrs.
4. Compare the number of tube giving positive reaction to a standard chart and record the number of bacteria present in it.
   For example: a water sample tested shows a result of 1-4 (1 × 50 ml positive, 4 × 10 ml positive) gives an MPN value of 16, i.e. the water sample contains an estimated 16 coliforms per 100 ml

MPN values per 100 ml of sample and 95% confidence limits for various combinations of positive and negative results (when one 50-ml and five 10-ml test portions are used)

No. of tubes giving a positive reaction		MPN (per 100 ml)	95% confidence limits	
1 of 50 ml	5 of 10 ml		Lower	Upper
0	0	<1	—	—
0	1	1	<1	4
0	2	2	<1	6
0	3	4	<1	11
0	4	5	1	13
0	5	7	2	17
1	0	2	<1	6
1	1	3	<1	9
1	2	6	1	15
1	3	9	2	21
1	4	16	4	40
1	5	>18	—	—

2. Confirmed test:

Some microrganisms other than coliforms also produce acid and gas from lactose fermentation. In order to confirm the presence of coliform, confirmatory test is done.

From each of the fermentation tubes with positive results transfer one loopful of medium to:

1. 3 ml lactose-broth or brilliant green lactose fermentation tube,
2. to an agar slant and
3. 3 ml tryptone water.

Incubate the inoculated lactose-broth fermentation tubes at 37°C and inspect gas formation after 24 ± 2 hours. If no gas production is seen,further incubate up to maximum of 48 ±3 hours to check gas production.

The agar slants should be incubated at 37°C for 24± 2 hours and **Gram-stained preparations** made from the slants should be examined microscopically.

The formation of gas in lactose broth and the demonstration of Gram negative, non-spore-forming bacilli in the corresponding agar indicates the presence of **a member of the coliform group** in the sample examined.

The absence of gas formation in lactose broth or the failure to demonstrate Gram-negative, non-spore-forming bacilli in the corresponding agar slant constitutes a negative test *(absence of coliforms in the tested sample)*.

Tryptone water Test

1. Incubate the tryptone water at (44.5 ±0.2°C) for 18-24 hours
2. Following incubation, add approximately 0.1ml of Kovacs reagent and mix gently.
3. The **presence of indole** is indicated by a red colour in the Kovacs reagent, forming a film over the aqueous phase of the medium.

a. Confirmatory tests positive for indole, growth, and gas production show the presence of thermotolerant *E. coli.*
b. Growth and gas production in the absence of indole confirm thermotolerant coliforms.

3. Completed test:

Since some of the positive results from the confirmatory test may be false, it is desirable to do completed tests. For this inoculum from each positive tube of the confirmatory test is streaked on a plate of EMB or Endo agar.

In this process, a loopful of sample from each positive BGLB tubes is streaked onto selective medium like **Eosin Methylene Blue agar** or Endo's medium. One plate each is incubated at 37°C and another at 44.5± 0.2°C for 24 hours.

High temperature incubation (44.5 ±0.2) is for detection of thermotolerant E.coli.

Following incubation,all plates are examined for presence of typical colonies.

- Coliforms produce colonies with greenish metallic sheen which differentiates it from non-coliform colonies (show no sheen). Presence of typical colonies on high temperature (44.5 ±0.2) indicate presence of thermotolerant ***E.coli.***

Advantages of MPN :

- Ease of interpretation, either by observation or gas emission
- Sample toxins are diluted
- Effective method of analyzing highly turbid samples such as sediments, sludge, mud, etc.
- that cannot be analysed by membrane filtration.

Disadvantages of MPN:

- It takes a long time to get the results
- Results are not very accurate
- Requires more hardware (glassware) and media
- Probability of false positives

Lecture 7 & 8:
Food borne illnesses and diseases

Foodborne illness (also **foodborne disease** and colloquially referred to as **food poisoning**) is any illness resulting from the food spoilage of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food, as well as toxins such as poisonous mushrooms and various species of beans that have not been boiled for at least 10 minutes.

Symptoms vary depending on the cause, and are described below in this article. A few broad generalizations can be made, e.g.: The incubation period ranges from hours to days, depending on the cause and on how much was consumed. The incubation period tends to cause sufferers to not associate the symptoms with the item consumed, and so to cause sufferers to attribute the symptoms to gastroenteritis for example.

Symptoms often include vomiting, fever, and aches, and may include diarrhea. Bouts of vomiting can be repeated with an extended delay in between, because even if infected food was eliminated from the stomach in the first bout, microbes (if applicable) can pass through the stomach into the intestine and begin to multiply. Some types of microbes stay in the intestine, some produce a toxin that is absorbed into the bloodstream, and some can directly invade deeper body tissues.

Foodborne illness usually arises from improper handling, preparation, or food storage. Good hygiene practices before, during, and after food preparation can reduce the chances of contracting an illness. There is a consensus in the public health community that regular hand-washing is one of the most effective defenses against the spread of foodborne illness. The action of monitoring food to ensure that it will not cause foodborne illness is known as food safety. Foodborne disease can also be caused by a large variety of toxins that affect the environment.

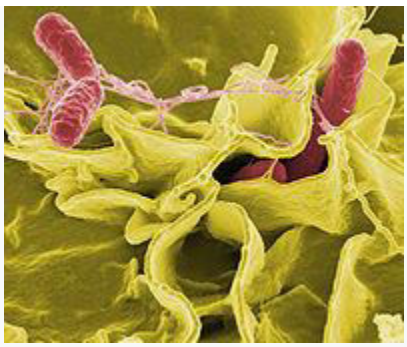
Furthermore, foodborne illness can be caused by pesticides or medicines in food and natural toxic substances such as poisonous mushrooms or reef fish.

Bacteria

Bacteria are a common cause of foodborne illness. In the United Kingdom during 2000, the individual bacteria involved were the following: *Campylobacter jejuni* 77.3%, *Salmonella* 20.9%, *Escherichia coli* O157:H7 1.4%, and all others less than 0.56%.[4] In the past, bacterial infections were thought to be more prevalent because few places had the capability to test for norovirus and no active surveillance was being done for this particular agent. Toxins from bacterial infections are delayed because the bacteria need time to multiply. As a result, symptoms associated with intoxication are usually not seen until 12–72 hours or more after eating contaminated food. However, in some cases, such as Staphylococcal food poisoning, the onset of illness can be as soon as 30 minutes after ingesting contaminated food.

Most common bacterial foodborne pathogens are:

- *Campylobacter jejuni* which can lead to secondary Guillain–Barré syndrome and periodontitis
- *Clostridium perfringens*, the "cafeteria germ"
- *Salmonella* spp. – its *S. typhimurium* infection is caused by consumption of eggs or poultry that are not adequately cooked or by other interactive human-animal pathogens



Salmonella

- *Escherichia coli O157:H7* enterohemorrhagic (EHEC) which can cause hemolytic-uremic syndrome

Other common bacterial foodborne pathogens are:

- *Bacillus cereus*
- *Escherichia coli*, other virulence properties, such as enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroaggregative (EAEC or EAgEC)
- *Listeria monocytogenes*
- *Shigella* spp.
- *Staphylococcus aureus*
- *Staphylococcal enteritis*
- *Streptococcus*
- *Vibrio cholerae*, including O1 and non-O1
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*
- *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Less common bacterial agents:

- *Brucella* spp.
- *Corynebacterium ulcerans*
- *Coxiella burnetii* or Q fever
- *Plesiomonas shigelloides*

Enterotoxins

In addition to disease caused by direct bacterial infection, some foodborne illnesses are caused by enterotoxins (exotoxins targeting the intestines). Enterotoxins can produce illness even when the microbes that produced them have been killed. Symptom appearance varies with the toxin but may be rapid in onset, as in the case of enterotoxins of *Staphylococcus aureus* in which symptoms appear in one to six hours. This causes intense vomiting including or not including diarrhea (resulting in staphylococcal enteritis), and staphylococcal enterotoxins (most commonly staphylococcal enterotoxin A but also including staphylococcal enterotoxin B) are the most commonly reported enterotoxins although cases of poisoning are likely underestimated. It occurs mainly in cooked and processed

foods due to competition with other biota in raw foods, and humans are the main cause of contamination as a substantial percentage of humans are persistent carriers of *S. aureus*. The CDC has estimated about 240,000 cases per year in the United States.

- *Clostridium botulinum*
- *Clostridium perfringens*
- *Bacillus cereus*

The rare but potentially deadly disease botulism occurs when the anaerobic bacterium *Clostridium botulinum* grows in improperly canned low-acid foods and produces botulin, a powerful paralytic toxin.

Pseudoalteromonas tetraodonis, certain species of Pseudomonas and Vibrio, and some other bacteria, produce the lethal tetrodotoxin, which is present in the tissues of some living animal species rather than being a product of decomposition.

Emerging foodborne pathogens

Many foodborne illnesses remain poorly understood.

- *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas sobria*

Preventing bacterial food poisoning

Proper storage and refrigeration of food help in the prevention of food poisoning

Prevention is mainly the role of the state, through the definition of strict rules of hygiene and a public services of veterinary surveying of animal products in the food chain, from farming to the transformation industry and delivery (shops and restaurants). This regulation includes:

- traceability: in a final product, it must be possible to know the origin of the ingredients (originating farm, identification of the harvesting or of the animal)

and where and when it was processed; the origin of the illness can thus be tracked and solved (and possibly penalized), and the final products can be removed from the sale if a problem is detected;
- enforcement of hygiene procedures such as HACCP and the "cold chain";
- power of control and of law enforcement of veterinarians.

At home, prevention mainly consists of good food safety practices. Many forms of bacterial poisoning can be prevented by cooking it sufficiently, and either eating it quickly or refrigerating it effectively. Many toxins, however, are not destroyed by heat treatment.

Module II 8L

Lecture 1:

Production of vinegar

Vinegar is the product of a two-stage fermentation. In the first stage, yeast convert sugars into ethanol anaerobically, while in the second ethanol is oxidized to acetic (ethanoic) acid aerobically by bacteria of the genera Acetobacter and Gluconobacter.

This second process is a common mechanism of spoilage in alcoholic beverages and the discovery of vinegar was doubtless due to the observation that this product of spoilage could be put to some good use as a flavouring and preservative.

The name vinegar is in fact derived from the French vin aigre for ‘sour wine’ and even today the most popular types of vinegar in a region usually reflect the local alcoholic beverage; for example, malt vinegar in the UK, wine vinegar in France, and rice vinegar in Japan.

In vinegar brewing, the alcoholic substrate, known as vinegar stock, is produced using the same or very similar processes to those used in alcoholic beverage production. Where differences occur they stem largely from the vinegar brewer’s relative disinterest in the flavour of the intermediate and his concern to maximize conversion of sugar into ethanol.

In the production of malt vinegar for example, hops are not used and the wort is not boiled so the activity of starch-degrading enzymes continues into the fermentation. Here we will concentrate on describing the second stage in the process, acetification.

Acetification, the oxidation of ethanol to acetic acid is performed by members of the genera Acetobacter and Gluconobacter. These are Gram-negative, catalase-positive, oxidase-negative, strictly aerobic bacteria.

Acetobacter spp. are the better acid producers and are more common in commercial vinegar production, but their ability to oxidize acetic acid to carbon dioxide and water, a property which distinguishes them from Gluconobacter, can cause problems in some circumstances when the vinegar brewer will see his key component disappearing into the air as CO_2.
Fortunately over-oxidation, as it is known, is repressed by ethanol and can be controlled by careful monitoring to ensure that ethanol is not completely exhausted during acetification.

Most acetifications are run on a semi-continuous basis; when acetification is nearly complete and acetic acid levels are typically around 10-14% w/v, a proportion of the fermenter's contents is removed and replaced with an equal volume of fresh alcoholic vinegar stock.

Since a substantial amount of finished vinegar is retained in the fermenter, this conserves the culture and means that a relatively high level of acidity is maintained throughout the fermentation, protecting against contamination.

Vinegar fermentations are usually initiated with seed or mother vinegar, an undefined culture obtained from previous fermentations. Depending on the type of acetification, the culture can be quite heterogeneous and A. europaeus, A. hansenii, A. acidophilum, A. polyoxogenes, and A. pasteurianus have all been isolated from high-acidity fermentations.

Oxidation of ethanol to acetic acid is the relatively simple pathway by which acetic acid bacteria derive their energy. It occurs in two steps mediated by an alcohol dehydrogenase and an aldehyde dehydrogenase . Both enzymes are associated with

the cytoplasmic membrane and have pyrroloquinoline quinone (PQQ) as a coenzyme.

PQQ acts as a hydrogen acceptor which then reduces a cytochrome. The consequent electron transport establishes a proton motive force across the membrane which can be used to synthesize ATP.

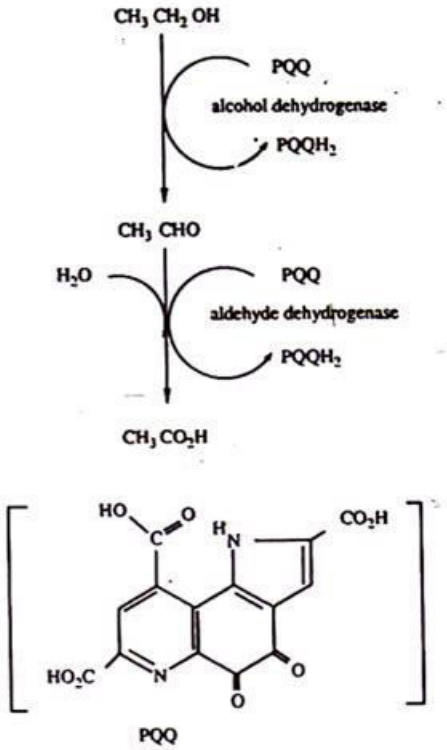


Figure *Oxidation of ethanol by acetic acid bacteria*

Overall acetification is expressed by

$C_2H_5OH + O_2 = CH_3COOH + H_2O$

From the stoichiometry of the equation it can be calculated that 1 l of ethanol should yield 1.036 kg of acetic acid and 0.313 kg of water. This leads to the approximate relationship that 1 % v/v ethanol will give 1 % w/v acetic acid, and this is used to predict the eventual acidity of a vinegar and to calculate fermentation efficiency.

The quick vinegar process derives its name from the faster rates of acetification achieved by increasing the area of active bacterial film and improving oxygen

transfer to the acetifying stock. The acetic acid bacteria grow as a surface film on an inert support material packed into a false-bottomed vat.

A possible cause of fermentation failure in submerged acetification is phage infection. The presence of bacteriophage particles has been demonstrated in disturbed vinegar fermentations both in submerged acetifiers and the quick vinegar process.

Lecture 2:

Production of lactic acid

Lactic acid fermentation is a metabolic process by which glucose and other six-carbon sugars (also, disaccharides of six-carbon sugars, e.g. sucrose or lactose) are converted into cellular energy and the metabolite lactate, which is lactic acid in solution. It is an anaerobicfermentation 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.

Homofermentative process

Overall, the homofermentative lactic acid fermentation converts a six-carbon sugar molecule to two lactic acid molecules, storing the released energy into two ATP molecules. The following equation describes this net result:

Heterofermentative process

Heterofermentative bacteria produce one mole of lactate from one mole of glucose as well as CO2 and acetic acid or ethanol. Examples

include Leuconostoc mesenteroides, Lactobacillus bifermentous, and Leconostoc lactis.

Applications

Lactic acid fermentation is used in many areas of the world to produce foods that cannot be produced through other methods. The most commercially important genus of lactic acid-fermenting bacteria is *Lactobacillus*, though other bacteria and even yeast are sometimes used. Two of the most common applications of lactic acid fermentation are in the production of yogurt and sauerkraut.

Pickle

A product prepared by lactic acid bacteria (LAB) fermentation of sugars present in the pieces of fruits and vegetables. The prepared product is rich in lactic acid, and only the beneficial bacteria that can tolerate lactic acid pH survive. It not only assures good quality of nutrients, but it is also a good source of probiotics.

Kimchi

Kimchi also uses lactic acid fermentation.

Sauerkraut

Lactic acid fermentation is also used in the production of sauerkraut. The main type of bacteria used in the production of sauerkraut is of the genus *Leuconostoc*.

As in yogurt, when the acidity rises due to lactic acid-fermenting organisms, many other pathogenic microorganisms are killed. The bacteria produce lactic acid, as well as simple alcohols and other hydrocarbons. These may then combine to form esters, contributing to the unique flavor of sauerkraut.

Yogurt[

The main method of producing yogurt is through the lactic acid fermentation of milk with harmless bacteria. The primary bacteria used are typically *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and United States as well as European law requires all yogurts to contain these two cultures (though others may be added as probiotic cultures). These bacteria produce lactic acid in the milk culture, decreasing its pH and causing it to congeal. The bacteria also produce compounds that give yogurt its distinctive flavor. An additional effect

of the lowered pH is the incompatibility of the acidic environment with many other types of harmful bacteria.

For a probiotic yogurt, additional types of bacteria such as *Lactobacillus acidophilus* are also added to the culture.[

Lecture 3:

Production of alcoholic beverages (beer)

The basic ingredients of beer are water; a starch source, such as malted barley, able to be fermented (converted into alcohol); a brewer's yeast to produce the fermentation; and a flavouring, such as hops, to offset the sweetness of the malt. A mixture of starch sources may be used, with a secondary saccharide, such as maize (corn), rice, or sugar, often being termed an adjunct, especially when used as a lower-cost substitute for malted barley. Less widely used starch sources include millet, sorghum, and cassava root in Africa, potato in Brazil, and agave in Mexico, among others.

Water

Beer is composed mostly of water. Regions have water with different mineral components; as a result, different regions were originally better suited to making certain types of beer, thus giving them a regional character.

Starch source

The starch source in a beer provides the fermentable material and is a key determinant of the strength and flavour of the beer. The most common starch source used in beer is malted grain. Grain is malted by soaking it in water, allowing it to begin germination, and then drying the partially germinated grain in a kiln. Malting grain produces enzymes that will allow conversion from starches in the grain into fermentable sugars during the mash process. Different roasting times and temperatures are used to produce different colours of malt from the same grain. Darker malts will produce darker beers.

Nearly all beer includes barley malt as the majority of the starch. This is because of its fibrous husk, which is important not only in the sparging stage of brewing (in which water is washed over the mashed barley grains to form the wort) but also as a rich source of amylase, a digestive enzyme that facilitates conversion of starch into sugars. Other malted and unmalted grains (including wheat, rice, oats, and rye, and, less frequently, maize (corn) and sorghum) may be used. In recent years, a few brewers have produced gluten-free beermade with sorghum with no barley

malt for people who cannot digest gluten-containing grains like wheat, barley, and rye.

Hops

Hops are the female flower clusters or seed cones of the hop vine *Humulus lupulus*, which are used as a flavouring and preservative agent in nearly all beer made today. Hops contain several characteristics that brewers desire in beer: they contribute a bitterness that balances the sweetness of the malt; they provide floral, citrus, and herbal aromas and flavours; they have an antibiotic effect that favours the activity of brewer's yeast over less desirable microorganisms; and they aid in "head retention", the length of time that a foamy head will last. The preservative in hops comes from the lupulin glands which contain soft resins with alpha and beta acids.

Yeast

Yeast is the microorganism that is responsible for fermentation in beer. Yeast metabolises the sugars extracted from grains, which produces alcohol and carbon dioxide, and thereby turns wort into beer. In addition to fermenting the beer, yeast influences the character and flavour.

Clarifying agent

Some brewers add one or more clarifying agents to beer, which typically precipitate (collect as a solid) out of the beer along with protein solids and are found only in trace amounts in the finished product. This process makes the beer appear bright and clean, rather than the cloudy appearance of ethnic and older styles of beer such as wheat beers.

Examples of clarifying agents include isinglass, obtained from swimbladders of fish; Irish moss, a seaweed; kappa carrageenan, from the seaweed *Kappaphycus cottonii*; Polyclar(artificial); and gelatin.

Brewing process

The process of brewing beer

Hot water tank

Mash tun

Malt

Hops

Copper

Hopback

Add **yeast** to **fermenter**

Heat exchanger

Bottling

Cask or **keg**

There are several steps in the brewing process, which may include malting, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging.

Malting is the process where barley grain is made ready for brewing. Malting is broken down into three steps in order to help to release the starches in the barley. First, during steeping, the grain is added to a vat with water and allowed to soak for approximately 40 hours. During germination, the grain is spread out on the floor of the germination room for around 5 days. The final part of malting is kilning when the malt goes through a very high temperature drying in a kiln; with gradual temperature increase over several hours.

When kilning is complete, the grains are now termed malt, and they will be milled or crushed to break apart the kernels and expose the cotyledon, which contains the majority of the carbohydrates and sugars; this makes it easier to extract the sugars during mashing. Milling also separates the seed from the husk. Care must be taken when milling to ensure that the starch reserves are sufficiently milled without damaging the husk and providing coarse enough grits that a good filter bed can be formed during lautering. Grains are typically dry-milled with roller mills or hammer mills. Hammer mills, which produce a very fine mash, are often used when mash filters are going to be employed in the lautering process because the grain does not have to form its own filter bed. In modern plants, the grain is often conditioned with water before it is milled to make the husk more pliable, thus reducing breakage and improving lauter speed.

Mashing converts the starches released during the malting stage into sugars that can be fermented. The milled grain is mixed with hot water in a large vessel known as a mash tun. In this vessel, the grain and water are mixed together to create a cereal mash. During the mash, naturally occurring enzymes present in the malt convert the starches (long chain carbohydrates) in the grain into smaller molecules or simple sugars (mono-, di-, and tri-saccharides). This "conversion" is called saccharification which occurs between the temperatures 140 - 158 degrees F. The result of the mashing process is a sugar-rich liquid or "wort", which is then strained through the bottom of the mash tun in a process known as lautering. Prior to lautering, the mash temperature may be raised to about 75–78 °C (167–172 °F) (known as a mashout) to free up more starch and reduce mash viscosity. Additional water may be sprinkled on the grains to extract additional sugars (a process known as sparging).

The wort is moved into a large tank known as a "copper" or kettle where it is boiled with hops and sometimes other ingredients such as herbs or sugars. This stage is where many chemical reactions take place, and where important decisions about the flavour, colour, and aroma of the beer are made. The boiling process serves to terminate enzymatic processes, precipitate proteins, isomerize hop resins, and concentrate and sterilize the wort. Hops add flavour, aroma and bitterness to the beer. At the end of the boil, the hopped wort settles to clarify in a vessel called a "whirlpool", where the more solid particles in the wort are separated out.

After the whirlpool, the wort is drawn away from the compacted hop trub, and rapidly cooled via a heat exchanger to a temperature where yeast can be added. A variety of heat exchanger designs are used in breweries, with the most common a plate-style. Water or glycol run in channels in the opposite direction of the wort, causing a rapid drop in temperature. It is very important to quickly cool the wort to a level where yeast can be added safely as yeast is unable to grow in very high temperatures, and will start to die in temperatures above 60 °C (140 °F). After the wort goes through the heat exchanger, the cooled wort goes into a fermentation tank. A type of yeast is selected and added, or "pitched", to the fermentation tank. When the yeast is added to the wort, the fermenting process begins, where the sugars turn into alcohol, carbon dioxide and other components. When the fermentation is complete the brewer may rack the beer into a new tank, called a conditioning tank. Conditioning of the beer is the process in which the beer ages, the flavour becomes smoother, and flavours that are unwanted dissipate. After conditioning for a week to several months, the beer may be filtered and force carbonated for bottling, or fined in the cask.

Mashing

Mashing is the process of combining a mix of milled grain (typically malted barley with supplementary grains such as corn, sorghum, rye or wheat), known as the "grain bill", and water, known as "liquor", and heating this mixture in a vessel called a "mash tun". Mashing is a form of steeping, and defines the act of brewing, such as with making tea, sake, and soy sauce.Technically, wine, cider and mead are not brewed but rather vinified, as there is no steeping process involving solids.Mashing allows the enzymes in the malt to break down the starch in the grain into sugars, typically maltose to create a malty liquid called wort.

Mashing usually takes 1 to 2 hours, and during this time the various temperature rests activate different enzymes depending upon the type of malt being used, its modification level, and the intention of the brewer. The activity of these enzymes convert the starches of the grains to dextrins and then to fermentable sugars such as maltose. A mash rest from 49–55 °C (120–131 °F) activates various proteases, which break down proteins that might otherwise cause the beer to be hazy. This rest is generally used only with undermodified (i.e. undermalted) malts which are decreasingly popular in Germany and the Czech Republic, or non-malted grains such as corn and rice, which are widely used in North American beers. A mash rest at 60 °C (140 °F) activates β-glucanase, which breaks down gummy β-glucans in the mash, making the sugars flow out more freely later in the process. In the modern mashing process, commercial fungal based β-glucanase may be added as a supplement. Finally, a mash rest temperature of 65–71 °C (149–160 °F) is used to convert the starches in the malt to sugar, which is then usable by the yeast later in the brewing process. Doing the latter rest at the lower end of the range favours β-amylase enzymes, producing more low-order sugars like maltotriose, maltose, and glucose which are more fermentable by the yeast. This in turn creates a beer lower in body and higher in alcohol. A rest closer to the higher end of the range favours α-amylase enzymes, creating more higher-order sugars and dextrins which are less fermentable by the yeast, so a fuller-bodied beer with less alcohol is the result. Duration and pH variances also affect the sugar composition of the resulting wort.

Lautering

Lautering is the separation of the wort (the liquid containing the sugar extracted during mashing) from the grains.

Boiling

After mashing, the beer wort is boiled with hops (and other flavourings if used) in a large tank known as a "copper" or brew kettle – though historically the mash vessel was used and is still in some small breweries. The boiling process is where chemical reactions take place, including sterilization of the wort to remove unwanted bacteria, releasing of hop flavours, bitterness and aroma compounds through isomerization, stopping of enzymatic processes, precipitation of proteins, and concentration of the wort. Finally, the vapours produced during the boil volatilise off-flavours, including dimethyl sulfide precursors

Copper is the traditional material for the boiling vessel, because copper transfers heat quickly and evenly, and because the bubbles produced during boiling, and which would act as an insulator against the heat, do not cling to the surface of copper, so the wort is heated in a consistent manner.The simplest boil kettles are direct-fired, with a burner underneath. These can produce a vigorous and favourable boil, but are also apt to scorch the wort where the flame touches the kettle, causing caramelisation and making cleanup difficult. Most breweries use a steam-fired kettle, which uses steam jackets in the kettle to boil the wort.

Hopback

A hopback is a traditional additional chamber that acts as a sieve or filter by using whole hops to clear debris (or "trub") from the unfermented (or "green") wort,

Wort cooling

After the boiling, the wort must be brought down to fermentation temperatures 20–26 °C (68–79 °F) before yeast is added. In modern breweries this is achieved through a plate heat exchanger

Fermenting

Fermentation takes place in fermentation vessels which come in various forms, from enormous cylindroconical vessels, through open stone vessels, to wooden vats After the wort is cooled and aerated – usually with sterile air – yeast is added to it, and it begins to ferment. It is during this stage that sugars won from the malt are converted into alcohol and carbon dioxide, and the product can be called beer for the first time.

Fermentation tanks are typically made of stainless steel. If they are simple cylindrical tanks with beveled ends, they are arranged vertically, as opposed to conditioning tanks which are usually laid out horizontally

Fermentation methods

In general, yeasts such as *Saccharomyces cerevisiae* are fermented at warm temperatures between 15 and 20 °C (59 and 68 °F), occasionally as high as 24 °C (75 °F),[103] while the yeast used by Brasserie Dupont for saison ferments even higher at 29 to 35 °C (84 to 95 °F).[104] They generally form a foam on the surface of the fermenting beer, which is called barm, as during the fermentation process its hydrophobic surface causes the flocs to adhere to CO_2 and rise; because of this, they are often referred to as "top-cropping" or "top-fermenting – though this distinction is less clear in modern brewing with the use of cylindro-conical tanks.

Filtering

A mixture of diatomaceous earthand yeast after filtering

Filtering the beer stabilizes the flavour, and gives beer its polished shine and brilliance. Not all beer is filtered. When tax determination is required by local laws, it is typically done at this stage in a calibrated tank. There are several forms of filters, they may be in the form of sheets or "candles", or they may be a fine powder such as diatomaceous earth, also called kieselguhr. The powder is added to the beer and recirculated past screens to form a filtration bed.

Filters range from rough filters that remove much of the yeast and any solids (e.g., hops, grain particles) left in the beer, to filters tight enough to strain colour and body from the beer. Filtration ratings are divided into rough, fine, and sterile. Rough filtration leaves some cloudiness in the beer, but it is noticeably clearer than unfiltered beer. Fine filtration removes almost all cloudiness. Sterile filtration removes almost all microorganisms.

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Packaging

Packaging is putting the beer into the containers in which it will leave the brewery. Typically, this means putting the beer into bottles, aluminium cans, kegs, or casks, but it may include putting the beer into bulk tanks for high-volume customers.

Lecture 4:

Production of alcoholic beverages (wine)

The process of **fermentation** in **winemaking** turns grape juice into an alcoholic beverage. During fermentation, yeaststransform sugars present in the juice into ethanol and carbon dioxide (as a by-product). In winemaking, the temperatureand speed of fermentation are important considerations as well as the levels of oxygen present in the must at the start of the fermentation. The risk of stuck fermentation and the development of several wine faults can also occur during this stage, which can last anywhere from 5 to 14 days for *primary fermentation* and potentially another 5 to 10 days for a *secondary fermentation*. Fermentation may be done in stainless steel tanks, which is common with many white wines like Riesling, in an open wooden vat, inside a wine barrel and inside the wine bottle itself as in the production of many sparkling wines.

During fermentation, there are several factors that winemakers take into consideration, with the most influential to ethanol production being sugar content in the must, the yeast strain used, and the fermentation temperature. The biochemical process of fermentation itself creates a lot of residual heat which can take the must out of the ideal temperature range for the wine. Typically, white wine is fermented between 18-20 °C (64-68 °F) though a wine maker may choose to use a higher temperature to bring out some of the complexity of the wine. Red wine is typically fermented at higher temperatures up to 29 °C (85 °F). Fermentation at higher temperatures may have adverse effect on the wine in stunning the yeast to inactivity and even "boiling off" some of the flavors of the wines. Some winemakers may ferment their red wines at cooler temperatures, more typical of white wines, in order to bring out more fruit flavors.

To control the heat generated during fermentation, the winemaker must choose a suitable vessel size or else use a cooling device. Various kinds of cooling devices are available, ranging from the ancient Bordeaux practice of placing the fermentation vat atop blocks of ice to sophisticated fermentation tanks that have built-in cooling rings.

A risk factor involved with fermentation is the development of chemical residue and spoilage which can be corrected with the addition of sulfur dioxide (SO_2), although excess SO_2 can lead to a wine fault. A winemaker who wishes to make a wine with high levels of residual sugar (like a dessert wine) may stop fermentation

early either by dropping the temperature of the must to stun the yeast or by adding a high level of alcohol (like brandy) to the must to kill off the yeast and create a fortified wine.

The ethanol produced through fermentation acts as an important co-solvent to the non-polar compound that water cannot dissolve, such as pigments from grape skins, giving wine varieties their distinct color, and other aromatics. Ethanol and the acidity of wine act as an inhibitor to bacterial growth, allowing wine to be safely kept for years in the absence of air.

Lecture 5:

Production of alcoholic beverages (distilled alcoholic beverages such as whiskey)

Whisky or **whiskey** is a type of distilled alcoholic beverage made from fermented grain mash. Various grains (which may be malted) are used for different varieties, including barley, corn, rye, and wheat. Whisky is typically aged in wooden casks, generally made of charred white oak.

Whisky is a strictly regulated spirit worldwide with many classes and types. The typical unifying characteristics of the different classes and types are the fermentation of grains, distillation, and aging in wooden barrels.

Whisky or whisky-like products are produced in most grain-growing areas. They differ in base product, alcoholic content, and quality.

- Malt whisky is made primarily from malted barley.
- Grain whisky is made from any type of grain.

Malts and grains are combined in various ways:

- *Single malt whisky* is whisky from a single distillery made from a mash that uses only one particular malted grain. Unless the whisky is described as *single-cask*, it contains whisky from many casks, and different years, so the blender can achieve a taste recognisable as typical of the distillery. In most cases, single malts bear the name of the distillery, with an age statement and perhaps some indication of some special treatments such as maturation in a port wine cask.
- *Blended malt whisky* is a mixture of single malt whiskies from different distilleries. If a whisky is labelled "pure malt" or just "malt" it is almost

certainly a blended malt whisky. This was formerly called a "vatted malt" whisky.

- *Blended whisky* is made from a mixture of different types of whisky. A blend may contain whisky from many distilleries so that the blender can produce a flavour consistent with the brand. The brand name may, therefore, omit the name of a distillery. Most Scotch, Irish and Canadian whisky is sold as part of a blend, even when the spirits are the product of one distillery, as is common in Canada.[33] American blended whisky may contain neutral spirits.
- *Cask strength* (also known as *barrel proof*) whiskies are rare, and usually only the very best whiskies are bottled in this way. They are bottled from the cask undiluted or only lightly diluted.
- *Single cask* (also known as *single barrel*) whiskies are bottled from an individual cask, and often the bottles are labelled with specific barrel and bottle numbers. The taste of these whiskies may vary substantially from cask to cask within a brand.
  - Bourbon whiskey—made from mash that consists of at least 51% corn (maize) and aged in new charred oak barrels.
  - Corn whiskey—made from mash that consists of at least 80% corn and is not aged, or, if aged, is aged in uncharred or used barrels.
  - Malt whiskey—made from mash that consists of at least 51% malted barley
  - Rye whiskey—made from mash that consists of at least 51% rye
  - Rye malt whiskey—made from mash that consists of at least 51% malted rye
  - Wheat whiskey—made from mash that consists of at least 51% wheat

Production of Whiskey

.**Fermentation**

Malting

Barley contains starch and it is this starch which needs to be converted into soluble sugars to make alcohol. For this to occur, the barley must undergo germination and this first part of the prodess is called 'malting'. Each distiller has their own preference about the type of barley they buy, but they need a type that produce high yields of soluble sugar. The barley is soaked for 2-3 days in warm water and then traditionally spread on the floor of a building called a malting house. It is

turned regularly to maintain a constant temperature. This is also carried out on a commercial scale in large drums which rotate.

When the barley has started to shoot, the germination has to be stopped by drying it in a kiln. Traditionally peat is used to power the kiln and it is at this point where the type of peat used and length of drying in the peat smoke can influence the flavour of the final spirit. The barley is now called 'malt' and this is ground down in a mill, with any husks and other debris being removed.

Mashing

The ground down malt, which is called 'grist', is now added to warm water to begin the extraction of the soluble sugars. The water is normally from a pure, reliable, local source - this is why most distilleries around the world are next to a river or lake. The character of this water can influence the final spirit as it can contain minerals from passing over or though granite, peat or other rock. The liquid combination of malt and water is called the 'mash'. It is put into a large vessel called a mash tun and stirred for several hours.

During this process, the sugars in the malt dissolve and these are drawn off through the bottom of the mash tun. The resulting liquid is called 'wort'. This process is normally carried out three times with the water temperature being increased each time to extract the maximum amount of sugar. Only wort from the first two times is used. The third lot is put back into the next batch of new grist. Any residue, such as husks, is called 'draff'. This is collected and used in the production of farm feed.

Fermentation

The wort is cooled and passed into large tanks called washbacks. These are traditionally made of wood, but now a number of distilleries use stainless steel. Here the yeast is added and the fermentation begins. The yeast turns the sugars that are present into alcohol. As with the barley and water, the distiller will carefully select the strain of yeast that they use and it can also have a small effect on the final flavour of the spirit. The fermentation normally takes around 48 hours to run its natural course, although some distilleries will let it go for longer so as to create further characteristics that they require. The liquid at this stage is called 'wash' and is low in alcohol strength (between 5-10% ABV), like beer or ale. You could make

beer from the liquid at this point, but the difference with whisky is that the liquid is now distilled rather than brewed.

Distillation

A still for making whisky is usually made of copper, since it removes sulfur-based compounds from the alcohol that would make it unpleasant to drink. Modern stills are made of stainless steel with copper innards (piping, for example, will be lined with copper along with copper plate inlays along still walls). The simplest standard distillation apparatus is commonly known as a pot still, consisting of a single heated chamber and a vessel to collect purified alcohol.

Column stills are frequently used in the production of grain whisky and are the most commonly used type of still in the production of bourbon and other American whiskeys. Column stills behave like a series of single pot stills, formed in a long vertical tube. Whereas a single pot still charged with wine might yield a vapour enriched to 40–60% alcohol, a column still can achieve a vapour alcohol content of 95.6%; an azeotropic mixture of alcohol and water.

Aging

Whiskies do not mature in the bottle, only in the cask, so the "age" of a whisky is only the time between distillation and bottling. This reflects how much the cask has interacted with the whisky, changing its chemical makeup and taste. Whiskies that have been bottled for many years may have a rarity value, but are not "older" and not necessarily "better" than a more recent whisky that matured in wood for a similar time. After a decade or two, additional aging in a barrel does not necessarily improve a whisky.

While aging in wooden casks, especially American oak and French oak casks, whisky undergoes six processes that contribute to its final flavor: extraction, evaporation, oxidation, concentration, filtration, and colouration. Extraction in particular results in whisky acquiring a number of compounds, including aldehydes and acids such as vanillin, vanillic acid, and syringaldehyde. Distillers will sometimes age their whiskey in barrels previously used to age other spirits, such as rum or sherry, to impart particular flavours.

Packaging

Most whiskies are sold at or near an alcoholic strength of 40% abv, which is the statutory minimum in some countries[8] – although the strength can vary, and cask-strength whisky may have as much as twice that alcohol percentage.

Lecture 6:

Production of alcoholic beverages (distilled alcoholic beverages such as rum)

Rum is a distilled alcoholic drink made from sugarcane byproducts, such as molasses, or directly from sugarcane juice, by a process of fermentation and distillation. The distillate, a clear liquid, is then usually aged in oak barrels. ... **Rums** are **produced** in various grades.

Step 1 – Sugar cane juice or molasses

As a general rule, rum can be divided into those produced by distilling sugar cane juice, and those made from molasses. Obtained by crushing sugar cane, sugar cane juice deteriorates very quickly, and so needs to be fermented as soon as possible, and then distilled to produce agricultural rum. A thick, residual syrup that results from the refinement of cane sugar, molasses are used as an ingredient in desserts and sweets, and in the production of numerous types of rum.

Sugar cane ('*Saccharum officinarum*') grows in tropical regions in the equatorial zone. It can be found in Florida, Texas, Louisiana, the West Indies, in Hawaii, in Central and South America, and also in Indonesia, Thailand, the Philippines, China, India, islands in the Indian Ocean, Australia, and in southern Spain. There are many varieties with various degrees of resistance to disease and levels of sugar content. The cane consists of water, fibres and saccharose. The saccharose is concentrated at the base of the cane.

The canes are harvested manually or mechanically at the age of 11 months, before they flower. The leaves and tops of the sugar canes are left in the fields. The bases are quickly transported to the sugar refinery to avoid any of the sugar being lost. The bases of the canes are then ground into fibres and hot water is added to extract the sweet juice. The pressing process results in two products: cane juice for the production of rum, and the bagasse, consisting of the fibrous residue, which is used as fuel.

In the case of molasses-based rum, the sugar is extracted from the cane juice and then transformed into molasses.

Step 2 – Cane wine and fermentation

Yeast is then added to the mash (molasses diluted with water or sugar cane juice), which is then fermented and gradually converted into alcohol until it eventually produces a sugar cane wine containing around 8% to 10% alcohol. A fundamental stage in the development of the flavours of the future rum, the fermentation of the sugar cane wine is conducted in various ways in different parts of the world, resulting in a very broad aromatic range.

There are three types of fermentation:

- **Spontaneous fermentation**

This relies on the yeasts and micro-organisms naturally present in the atmosphere or in the sugar cane juice. Such fermentation takes place in open vats and takes between 1 and 2 weeks. Small distilleries, especially those in Haiti, still practise spontaneous fermentation.

- **Controlled fermentation (batch)**

Usually carried out in batches, this kind of fermentation uses laboratory-grown yeasts that are added to the sweet liquid. Some distilleries cultivate and maintain their own strains of yeast that they protect, like a trademark. Such fermentation is spread over 2 to 3 days and enables the production of a consistent percentage of alcohol and range of flavours.

- **Controlled fermentation (continuous)**

This is a growing trend in the rum industry. It involves keeping a fermentation vat permanently filled, with the continuous addition of more molasses. This enables the yeasts to be kept active by removing a quantity of the mash, whose sugars have already been digested by the yeasts, from different sections of the vat.

Step 3 – Choice of still

Like many other spirits, rum can be distilled in a column still, continuously, or in a pot still (used by the more traditional producers). The type of distillation practised is often influenced by the country's colonial history. Former British and French colonies still use copper pot stills, while those of Spanish origin tend to prefer column stills. The type of rum produced very much depends on the distillation method: in basic terms, heavier rums tend to come from pot stills (due to the middle cut of the distillate being collected at between 68% and 70% alcohol) while lighter rums are produced by column stills (the distillate collected has an alcohol content above 90%, thereby eliminating the heavier vapour).

Pot still - batch distillation: this 'batch' distillation technique requires regular stopping of the still so that it can be cleaned and allowed to rest before loading with another batch for distillation. This is the most traditional of distillation methods.

Column still - continuous distillation: usually consisting of two or four columns, each one feeding the next, this type of distillation does not require any interruption, with the columns being continuously supplied. Consisting of different levels of concentration, through which the steam circulates, this technique enables the rum's aromatic profile to be monitored and controlled. Only its lightest vapour in terms of flavour reaches the column's final level. The heavier vapour remains at the lower levels.

Step 4 – Ageing

Since there are no legal regulations, the ageing process and associated designations vary from one producer to another. While most ageing takes place in old bourbon barrels, rum can also be aged in cognac barrels and new oak barrels.

Lecture 7:

Production of alcoholic beverages (distilled alcoholic beverages such as vodka)

Vodka is an alcoholic beverage distilled at a high proof from a fermented vegetable or grain mash. Proof is a measurement of the alcohol content. Each degree of proof equals a half percent of alcohol. Thus, 100 proof is that which contains 50% alcohol, 90 proof contains 45%, and so on. Because distilled vodka can have a proof as high as 145, all taste and odor has been eliminated, making vodka a neutral spirit. Water is added to bring the proof down to a range between 80 and

The practice of allowing certain grains, fruits, and sugars to ferment so that they produce an intoxicating beverage has been around since ancient times. Fermentation is a chemical change brought about by the yeast, bacteria, and mold in an animal or vegetable organism. In the production of alcoholic beverages, yeast enzymes act on the sugars in the mash (usually dextrose and maltose) and convert them to ethyl alcohol.

Raw Materials

Vegetables or grains

Because it is a neutral spirit, devoid of color and odor, vodka can be distilled from virtually any fermentable ingredients. Originally, it was made from potatoes. Although some eastern European vodkas are still made from potatoes and corn, most of the high quality imports and all vodka made in the United States are distilled from cereal grains, such as wheat. Distillers either purchase the grain from suppliers, or grow it in company-owned fields.

Water

Water is added at the end of the distillation process to decrease the alcohol content. This is either purchased from outside suppliers or brought in from company-owned wells.

Malt meal

Because vegetables and grains contain starches rather than sugars, an active ingredient must be added to the mash to facilitate the conversion of starch to sugar. These particular converted sugars, maltose, and dextrin respond most effectively to the enzyme diastase that is found in malt. Therefore, malt grains are soaked in water and allowed to germinate. Then, they are coarsely ground into a meal and added during the mash process.

Yeast

A microscopic single-celled fungus, yeast contains enzymes that allow food cells to extract oxygen from starches or sugars, producing alcohol. In the manufacturing of alcoholic beverages, the yeast species *Saccharomyces cerevisiae* is used. It is purchased from outside suppliers.

Flavorings

In the latter part of the twentieth century, flavored vodkas became popular. Thus, herbs, grasses, spices, and fruit essences may be added to the vodka after distillation. These are usually purchased from an outside supplier.

Manufacturing Process

Mash preparation

- 1 The grain or vegetables are loaded into an automatic mash tub. Much like a washing machine, the tub is fitted with agitators that break down the grain as the tub rotates. A ground malt meal is added to promote the conversion of starches to sugar.

Sterilization and inoculation

- 2 Preventing the growth of bacteria is very important in the manufacture of distilled spirits. First, the mash is sterilized by heating it to the boiling point. Then, it is injected with lactic-acid bacteria to raise the acidity level needed for fermentation. When the desired acidity level is reached, the mash is inoculated once again.

Fermentation

- 3 The mash is poured into large stainless-steel vats. Yeast is added and the vats are closed. Over the next two to four days, enzymes in the yeast convert the sugars in the mash to ethyl alcohol.

Distillation and rectification

- 4 The liquid ethyl alcohol is pumped to stills, stainless steel columns made up of vaporization chambers stacked on top of each other. The alcohol is continuously cycled up and down, and heated with steam, until the vapors are released and condensed. This process also removes impurities. The vapors rise into the upper chambers (still heads) where they are concentrated. The extracted materials flow into the lower chambers and are discarded. Some of the grain residue may be sold as livestock feed.

Water added

- 5 The concentrated vapors, or fine spirits, contain 95-100% alcohol. This translates to 190 proof. In order to make it drinkable, water is added to the spirits to decrease the alcohol percentage to 40, and the proof to 80.

Bottling

- 6 Alcoholic beverages are stored in glass bottles because glass is non-reactive. Other receptacles, such as plastic, would cause a chemical change in the beverage. The bottling procedure is highly mechanized as the bottles are cleaned, filled, capped, sealed, labeled, and loaded into cartons. This can be done at rates as high as 400 bottles per minute.

Lecture 8:

Production of Glycerol

An osmophilic yeast, Candida Krusei, was used for glycerol production with various starchy substrates. The results showed that cassava

starch and corn starch were easily liquefied and saccharified compared with corn meal and sweet potato meal. The optimal concentrations of corn steep liquor (CSL) added to the liquefied and saccharified media from corn meal, sweet potato meal, cassava starch, and corn starch were 0, 0, 0.15%, and 0.1%, respectively. Correspondingly, the maximal glycerol productions were 3.8%, 3.5%, 5.5%, and 7.1%, respectively. Furthermore, it was found that glycerol produced was utilized as the second carbon source at 24 h, 24 h, 72 h, and 84 h, respectively. in addition, accompanying the lower rate of glycerol production (RGP), the maximal glycerol production (GP) and glycerol yield (GY) were reached when corn starch was selected as starchy substrate. in the case of corn meal and sweet potato used as starchy substrates, biomass increased rapidly and residual glucose decreased quickly, compared with corn starch and cassava starch.

Glycerol, an important chemical product, has been widely used in the cosmetic, paint, tobacco, food, and pharmaceutical industries. Traditionally, three kinds of processes, chemical synthesis from petrochemical materials, recovery from soap manufacture, and microbial fermentation process, are employed for glycerol production. However, the gradual replacement of soaps by detergents and the

petroleum crisis of the last decade have absorbed worldwide attention back to the fermentation route for glycerol production .
Therefore, much works toward glycerol fermentation have been made. Spencer et al. had ever reported glycerol production using Aerobacter aerogenes under high glucose concentration and 3.2–4.8% glycerol were reached . A strain of Torulopsis magnoliae was isolated for glycerol production and it was found that glycerol was the only accumulating product during fermentation, and 17% glycerol were obtained .

Microorganism and inoculums preparation

An osmophilic yeast, Candida krusei ICM-Y-05, used was obtained from State Key Laboratory of Biochemica Engineering, Institute of Chemical Metallurgy, Chinese Academy of Sciences (Beijing, P.R.China). The stock culture was grown on agar slants containing 20% (w/v) glucose, 0.4% (w/v) CSL, 0.3% (w/v) urea and 2% (w/v) agar (pH 4.0–4.5) at 35°C, then washed with 10 ml sterile water to a Erlenmlyer flask. The inocula were cultured in 100 ml medium consisting of 10% (w/v) glucose, 0.4% (w/v) CSL and 0.3% (w/v) urea (pH 4.0–4.5) in a 500 ml flask on a rotary shaker (160 rpm) at 35°C for 24 h.

Materials and reagents

Corn meal, corn starch, cassava starch, SCL, and sweet potato meal, were easily obtained on the market in China. The amylase and glucoamylase used were commercial fungal extract for Aspergillus niger with standard activity.

Liquefaction and saccharification with different starchy substrates

The starchy substrates (80 mesh) were first modulated to 35% slurry (w/v) with water in flasks (500 ml), followed by addition of amylase starchy substrate, and adjusted pH within 6.0–6.5, then liquefied at 92°C in a water bath till liquefactions was completed. After cooled to 58°C, glucoamylase was added to starchy substrate, followed by adjustment of pH to 4.5. Saccharification process was carried out at 58°C. When saccharification process was finished.
Then, the resultant media was used as a fermentation broth for glycerol fermentation.

Fermentation

Batch fermentation experiments at optimization of variable concentrations of corn steep liquor (CSL) were carried out at 35°C with 100 ml fermentation medium in flasks containing 25% (w/v) glucose in saccharification liquors from starchy

substrate and 0.2% (w/v) urea. After sterilization (110°C, 20 min) and cooling to room temperature, 10%(v/v) inocula were aseptically transferred in fermentation medium, and fermentation mediums were incubated at 35°C. Glycerol fermentations with saccharification liquors from corn meal, sweet potato meal, cassava starch, and cornstarch, respectively, were performed at 35°C with 100 ml fermentation medium in flasks.

Module III 8L

Lecture 1:

Baker's yeast fermentation

For a successful industrial fermentation, the following factors should be considered.

Selection of microorganism

The selected organism

1. Should be a potent strain
2. Should be able to ferment cheap raw materials
3. Should not produce any toxic material
4. Should be genetically stable
5. Should not undergo autolysis.

INTRODUCTION

Saccharomyces cerevisiae (Baker's yeast) has a very long history in industrial fermentation. It has been extensively used for the production of ethanol and Single-cell Protein (SCP). In addition, it is also employed in the leavening of dough because of its ability to produce ethanol and carbon dioxide from sugars, e.g.,

maltose, present in the dough. Furthermore, it is also used in the leavening process because of its contribution to the flavour and aroma of bread .

Yeasts (*S. cerevisiae* inclusive) cannot degrade starch naturally. Hence, the need to first hydrolyze the starch and then use the reducing sugars obtained as substrate (i.e., **carbon source**) for the cultivation of *S. cerevisiae*.

Types of baker's yeast

Yeast, baker's, active dry	
Nutritional value per 100 g (3.5 oz)	
Energy	1,361 kJ (325 kcal)
Carbohydrates	41.22 g
Sugars	0 g
Dietary fiber	26.9 g
Fat	7.61 g
Protein	40.44 g
Vitamins	**Quantity %DV**†
Thiamine (B1)	956% 10.99 mg
Riboflavin (B2)	333% 4 mg
Niacin (B3)	268%

	40.2 mg
Pantothenic acid (B5)	270% 13.5 mg
Vitamin B6	115% 1.5 mg
Folate (B9)	585% 2340 µg
Choline	7% 32 mg
Vitamin C	0% 0.3 mg
Minerals	**Quantity %DV**[†]
Calcium	3% 30 mg
Iron	17% 2.17 mg
Magnesium	15% 54 mg
Manganese	15% 0.312 mg
Phosphorus	91% 637 mg

Potassium	20% 955 mg
Sodium	3% 51 mg
Zinc	84% 7.94 mg
Other constituents	**Quantity**
Water	5.08 g

Link to USDA Database entry

- Units
- µg = micrograms • mg = milligrams
- IU = International units

Baker's yeast is available in a number of different forms, the main differences being the moisture contents Though each version has certain advantages over the others, the choice of which form to use is largely a question of the requirements of the recipe at hand and the training of the cook preparing it. Dry yeast forms are good choices for longer-term storage, often lasting more than a year at room temperatures without significant loss of viability. In general, with occasional allowances for liquid content and temperature, the different forms of commercial yeast are considered interchangeable.

- **Cream yeast** is the closest form to the yeast slurries of the 19th century, in essence being a suspension of yeast cells in liquid, siphoned off from the growth medium. Its primary use is in industrial bakeries with special high-volume dispensing and mixing equipment, and it is not readily available to small bakeries or home cooks.
- **Compressed yeast** is, in essence, cream yeast with most of the liquid removed. It is a soft solid, beige in color, and best known in the consumer form as small, foil-wrapped cubes of **cake yeast**. It is also available in larger-block form for bulk usage. It is highly perishable; though formerly widely available for the

consumer market, it has become less common in supermarkets in some countries due to its poor keeping properties, having been superseded in some such markets by active dry and instant yeast. It is still widely available for commercial use, and is somewhat more tolerant of low temperatures than other forms of commercial yeast; however, even there, instant yeast has made significant market inroads.

- **Active dry yeast** is the form of yeast most commonly available to non-commercial bakers in the United States. It consists of coarse oblong granules of yeast, with live yeast cells encapsulated in a thick jacket of dry, dead cells with some growth medium. Under most conditions, active dry yeast must first be proofed or rehydrated. It can be stored at room temperature for a year, or frozen for more than a decade, which means that it has better keeping qualities than other forms, but it is generally considered more sensitive than other forms to thermal shock when actually used in recipes.

- **Instant yeast** appears similar to active dry yeast, but has smaller granules with substantially higher percentages of live cells per comparable unit volumes. It is more perishable than active dry yeast but also does not require rehydration, and can usually be added directly to all but the driest doughs. In general, instant yeast has a small amount of ascorbic acid added as a preservative. Some producers provide specific variants for doughs with high sugar contents, and such yeasts are more generally known as **osmotolerant** yeasts.
- **Rapid-rise yeast** is a variety of dried yeast (usually a form of instant yeast) that is of a smaller granular size, thus it dissolves faster in dough, and it provides greater carbon dioxide output to allow faster rising Rapid-rise yeast is often marketed specifically for use in bread machines.
- **Deactivated yeast** is dead yeast which has no leavening value and is not interchangeable with other yeast types. Typically used for pizza and pan bread doughs, it is used at a rate of 0.1% of the flour weight, though manufacturer specifications may vary. It is a powerful reducing agent used to increase the extensibility of a dough.

Lecture 2:

Riboflavin–vitamin b2 fermentation process

1. 1. Riboflavin–Vitamin B2 Production MAHYAR M AGHEGH UNIVERSITY OF TEHRAN – FACULTY OF CHEMICAL ENGINEERING
2. 2. Introduction • Riboflavin was first isolated by Blyth in 1879 from whey, and the water-soluble, yellow, fluorescent material was named lactochrome. • According to IUPAC rules, riboflavin [83-88-5] is called 7,8- dimethyl-10-(d-1'-ribityl)isoalloxazine, also known as vitamin B2 or lactoflavin. • The daily human demand for riboflavin is around 1.7 mg, and deficiencies lead to various symptoms such as, e.g., versions of dermatitis. • The vitamin cannot be stored in the body and a constant intake is required.
3. 3. A solution of riboflavin.
4. 4. Introduction • Chemical synthesis was the first production method to be established and is still dominating, but in recent years the production is shifting more and more to fermentation. • Green plants, most bacteria, and moulds, however, can produce their own riboflavin. • At present, three organisms are used for the industrial production of riboflavin by fermentation: • The filamentous fungus Ashbya gossypii (BASF, Germany) • The yeast Candida famata (ADM, USA) • A genetically engineered strain of Bacillus subtilis (DSM, Germany)
5. 5. Upstream Downstream
6. 6. Production Process • In this case study, a batch process using E. Ashbyii with a capacity of around 1000 tons/year is analyzed. • Upstream processing consists of preparation of medium and associated continuous counter-current sterilization. • Feed components are: 70% glucose syrup, yeast and malt extract, sunflower oil, sulfuric acid, and concentrated salt solution at room temperature. • Fermentation is operated batch-wise with 10% inoculum ratios. • Downstream processing starts with harvesting followed by crystallization, centrifugation (decanter), and final drying (spray dryer) . • The requested purity of riboflavin is 70%. The residual 30% consists of salts and biomass. The product is obtained as dry powder or as granulate.
7. 7. Upstream • The upstream processes include preparation and sterilization of the medium. • The medium's composition does not allow sterilization of all components mixed together and using classical batch conditions (121◦C, 20 minutes). Therefore, the medium would be divided into several groups: I. glucose and sunflower oil, II. peptone, yeast and malt extracts, III. salts in water IV. methionine. The latter is sterilized by filtration. Sulfuric acid does not require sterilization. • Only two separate solutions have to be prepared: 1. 70% glucose (P-1) 2. other nutrients (Nutrients Tank / P-4)

8. 8. Fermentation • In several steps the necessary seed cultures are prepared in different seed fermenters. • The last seed culture is the start inoculum for the main fermentation. • The duration of a seed-fermentation is around 50 hours, while the main fermentation lasts about 500 hours. • During this time the strain produces 27 g/L riboflavin. • Fermentation requires aeration accomplished by a gas compressor (P-7) and a sterile filter (P-8). • Exhaust gases are filtered by a second filter (P-10). • A small fraction of the harvested broth is put into another tank and is used as inoculum for the next batch (P-13).
9. 9. Downstream • After fermentation the broth is harvested into the harvest tank (P-15). • Part of the product crystallizes in the fermenter and also in the harvesting tank. Crystallization is completed in the crystallizer (P-16) by evaporation of some of the water. • Afterwards the suspension is stored in tank P-17. • From the decanter three streams are harvested, two liquid phases and the cell/crystal suspension (P-18). To achieve higher purity, a washing step is used with a second separation. • The last step is drying, either using a spray dryer to obtain a powdered product or applying a spray granulation to obtain granulate. Granulate can be dosed more precisely(P-20)

Lecture 3:

Vitamin fermentation B_{12}

Vitamin B_{12}, also called **cobalamin**, is a water-soluble vitamin that is involved in the metabolism of every cell of the human body: it is a cofactor in DNA synthesis, and in both fatty acid and amino acid metabolism. It is particularly important in the normal functioning of the nervous system via its role in the synthesis of myelin, and in the maturation of developing red blood cells in the bone marrow.

Vitamin B_{12} is one of eight B vitamins; it is the largest and most structurally complicated vitamin. It consists of a class of chemically related compounds (vitamers), all of which show physiological activity. It contains the biochemically rare element cobalt (chemical symbol **Co**) positioned in the center of a corrin ring. The only organisms to produce vitamin B_{12} are certain bacteria, and archaea. Some of these bacteria are found in the soil around the grasses that ruminants eat; they are taken into the animal, proliferate, form part of their gut flora, and continue to produce vitamin B_{12}.

The most common cause of vitamin B_{12} deficiency in developed countries is impaired absorption due to a loss of gastric intrinsic factor, which must be bound

to food-source B_{12} in order for absorption to occur. Another group affected are those on long term antacid therapy, using proton pump inhibitors, H2 blockers or other antacids. This condition may be characterised by limb neuropathy or a blood disorder called pernicious anemia, a type of megaloblastic anemia. Folate levels in the individual may affect the course of pathological changes and symptomatology. Deficiency is more likely after age 60, and increases in incidence with advancing age. Dietary deficiency is very rare in developed countries due to access to dietary meat and fortified foods, but children in some regions of developing countries are at particular risk due to increased requirements during growth coupled with lack of access to dietary B_{12}; adults in these regions are also at risk. Other causes of vitamin B_{12} deficiency are much less frequent.

Deficiency

Vitamin B_{12} deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system. At levels only slightly lower than normal, a range of symptoms such as fatigue, lethargy, depression, poor memory, breathlessness, headaches, and pale skin, among others, may be experienced, especially in elderly people (over age 60) who produce less stomach acid as they age, thereby increasing their probability of B_{12} deficiencies. Vitamin B_{12} deficiency can also cause symptoms of mania and psychosis.

Industrial production

Industrial production of B_{12} is achieved through fermentation of selected microorganisms. *Streptomyces griseus*, a bacterium once thought to be a fungus, was the commercial source of vitamin B_{12} for many years. The species *Pseudomonas denitrificans* and *Propionibacterium freudenreichii* subsp. *shermanii* are more commonly used today.These are frequently grown under special conditions to enhance yield, and at least one company uses genetically engineered versions of one or both of these species. Since a number of species of Propionibacterium produce no exotoxins or endotoxins and are generally recognized as safe (have been granted GRAS status) by the Food and Drug Administration of the United States, they are presently the FDA-preferred bacterial fermentation organisms for vitamin B_{12} production.

Species from the following genera and species are known to synthesize B_{12}: *Propionibacterium shermanii*, *Pseudomonas denitrificans*, *Streptomyces griseus*,[92] *Acetobacterium*, *Aerobacter*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Baci*

llus, *Clostridium*, *Corynebacterium*, *Flavobacterium*, *Lactobacillus*, *Micromonospora*, *Mycobacterium*, *Nocardia*, *Protaminobacter*, *Proteus*, *Rhizobium*, *Salmonella*, *Serratia*, *Streptococcus* and *Xanthomonas*

Lecture 4:

Penicillin Fermentation

Penicillin is a secondary metabolite of certain species of Penicillium and is produced when growth of the fungus is inhibited by stress.

Penicillin (**PCN** or **pen**) is a group of antibiotics which include penicillin G (intravenous use), penicillin V (use by mouth), procaine penicillin, and benzathine penicillin (intramuscular use). Penicillin antibiotics were among the first medications to be effective against many bacterial infections caused by staphylococci and streptococci. They are still widely used today, though many types of bacteriahave developed resistance following extensive use.

About 10% of people report that they are allergic to penicillin; however, up to 90% of this group may not actually be allergic. Serious allergies only occur in about 0.03%. All penicillins are β-lactam antibiotics.

Penicillin was discovered in 1928 by Scottish scientist Alexander Fleming. People began using it to treat infections in 1942. There are several enhanced penicillin families which are effective against additional bacteria; these include the antistaphylococcal penicillins, aminopenicillins and the antipseudomonal penicillins. They are derived from *Penicillium* fungi.

The term "penicillin" is often used generically to refer to benzylpenicillin (penicillin G, the original penicillin found in 1928), procaine benzylpenicillin (procaine penicillin), benzathine benzylpenicillin (benzathine penicillin), and phenoxymethylpenicillin (penicillin V). Procaine penicillin and benzathine penicillin have the same antibacterial activity as benzylpenicillin but act for a longer period of time. Phenoxymethylpenicillin is less active against gram-negative bacteria than benzylpenicillin. Benzylpenicillin, procaine penicillin and benzathine penicillin can only be given by intravenous or intramuscular injections, but phenoxymethylpenicillin can be given by mouth because of its acidic stability.

Susceptibility

As an antibiotic, benzylpenicillin is noted to possess effectiveness mainly against Gram-positive organisms. Some Gram-negativeorganisms such

as *Neisseria gonorrhoeae* and *Leptospira weilii* are also reported to be susceptible to benzylpenicillin.

While the number of penicillin-resistant bacteria is increasing, penicillin can still be used to treat a wide range of infections caused by certain susceptible bacteria, including Streptococci, Staphylococci, Clostridium, Neisseria, and Listeria genera. The following list illustrates minimum inhibitory concentration susceptibility data for a few medically significant bacteria

- *Listeria monocytogenes*: from less than or equal to 0.06 μg/ml to 0.25 μg/ml
- *Neisseria meningitidis*: from less than or equal to 0.03 μg/ml to 0.5 μg/ml
- *Staphylococcus aureus*: from less than or equal to 0.015 μg/ml to more than 32 μg/ml

Side effect

Common (≥ 1% of people) adverse drug reactions associated with use of the penicillins include diarrhoea, hypersensitivity, nausea,
rash, neurotoxicity, urticaria, and superinfection(including candidiasis). Infrequent adverse effects (0.1–1% of people) include fever,
vomiting, erythema, dermatitis, angioedema, seizures (especially in people with epilepsy), and pseudomembranous colitis. Penicillin can also induce serum sickness or a serum sickness-like reaction in some individuals. Serum sickness is a type III hypersensitivity reaction that occurs one to three weeks after exposure to drugs including penicillin. It is not a true drug allergy, because allergies are type I hypersensitivity reactions, but repeated exposure to the offending agent can result in an anaphylactic reaction. Anaphylaxis will occur in approximately 0.01% of patients.

Pain and inflammation at the injection site is also common
for parenterally administered benzathine benzylpenicillin, benzylpenicillin, and, to a lesser extent, procaine benzylpenicillin.

Natural penicillins

- Penicillin G
- Penicillin K
- Penicillin N
- Penicillin O
- Penicillin V

Lecture 5:

Streptomycin Fermentation

Introduction to Streptomycin:

Streptomycin, produced by streptomyces griseus is active against Gram (-) ve bacteria and against tuberculosis bacterium, Mycobacterium tuberculosis. However, it proved to be useful in the treatment of infections caused by Gram (+) ve specially resistant to penicillin. It is also useful in the control of plant diseases caused by bacteria as it acts systemically in plants.
One of the disadvantages of streptomycin is its neurotoxicity due to which hearing impairment and balance maintenance is lost in man due to prolonged streptomycin treatment at high dosage. Its reduction to dihydrostreptomycin results in the decreased toxicity.
For this reason in recent times only dihydrostreptomycin is being produced due to ready development of resistance against streptomycin. It is used mostly in conjunction with para aminosalicyclic acid or isoniazid (isonicotinic acid hydrazide) which minimizes resistance build up in sensitive microorganisms.

Chemical Structure of Streptomycin:

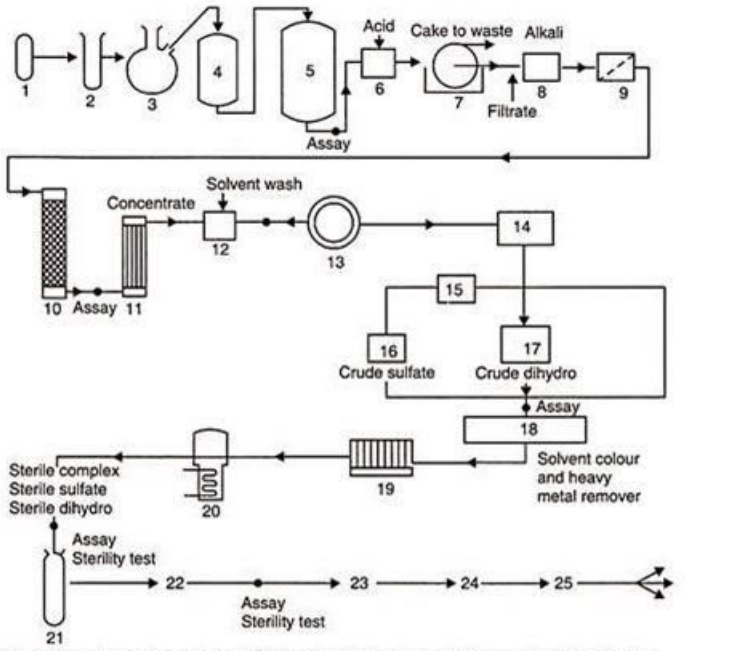
Streptomycin and dihydrostreptomycin is an aminoglycoside antibiotic and basic compound which is available as hydrochloride, $C_{21}H_{39}N_7O_{12}$. 3 HCl, as a crystalline hydrochloride double salt with calcium chloride or as phosphate or sulphate and dihydrostreptomycin as the hydrochloride or sulfate.
Unit of streptomycin activity is equal to one microgram of the free base. Use of precursor does not increase yields of streptomycin.

Biosynthesis of Streptomycin:

Streptomycin is directly derived from glucose. Though the enzymes involved in the synthesis of N-methyl glucosamine are not yet known, it is expected that about 28 enzymes take part in the conversion of glucose into streptomycin.

Fermentation Process of Streptomycin:

Industrially streptomycin is produced by submerged culture method, whose flow sheet is given in Fig.



1. Master culture 2. Agar slopes 3. Shaker flask 4. Seed vessel 5. Fermentor 6. Acidification
7. Filtration 8. Neutralization 9. Filter clarification 10. Ion-exchange reagent 11. Evaporator
12. Crystallization 13. Vacuum oven 14. Calcium chloride crude complex 15. Calcium chloride removal
16. Crystallization 17. Catalytic hydrogenation 18. Finishing 19. Seitz filter 20. Freeze drying
21. Vial filling machine 22. Capping 23. Labelling 24. Packing 25. Despatch

Fig. Flow sheet of streptomycin production by submerged-method

When Woodruff and Mc Daniel (1954) suggested medium consisting of soyabean meal (1%), glucose 1% and sodium chloride (0.5%), Hocken hull (1963) recommended the medium consisting of glucose (2.5%), soyabean meal (4.0%), distillers dry soluble (0.5%) and sodium chloride (0.25%) and pH 7.3-7.5 for production of streptomycin by *S. griseus.*

(i) The Inoculum Production:

Spores of S. griseus maintained as soil stocks or lyophilized in a carrier such as sterile skimmed milk, is employed as stock culture. The spores from these stock cultures are then transferred to a sporulation medium to provide enough sporulated growth to initiate liquid culture build-up of mycelial inoculum in flasks or inoculum tanks. After sufficient mycelial growth, it is fed to production fermenter.

(ii) Preparation of the Medium:

A production medium contains carbon source and nitrogen source. Glucose is one of the best carbon sources which helps in the greater yield of streptomycin, because it provides basic carbon skeleton for the streptomycin production. Apart from glucose, fructose, maltose, lactose, galactose, mannitol, xylose and starch can also be used as carbon source. Polysaccharides and oligosaccharides generally give low yields.

Peptones, soya extracts, meat extract, the residue from alcohol distillation, ammonium salts, nitrates and glycine may be used as nitrogen source. Magnesium, calcium, potassium, boron and molybdenum may be used as mineral source along with sulphates, phosphates and chlorides.

Phenylacetic acid, L-naphthalene acetic acid may be added as growth stimulating compounds. It is better to add proline into the medium which helps in high streptomycin production. Fats, oils and fatty acids may also be used along with glucose. If necessary antioxidants such as sodium sulphate or starch or agar may also be added into the medium. There is no need of precursor in the production of streptomycin.

(iii) Fermentation:
Sterilized liquid medium with all the above substances is fed to the production fermenter. Appropriate volume of inoculum (4-5%) is introduced into it. The optimum fermentation temperature is in the range of 25 to 30°C and the optimum pH range is between 7.0 and 8.0. High rate of streptomycin production, however, occurs in the pH range of 7.6 to 8.0.

The process of fermentation is highly aerobic and lasts approximately for 5 to 7 days and passes through 3 phases:
(a) The First Phase:
It takes about 24 hours to 48 hours. Rapid growth and formation of abundant mycelium occurs during this phase. The pH rises to 8.0 due to release of ammonia into medium, due to proteolytic activity of S. griseus. Glucose is utilized slowly and little production of streptomycin is witnessed.

(b) The Second Phase:
It lasts for 2 days. Streptomycin production takes place at a rapid rate without increase in the mycelial growth. The ammonia released in the first phase is utilized,

which results in the decrease of pH to 7.6-8.0. Glucose and oxygen are required in large quantity during this phase.

(c) Third Phase:
Cells undergo lysis, releasing ammonia and increase in the pH, which falls again after a period of continuous streptomycin production. Requirement of oxygen decreases and the contents of the medium including sugar get exhausted. Finally streptomycin production ceases. A yield of 1200 micrograms per milliliter of streptomycin is obtained.

(iv) Harvest and Recovery:
After completion of fermentation the mycelium is separated from the broth by filtration. Streptomycin is recovered by several methods.

But the one which is generally employed is described below:
The fermentation broth is acidified, filtered and neutralized. It is then passed through a column containing a cation exchange resin to adsorb the streptomycin from the broth. The column is then washed with water and the antibiotic is eluted with hydrochloric acid or cyclohexanol or phosphoric acid. It is then concentrated at about 60°C under vacuum.

The streptomycin is then dissolved in methanol and filtered and acetone is added to the filtrate to precipitate the antibiotic. The precipitate is again washed with acetone and vacuum dried. It is purified further by dissolving in methanol. The streptomycin in pure form is extracted as calcium chloride complex.

(v) Byproduct Vitamin B_{12}:
Vitamin B_{12} is produced as a byproduct which will not affect adversely the yield of streptomycin.

Uses of Streptomycin:

1. Streptomycin is active against Gram (-) negative bacteria. It is used in the treatment of tuberculosis caused by Mycobacterium tuberculi.

2. It is also used therapeutically in the treatment of infectious diseases caused by Gram (-) negative bacteria, specially those organisms which are resistant to penicillin.

3. Prolonged treatment by streptomycin at high dosage can produce neurotoxic reactions such as hearing impairment, loss of balance maintenance in man.

Lecture 6:

Tetracycline

Tetracycline, is an antibiotic used to treat a number of infections. This includes acne, cholera, brucellosis, plague, malaria, and syphilis. It is taken by mouth.

Common side effects include vomiting, diarrhea, rash, and loss of appetite. Other side effects include poor tooth development if used by children less than eight years of age, kidney problems, and sunburning easily. Use during pregnancy may harm the baby. Tetracycline is in the tetracyclines family of medications. It works by blocking the ability of bacteria to make proteins.

Tetracycline was originally made from bacteria of the *Streptomyces* type.

etracyclines have a broad spectrum of antibiotic action. Originally, they possessed some level of bacteriostatic activity against almost all medically relevant aerobic and anaerobic bacterial genera, both Gram-positive and Gram-negative, with a few exceptions, such as *Pseudomonas aeruginosa* and *Proteus* spp., which display intrinsic resistance. However, acquired (as opposed to inherent) resistance has proliferated in many pathogenic organisms and greatly eroded the formerly vast versatility of this group of antibiotics. Resistance amongst *Staphylococcus* spp., *Streptococcus* spp., *Neisseria gonorrhoeae*, anaerobes, members of the Enterobacteriaceae, and several other previously sensitive organisms is now quite common. Tetracyclines remain especially useful in the management of infections by certain obligately intracellular bacterial pathogens such as *Chlamydia*, *Mycoplasma*, and *Rickettsia*. They are also of value in spirochaetal infections, such as syphilis, leptospirosis, and Lyme disease. Certain rare or exotic infections, including anthrax, plague and brucellosis, are also susceptible to tetracyclines. These agents also have activity against certain eukaryotic parasites, including those responsible for diseases such as malaria and balantidiasis.

Fermentation Process of Tetracycline

Streptomyces lusitanus var tetracyclini 106-T NCIB9500 is cultivated in an aq. nutrient medium contg. cornsteep liquor, $CaCO_3$, starch or semi-hydrolyzate,

ammonium salts, trace elements, peanut meal, and lard oil at a pH 6.7-6.8 for 140 hrs. at 26-8.degree. with aeration to obtain tetracycline in very high yields. There is no chlortetracycline produced. Thus, a prefermentor contg. 150 l. of medium is inoculated with starter culture and fermented at 26.degree. with agitation for 24 hrs. Then, a fermentor having a useful capacity of 6000 l. and contg. a medium of the following compn./l. of tap water: cornsteep liquor, $CaCO_3$, starch, $(NH_4)_2SO_4$, NH_4Cl, $MnSO_4.bul.4H_2O$, $CoCl_2.bul.6H_2O$, $ZnSO_4$, peanut meal, and lard oil 28, 14, 38, 5.7, 1.5, 0.05, 0.002, 0.05, 25, and 35 g., resp., is inoculated with the 24-hr. culture and fermented at 30.degree. for an addnl. 24 hrs., with aeration of 1.5 l./min./l. of broth. Then, the temp. is lowered to 26.degree. and the aeration increased to 4 l./min./l. of broth. After 140 hrs. of fermentation, 11.1 g. tetracycline/l. was obtained.

Lecture 7:

Single Cell Protein or SCP refers to protein derived from cells of microbes such as bacteria, yeast, mold and algae. They are grown on various carbon sources. The dried cells of microorganisms is harvested and consumed. As a source of protein it is very promising with potential to satisfy the word shortage of food while population increases.

This module focuses on single cell protein production and its importance. SCPs with rich protein (60-70%) with high concentration of vitamins B complex and **low fat** values suit them as a good for human and animal consumption. The use of SCP as food ingredient is still in stages of development.

Production of SCP

Single-cell protein (SCP) refers to the microbial cells or total protein extracted from pure microbial cell culture (monoculture) which can be used as protein supplement for humans or animals. The word SCP is considered to be appropriate, since most of the microorganisms grow as single or filamentous individuals. This is in contrast to complete multicellular plants and animals.

If the SCP is suitable for human consumption, it is considered as food grade. SCP is regarded as feed grade, when it is used as animal feed supplement, but not suitable for human consumption. Single-cell protein broadly refers to the microbial biomass or protein extract used as food or feed additive. Besides high protein content (about 60-80% of dry cell weight), SCP also contains fats, carbohydrates, nucleic acids, vitamins and minerals.

Several microorganisms that include bacteria, yeasts, fungi, algae and actiomycetes utilizing a wide range of substrates are used for the production of SCP. A selected list is given below.

A Selected list of microorganisms and substrates used for single-cell protein production

Microorganism	*Substrate(s)*
Bacteria	
Methylophilus methylotrophus	Methane, methanol
Methylomonas sp	Methanol
Pseudomonas sp	Alkanes
Brevibacterium sp	C_1–C_4 hydrocarbons
Yeasts	
Saccharomycopsis lipolytica (previous name—*Candida lipolytica*)	Alkanes
Candida utilis	Sulfite liquor
Kluyveromyces fragilis	Whey
Saccharomyces cerevisiae (baker's yeast)	Molasses
Lactobacillus bulgaricus	Whey
Tosulopsis sp	Methanol
Fungi	
Chaetomium cellulolyticum	Cellulosic wastes
Paecilomyces varioti	Sulfite liquor
Aspergillus niger	Molasses
Trichoderma viride	Straw, starch
Algae	
Spirulina maxima	Carbon dioxide
Chlorella pyrenoidosa	Carbon dioxide
Scenedesmus acutus	Carbon dioxide
Actinomycetes	
Nocardia sp	Alkanes
Thermomonospora fusca	Cellulose
Mushrooms (a type of fungi)	
Agaricus biosporus	Compost, rice straw
Morchella crassipes	Whey, sulfite liquor
Auricularia sp	Saw dust, rice bran
Lentinus edodes	Saw dust, rice bran
Volvariella volvaceae	Cotton, straw

The selection of microorganisms for SCP production is based on several criteria. These include their nutritive value, non-pathogenic nature, production cost, raw materials used and growth pattern.

Substrates:
The nature of the raw materials supplying substrates is very crucial for SCP production. The cost of raw material significantly influences the final cost of SCP. The most commonly used raw materials may be grouped in the following categories.

1. High-energy sources e.g. alkanes, methane, methanol, ethanol, gas oil.

2. Waste products e.g. molasses, whey, sewage, animal manures, straw, bagasse.

3. Agricultural and forestry sources e.g. cellulose, lignin.

4. Carbon dioxide, the simplest carbon source.

Production of SCP from High Energy Sources:

There are a large number of energy-rich carbon compounds or their derivatives which serve as raw materials for SCP production. These include alkanes, methane, methanol, and ethanol and gas oil. Bacteria and yeasts are mostly employed for SCP production from high energy sources. Some scientists question the wisdom of using (rather misusing) high-energy compounds for the production of food, since they regard it as a wasteful exercise.

Production of SCP from alkanes:
Alkanes can be degraded by many yeasts, certain bacteria and fungi. The major limitation of alkanes is that they are not easily soluble, hence they cannot enter the cells rapidly. It is believed that the cells produce emulsifying substances which convert insoluble alkanes into small droplets (0.01-0.5 pm) that can enter the cells by passive diffusion.

It is observed that when cells are grown on a medium of alkanes enriched with lipids, the diffusion of alkanes into the cells is enhanced. Certain yeasts have been

successfully used for producing SCP from alkanes e.g. Saccharomycopsis lipolytica, Candida tropicalis, Candida oleophila.

Petroleum products for SCP production:

Several oil companies have developed fermentation systems, employing petroleum products for large scale manufacture of SCP by yeasts. Two types of petroleum products are mainly used for this purpose.

1. Gas oil or diesel oil containing 10-25% of alkanes with carbon length C_{15}-C_{30} (i.e. long chain alkanes).
2. Short chain alkanes with carbon length in the range of C_{10}-C_{17}, isolated from gas oil by use of molecular sieves.

Airlift bioreactor system with continuous operation was once used (in France and Britain) to produce SCP from gas oil employing the organism Saccharomycopsis lipolytica. But this is now discontinued for political reasons.

Degradation of alkanes:

Alkanes have to be first broken down to appropriate metabolites for their utilization to form SCP. The most important step in this direction is the introduction of oxygen into alkanes which can be brought out by two pathways- terminal oxidation and sub-terminal oxidation.

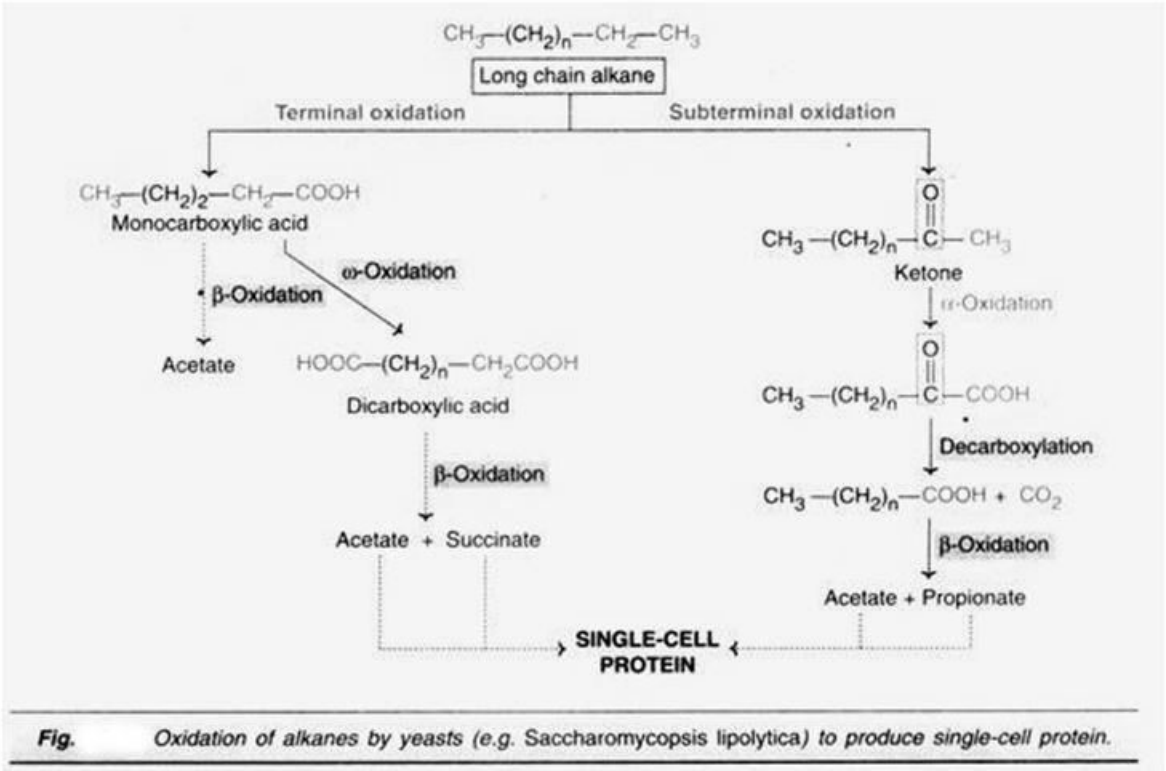


Fig. *Oxidation of alkanes by yeasts (e.g.* Saccharomycopsis lipolytica*) to produce single-cell protein.*

In terminal oxidation, the terminal carbon gets oxidized to the corresponding monocarboxylic acid. The latter then undergoes β -oxidation to form acetic acid. In some microorganisms, the oxidation may occur at both the terminal carbon atoms (by a process referred to as co-oxidation) to form a dicarboxylic acid. This can be further broken down to acetate and succinate by β-oxidation. Terminal oxidation is the predominant pathway occurring in majority of yeasts and bacteria.

Sub-terminal oxidation involves the oxidation of interminal carbon atoms (any carbon other than terminal i.e. C_2, C_3, C_4, and so on). The corresponding ketone produced undergoes a-oxidation, decarboxylation, and finally β-oxidation to form acetate and propionate. The individual enzymes responsible for terminal oxidation or sub-terminal oxidation have not been fully identified.

Limitations of SCP production from alkanes:

The production of SCP from alkanes is a very complex biotechnological process and has been extensively studied. The major drawback of alkanes as substrates is the formation of carcinogens, along with SCP which are highly harmful. For this reason, many countries have discontinued alkane-based production of SCP.

Production of SCP from methane:

Methane is the chief constituent of natural gas in many regions. Although methane can be isolated in pure gas form, it cannot be liquefied. The handling and transportation of methane (an explosive gas) are very difficult and expensive. Certain bacteria that can utilize methane for SCP production have been identified e.g. Methylococcus capsulatus, Methylomonas methanica, Methylovibrio soehngenii. So far, yeasts that can utilize methane have not been identified.

The bacterial enzyme methane oxygenase oxidizes methane to methanol, which can be converted to formaldehyde and then to formic acid. Although methane was extensively researched for its use as a source of SCP, it is not widely used due to technical difficulties.

Production of SCP from methanol:
Methanol is a good substrate for producing SCP. Methanol as a carbon source for SCP has several advantages over alkanes and methane. Methanol is easily soluble in aqueous phase at all concentrations, and no residue of it remains in the harvested biomass. Technically, methanol can be easily handled. The sources for methanol are natural gas, coal, oil and methane.

Many species of bacteria (Methylobacter, Arthrobacter, Bacillus, Pseudomonas, Vibrio) yeasts (Candida biodinii, Hansenula sp, Torulopsis sp) and fungi (Trichoderma lignorum, Gliocladium delinquescens) are capable of producing SCP from methanol. Bacteria are mostly preferred because they require simple fermentation conditions, grow rapidly and possess high content of protein.

Oxidation of methanol:
Methanol gets oxidized to formaldehyde, then to formic acid and finally to carbon dioxide.

The products obtained from methanol have to form C_3 compounds (such as pyruvate) for final production of SCP. Carbon dioxide formed from methanol can be utilized by photosynthetic organisms for the formation of ribulose diphosphate. Alternately, formaldehyde may condense with ribulose 5-phosphate to form 3-keto 6-phosphohexulose which then gives fructose 6-phosphate and finally pyruvate. This pathway is referred to as ribulose monophosphate (or Quayle) cycle.

Formaldehyde can condense with glycine to form serine which in a series of reactions forms phosphoenol pyruvate. This is referred to as serine pathway.

Advantages of SCP

Large scale SCP production has some advantages over the conventional food production, these advantages are :

1. Microorganisms have a high rate of multiplication to hence rapid succession of generation (algae: 2-6hours, yeast: 1-3 hours, bacteria: 0.5-2 hours)
2. They can be easily genetically modified for varying the amino acid composition.
3. A very high protein content 43-85 % in the dry mass.
4. They can utilize a broad spectrum of raw materials as carbon sources, which include even waste products. Thus they help in the removal of pollutants also.
5. Strains with high yield and good composition can be selected or produce relatively easily.
6. Microbial biomass production occurs in continuous cultures and the quality is consistent since the growth is independent of seasonal and climatic variations.
7. Land requirements is low and is ecologically beneficial.
8. It is not dependent on climate

Disadvantage of SCP

1. Many types of microorganisms produce some substances which are toxic to the

2. human and also to the animals. Therefore it has to be made sure that the produced microbial biomass does not contain any of these toxic substances.
3. Sometimes the microbial biomass when taken as diet supplement may lead to indigestion or allergic reactions in humans.

4. The high nucleic acid content of many types of microbial biomass products is also undesirable for human consumption as single cell protein. Sometimes this high level of nucleic acid content in microbial biomass will lead to kidney stone formation or gout.

5. The high nucleic acid content of many types of microbial biomass may lead to poor digestibility, gastrointestinal problem and also some skin reactions in humans.

6. The possibility of presence of toxins or carcinogenic compounds may lead to some serious health problems in humans as well as in animal stock.

7. Single cell protein production is a very expensive procedure as it needs high level of sterility control in the production unit or in the laboratory.

Lecture 8:

Introduction, Production, Storage, Limitation of mushroom

Mushrooms are fungi belonging to the classes basidiomycetes (Agaricus sp, Auricularia sp, Tremella sp) and ascomycetes (Morchella sp, Tuber sp). Majority of edible mushrooms are the species of basidomyces. It is estimated that there are around 4,000 species of basidiomyces. Of these, around 200 are edible, and a dozen of them are cultivated on large scale. Some of the most important edible mushrooms, their common names and the substrates used are given in Table.

TABLE A selected list of the edible mushrooms cultivated on commercial scale

Mushroom species	*Common name*	*Substrate(s)*
Agaricus bisporus	Button mushroom	Straw, horse manure, compost
Leutinule edodes	Oak or shiitake mushroom	Saw dust, wooden logs, rice bran
Pleurotus ostreatus	Oyster mushroom	Straw, saw dust, paper
Volvariella volvacea	Chinese mushroom or padi-straw mushroom	Straw, cotton
Auricularia sp	Wood-ear mushroom	Saw dust, rice bran
Coprinus sp	— — —	Straw

There are certain poisonous mushrooms also. They usually possess unpleasant taste and odour. These mushrooms produce some poisonous substances like phallin and muscarine. The examples of poisonous mushrooms are Amanita phalloides, A. muscaria, A. viraosa, Lepiota morgani and Boletus satanas.

Nutritive value of edible mushrooms:
Some people regard edible mushrooms as vegetable meat. Mushrooms contain 80-90% water, depending on the growth conditions (temperature, humidity). Edible mushrooms are rich sources of protein (35-45% of dry weight). However, all these proteins are not easily digestible by humans. Mushrooms also contain fats and free fatty acids (7-10%), carbohydrates (5-15%) and minerals in good concentration. Certain undesirable substances may also be present in edible mushrooms e.g. cadmium, chromium.

Many delicious recipes of edible mushrooms can be prepared. This actually depends on the dietary habits of the people. Some of the common recipes are mushroom soup, mushroom paneer, mushroom pulao, and mushroom omelets.

Production of Mushroom
There are the following steps of mushroom farming.

1. Composting
2. Spawning
3. Casing
4. Pinning
5. Cropping

1. Composting

The growing cycle of mushrooms starts with compost. Compost preparation starts with horse manure. The compost factories get the horse manure from large horse breeding companies that pay the compost factories to collect the manure. Straw, gypsum, chicken manure and water are added to the horse manure. The straw improves the structure, gypsum ensures the proper acidity and the two manures are the nutrients. The compost is produced in tunnels in order to prevent the smell from becoming a nuisance. As manure emits ammonia, compost factories purify the air with ammonia wash to prevent the emission of gases. The indoor fresh compost looks like earth from a forest. Dark brown, full of trampled bits of straw. The compost is steaming, due to the composting process: heat is generated which digests the components. What's left is a very fertile, nutritious source for mushrooms. On one batch of compost, two to three flushes of mushrooms can be grown. A square metre of compost (which is equal to 90 kilos) yields a maximum of 35 kilos of mushrooms. After that it's no longer lucrative to reuse the compost.

The leftover compost can still be used as a soil conditioner in other agricultural companies.

2. Spawning

In a tunnel, the indoor fresh compost is pasteurized at 57-60 degrees Celsius. This kills all possible bacteria. The compost stays in the tunnel to mature for six days, after which the compost is mixed with spawn that will produce the mushrooms: the mycelium. The compost is then moved to another tunnel where the mycelium can spread through the compost. The mycelium grows quickly; after two weeks it has completely permeated the compost, which means that is has reached the point that it is ready for the growers. At this time the compost looks like light brown peat. Most mushroom growers do not produce their own spawn, as it is a very sophisticated process. Specialized companies produce the spawn by inoculating grain with spores. The grain is sterilized first to prevent infection and it's kept moist, exactly the way mushrooms like it. Ten kilo of spores (22 pounds) provides about five hundred kilos of inoculated grain (1100 pounds). The grain is incubated in a bag for two weeks at 25 degrees Celsius (75 degrees Fahrenheit), then transferred to a refrigerator at 2 degrees Celsius (35 degrees Fahrenheit) to harden it. In this way, the spawn gets a shelf life of 6 months without the mycelium losing its vitality.

3. Casing

The matured compost is spread onto long stainless steel boxes, the mushroom beds. The beds are inside special dark rooms called cells. The temperature in the cells is kept nice and warm, at about 23 degrees Celsius. A layer of peat casing material is added on top of the compost to keep the compost moist. Over a period of six days, 20 to 25 litres of water is sprinkled on each m2 in each cell because more moisture is needed. After this, the fungus has two days to grow through the covering layer of casing soil.

4. Pinning

Mushrooms only grow in the wild in autumn. However, they can be cultivated year round by recreating autumn conditions. Therefore, the temperature in the cell is gradually lowered from 23 to 17 degrees Celsius over four days. The mushroom grower starts to lower the temperature once he sees that the mycelium has grown to its full extent. The temperature shock is a sign for the mycelium to start sprouting the mushrooms. The same thing happens in nature. Mycelium grows well in mild

autumn weather, and after an October storm, the mushrooms will start appearing. The mycelium starts to form little buds, which will develop into mushrooms. Those little white buds are called pins. In this phase, air temperature and humidity can influence growth. Low air temperature and low humidity produce more buds, which yield smaller mushrooms. Higher air temperature and humidity produce fewer but larger mushrooms.

5: Harvesting

After this, the temperature is kept steady at 18 degrees Celsius. Mushrooms grow best at this temperature; they' will grow 3 cm (1 inch) in a week, which is the normal size for harvesting. In week 3 the first flush is harvested. Mushrooms destined for selling fresh are still harvested by hand; mushrooms destined for preserving are being picked and sorted mechanically. Although hand-picking is a lot of work, it offers the best guarantee that the mushrooms will be removed from the beds undamaged. On average, a picker can harvest between 18 and 30 kilos of mushrooms an hour. The mushrooms are picked from the beds with a rotating motion and sorted by the pickers based on quality, size and weight. Nine days after the first flush, the second flush will be harvested. The second flush often consists of larger, but fewer mushrooms than the first flush.

After the second flush of mushrooms has been picked, the cells need to be cleaned. First the cell is pasteurized with steam to kill any remaining fungus to ensure that there is no transfer from cycle to cycle. During steam-cleaning, the temperature in the cells reaches 70 degrees Celsius for eight hours. After steam-pasteurization, the compost is removed from the beds. The empty cell is thoroughly cleaned one more time and then it is ready to be filled again.

Advantages of edible mushroom

1. Mushrooms can be produced by utilizing cheap and often waste substrates (industrial and wood wastes).

2. They are of high nutritive value being rich in proteins, vitamins and minerals.

3. Many delicious recipes can be prepared from mushrooms.

4. Due to low carbohydrate content, consumption of mushrooms is advocated to diabetic patients.

5. Mushrooms are rich in anti-oxidants that fight against the harmful free radicals in our body. If not acted upon, these free radicals tend to harm our body cells which might eventually lead to cancer. Selenium is a mineral found in mushrooms that facilitates the functioning of our liver enzymes and thus helps to detoxify certain cancer-causing compounds in our body. Vitamin D is also present in mushrooms which regulates the cell growth cycle and prevents the growth of cancer cells.

6. Mushroom contains a powerful anti-oxidant called ergothioneine that helps to prevent inflammation.

Side-Effects & Allergies of Mushroom

It is not easy for a layman to differentiate wild mushrooms from healthy mushrooms. Consumption of wild mushrooms may result in severe illnesses in humans and can even cause death. As beta-glucans present in mushroom stimulate immune function, people with autoimmune diseases like arthritis, lupus, asthma and multiple sclerosis should avoid consuming it.

Mushroom production is basically a fermentation process. This is mostly carried out by solid-substrate fermentation. A wide range of substrates (straw, saw dust, compost, wooden logs) depending the organism can be used. Mushroom production is a good example of a low technology utilization in an otherwise sophisticated modern biotechnology.

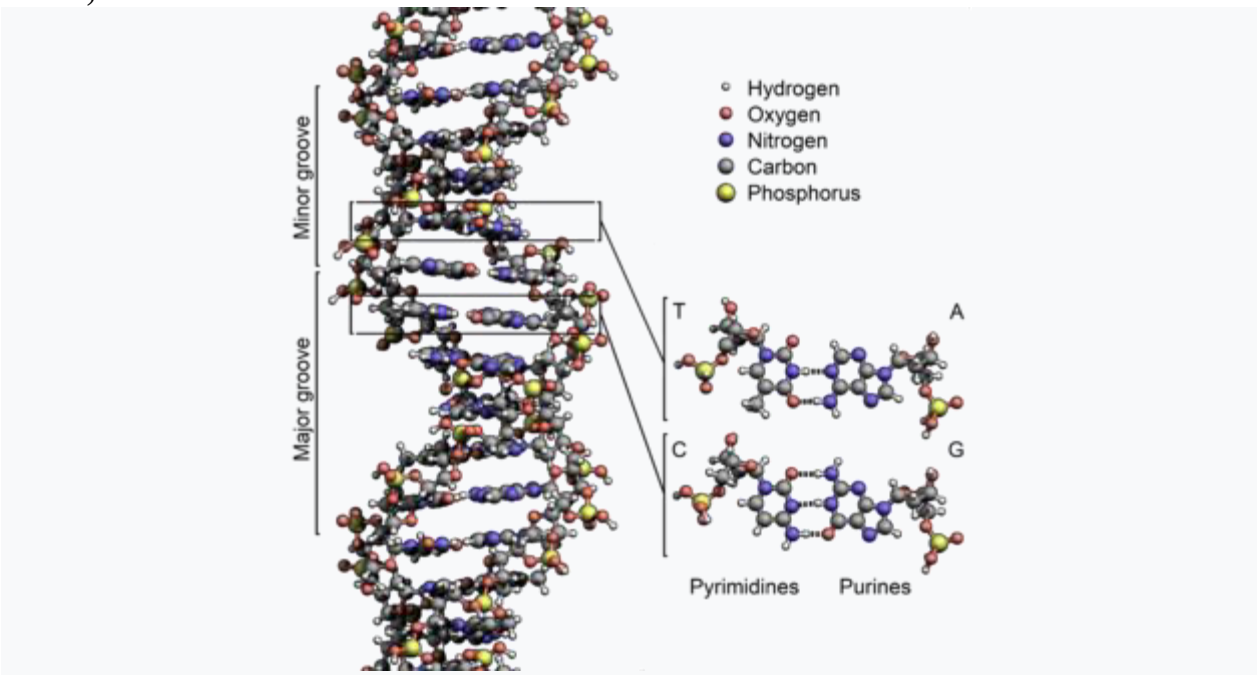
Module IV 8L

Lecture 1:

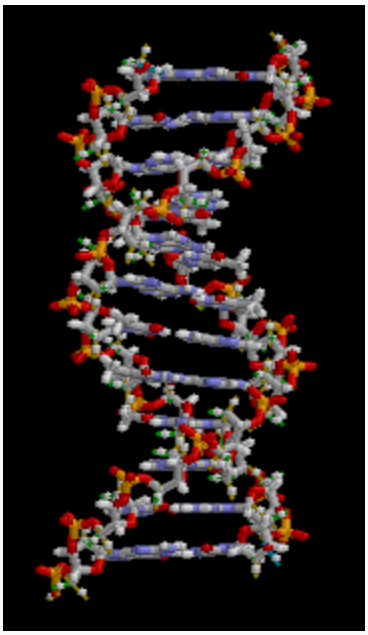
Introduction of Genetic Engineering

Genetics is a field of biology that studies how traits are passed from parents to their offspring. The passing of traits from parents to offspring is known as heredity, therefore, **genetics** is the study of heredity. DNA molecules hold all the **genetic** information for an organism. For some lower organisms or living materials RNA is the genetic material.

DNA, RNA



The structure of the DNA double helix. The atoms in the structure are colour-coded by element and the detailed structures of two base pairs are shown in the bottom right.



The structure of part of a DNA double helix

Deoxyribonucleic acid (**DNA**) is a molecule composed of two chains (made of nucleotides) that coil around each other to form a double helix carrying the genetic instructions used in the growth, development, functioning and reproduction of all known living organisms and many viruses. DNA and ribonucleic acid(RNA) are nucleic acids; alongside proteins, lipids and complex carbohydrates (polysaccharides), nucleic acids are one of the four major types of macromolecules that are essential for all known forms of life.

The two DNA strands are also known as polynucleotides since they are composed of simpler monomeric units called nucleotides. Each nucleotide is composed of one of four nitrogen-containing nucleobases (cytosine [C], guanine [G], adenine [A] or thymine [T]), a sugar called deoxyribose, and a phosphate group. The nucleotides are joined to one another in a chain by covalent bonds between the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. The nitrogenous bases of the two separate polynucleotide strands are bound together, according to base pairing rules (A with T and C with G), with hydrogen bonds to make double-stranded DNA.

The complementary nitrogenous bases are divided into two groups, pyrimidines and purines. In DNA, the pyrimidines are thymine and cytosine; the purines are adenine and guanine.

DNA stores biological information. The DNA backbone is resistant to cleavage, and both strands of the double-stranded structure store the same biological

information. This information is replicated as and when the two strands separate. A large part of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences.

The two strands of DNA run in opposite directions to each other and are thus antiparallel. Attached to each sugar is one of four types of nucleobases (informally, *bases*). It is the sequence of these four nucleobases along the backbone that encodes genetic information.

Ribonucleic acid (**RNA**) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes. RNA and DNA are nucleic acids, and, along with lipids, proteins and carbohydrates, constitute the four major macromoleculesessential for all known forms of life. Like DNA, RNA is assembled as a chain of nucleotides, but unlike DNA it is more often found in nature as a single-strand folded onto itself, rather than a paired double-strand. Cellular organisms use messenger RNA (***mRNA***) to convey genetic information (using the nitrogenous bases of guanine, uracil, adenine, and cytosine, denoted by the letters G, U, A, and C) that directs synthesis of specific proteins. Many viruses encode their genetic information using an RNA genome.

Some RNA molecules play an active role within cells by catalyzing biological reactions, controlling gene expression, or sensing and communicating responses to cellular signals. One of these active processes is protein synthesis, a universal function in which RNA molecules direct the assembly of proteins on ribosomes. This process uses transfer RNA (***tRNA***) molecules to deliver amino acids to the ribosome, where ribosomal RNA (***rRNA***) then links amino acids together to form proteins.

Lecture 2:

Mutation

A gene **mutation** is a permanent alteration in the DNA sequence that makes up a gene, such that the sequence differs from what is found in most people. **Mutations** range in size; they can affect anywhere from a single DNA building block (base pair) to a large segment of a chromosome that includes multiple genes.

Mutations may be (1) spontaneous mutations, (2) induced mutations.

Spontaneous mutation- When mutation takes place by an unidentifiable cause then that is known as spontaneous mutation.

Induced mutation- is caused by mutagens.

Mutagen- a **mutagen** is a physical or chemical agent that changes the genetic material, usually DNA, of an organism.

Types of Mutagens - Mutagens may be of physical, chemical or biological origin.

Physical mutagens-Ionizing radiations as X-rays, gamma rays and alpha particles are physical mutagenic agents.

The most common lab sources include cobalt-60 and cesium-137. Ultraviolet radiations with wavelength above 260 nm are absorbed strongly by bases, producing pyrimidine dimers, which can cause error in replication if left uncorrected. Radioactive decay, such as ^{14}C in DNA which decays into nitrogen

Chemical mutagens- A large number of chemicals may interact directly with DNA. NTG, Hydroxylamine, Nitrous acid etc are chemical mutagens. However, many such as PAHs, aromatic amines, benzene are not necessarily mutagenic by themselves, but through metabolic processes in cells they produce mutagenic compounds.

Biological mutagens- Transposons (a section of DNA that undergoes autonomous fragment relocation/multiplication. Its insertion into chromosomal DNA disrupts functional elements of the genes), Virus (Virus DNA may be inserted into the genome and disrupts genetic function. Infectious agents have been suggested to cause cancer).

Types of mutations –

Point Mutation Transition, Tranversion, Insertion/Deletion, Frame shift Mutation.

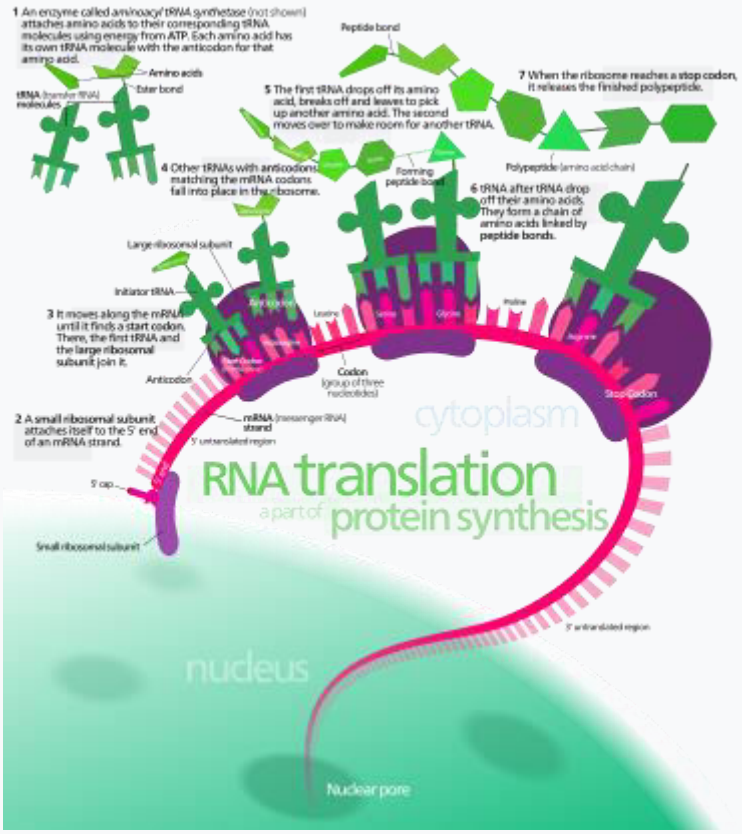
Lecture 3:

Replication

DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. This process occurs in all living

organisms and is the basis for biological inheritance. The cell possesses the distinctive property of division, which makes replication of DNA essential.

Translation



Overview of the translation of eukaryotic messenger RNA

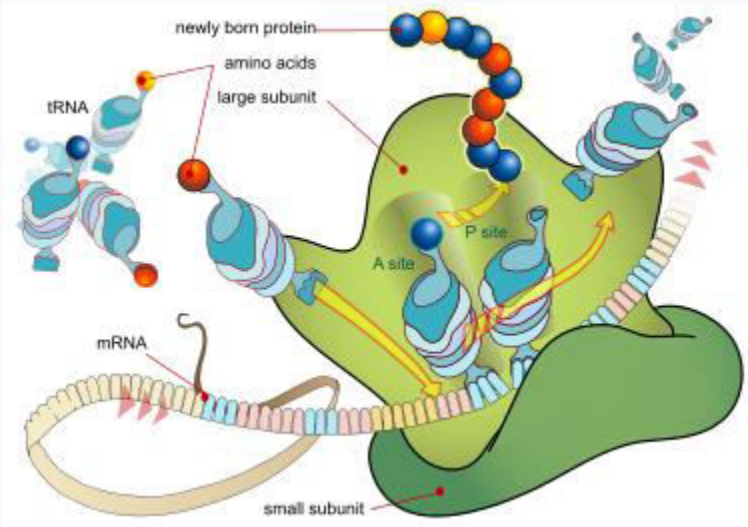


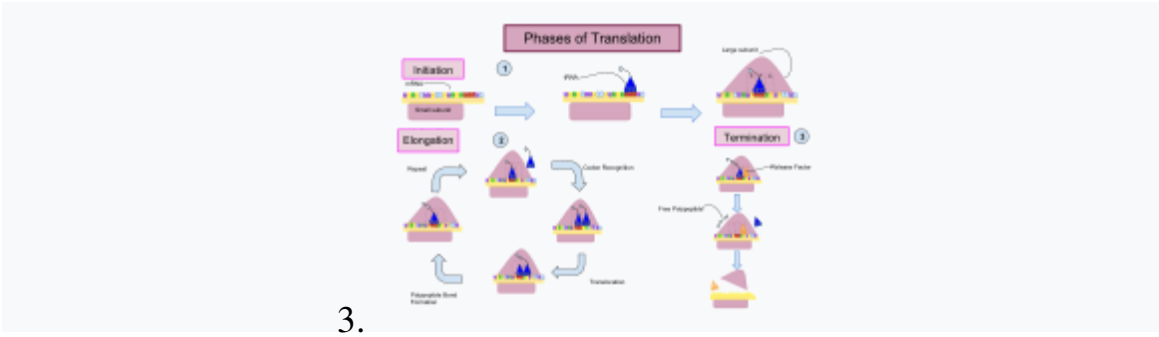
Diagram showing the translation of mRNA and the synthesis of proteins by a ribosome

In molecular biology and genetics, **translation** is the process in which ribosomes in the cytoplasm or ER synthesize proteins after the process of transcription of DNA to RNA in the cell's nucleus. The entire process is called gene expression.

In translation, messenger RNA (mRNA) is decoded in a ribosome to produce a specific amino acid chain, or polypeptide. The polypeptide later folds into an active protein and performs its functions in the cell. The ribosomefacilitates decoding by inducing the binding of complementary tRNA anticodon sequences to mRNA codons. The tRNAs carry specific amino acids that are chained together into a polypeptide as the mRNA passes through and is "read" by the ribosome.

Translation proceeds in three phases:

1. **Initiation**: The ribosome assembles around the target mRNA. The first tRNA is attached at the start codon.
2. **Elongation**: The tRNA transfers an amino acid to the tRNA corresponding to the next codon. The ribosome then moves (*translocates)* to the next mRNA codon to continue the process, creating an amino acid chain.



3.

The three phases of translation initiation polymerase binds to the DNA strand and moves along until the small ribosomal subunit binds to the DNA. Elongation is initiated when the large subunit attaches and termination end the process of elongation.

Termination: When a stop codon is reached, the ribosome releases the polypeptide.

In prokaryotes (bacteria), translation occurs in the cytosol[1], where the medium and small subunits of the ribosomebind to the tRNA. In eukaryotes, translation occurs in the cytosol or across the membrane of the endoplasmic reticulum in a process called co-translational translocation. In co-translational translocation, the entire ribosome/mRNA complex binds to the outer membrane of the rough endoplasmic reticulum (ER) and the new protein is synthesized and released into

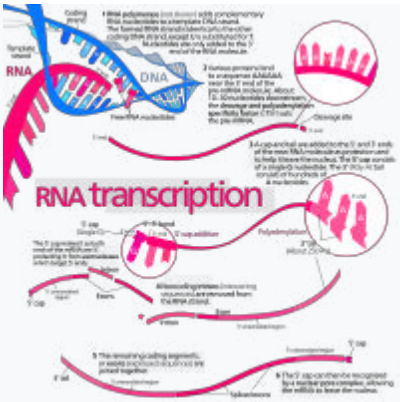
the ER; the newly created polypeptide can be stored inside the ER for future vesicle transport and secretion outside the cell, or immediately secreted.

Many types of transcribed RNA, such as transfer RNA, ribosomal RNA, and small nuclear RNA, do not undergo translation into proteins.

A number of antibiotics act by inhibiting translation. These include clindamycin, anisomycin, cycloheximide, chloramphenicol, tetracycline, st reptomycin, erythromycin, and puromycin. Prokaryotic ribosomes have a different structure from that of eukaryotic ribosomes, and thus antibiotics can specifically target bacterial infections without any harm to a eukaryotic host's cells

Lecture 4:

Transcription



Simplified diagram of mRNA synthesis and processing. Enzymes not shown.

Transcription is the first step of gene expression, in which a particular segment of DNA is copied into RNA (especially mRNA) by the enzymeRNA polymerase. Both DNA and RNA are nucleic acids, which use base pairs of nucleotides as a complementary language. During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand called a primary transcript.

Transcription proceeds in the following general steps:

1. RNA polymerase, together with one or more general transcription factors, binds to promoter DNA.

2. RNA polymerase creates a transcription bubble, which separates the two strands of the DNA helix. This is done by breaking the hydrogen bonds between complementary DNA nucleotides.
3. RNA polymerase adds RNA nucleotides (which are complementary to the nucleotides of one DNA strand).
4. RNA sugar-phosphate backbone forms with assistance from RNA polymerase to form an RNA strand.
5. Hydrogen bonds of the RNA–DNA helix break, freeing the newly synthesized RNA strand.
6. If the cell has a nucleus, the RNA may be further processed. This may include polyadenylation, capping, and splicing.
7. The RNA may remain in the nucleus or exit to the cytoplasm through the nuclear pore complex.

The stretch of DNA transcribed into an RNA molecule is called a *transcription unit* and encodes at least one gene. If the gene encodes a protein, the transcription produces messenger RNA (mRNA); the mRNA, in turn, serves as a template for the protein's synthesis through translation. Alternatively, the transcribed gene may encode for non-coding RNAsuch as microRNA, ribosomal RNA (rRNA), transfer RNA (tRNA), or enzymatic RNA molecules called ribozymes.[1] Overall, RNA helps synthesize, regulate, and process proteins; it therefore plays a fundamental role in performing functions within a cell.

In virology, the term may also be used when referring to mRNA synthesis from an RNA molecule (i.e., RNA replication). For instance, the genome of a negative-sense single-stranded RNA (ssRNA -) virus may be template for a positive-sense single-stranded RNA (ssRNA +). This is because the positive-sense strand contains the information needed to translate the viral proteins for viral replication afterwards. This process is catalyzed by a viral RNA replicase.

Major steps

Transcription is divided into *initiation*, *promoter escape*, *elongation,* and *termination*.

Initiation

Transcription begins with the binding of RNA polymerase, together with one or more general transcription factors, to a specific DNA sequence referred to as a "promoter" to form an RNA polymerase-promoter "closed complex". In the "closed complex" the promoter DNA is still fully double-stranded.

RNA polymerase, assisted by one or more general transcription factors, then unwinds approximately 14 base pairs of DNA to form an RNA polymerase-promoter "open complex". In the "open complex" the promoter DNA is partly unwound and single-stranded. The exposed, single-stranded DNA is referred to as the "transcription bubble".

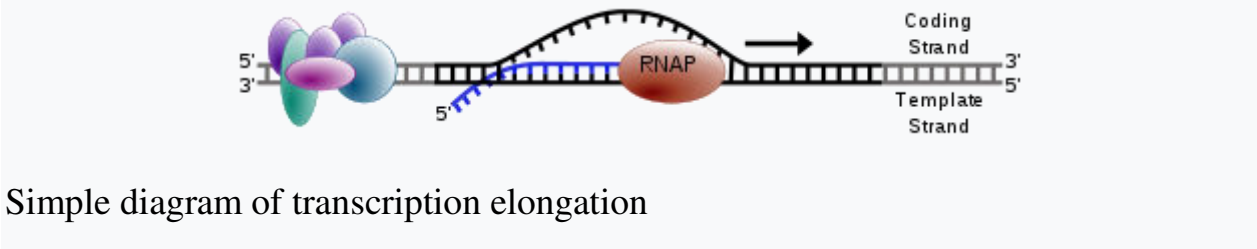
RNA polymerase, assisted by one or more general transcription factors, then selects a transcription start site in the transcription bubble, binds to an initiating NTP and an extending NTP (or a short RNA primer and an extending NTP) complementary to the transcription start site sequence, and catalyzes bond formation to yield an initial RNA product.

In bacteria, RNA polymerase holoenzyme consists of five subunits: 2 α subunits, 1 β subunit, 1 β' subunit, and 1 ω subunit. In bacteria, there is one general RNA transcription factor: sigma. RNA polymerase core enzyme binds to the bacterial general transcription factor sigma to form RNA polymerase holoenzyme and then binds to a promoter. (RNA polymerase is called a holoenzyme when sigma subunit is attached to the core enzyme which is consist of 2 α subunits, 1 β subunit, 1 β' subunit only)

In archaea and eukaryotes, RNA polymerase contains subunits homologous to each of the five RNA polymerase subunits in bacteria and also contains additional subunits. In archaea and eukaryotes, the functions of the bacterial general transcription factor sigma are performed by multiple general transcription factors that work together. In archaea, there are three general transcription factors: TBP, TFB, and TFE. In eukaryotes, in RNA polymerase II-dependent transcription, there are six general transcription factors: TFIIA, TFIIB (an ortholog of archaeal TFB), TFIID (a multisubunit factor in which the key subunit, TBP, is an ortholog of archaeal TBP), TFIIE (an ortholog of archaeal TFE), TFIIF, and TFIIH. In archaea and eukaryotes, the RNA polymerase-promoter closed complex is usually referred to as the "preinitiation complex.

Transcription initiation is regulated by additional proteins, known as activators and repressors, and, in some cases, associated coactivators or corepressors, which modulate formation and function of the transcription initiation complex.

Elongation



Simple diagram of transcription elongation

One strand of the DNA, the *template strand* (or noncoding strand), is used as a template for RNA synthesis. As transcription proceeds, RNA polymerase traverses the template strand and uses base pairing complementarity with the DNA template to create an RNA copy (which elongates during the traversal). Although RNA polymerase traverses the template strand from 3' → 5', the coding (non-template) strand and newly formed RNA can also be used as reference points, so transcription can be described as occurring 5' → 3'. This produces an RNA molecule from 5' → 3', an exact copy of the coding strand (except that thymines are replaced with uracils, and the nucleotides are composed of a ribose (5-carbon) sugar where DNA has deoxyribose (one fewer oxygen atom) in its sugar-phosphate backbone). mRNA transcription can involve multiple RNA polymerases on a single DNA template and multiple rounds of transcription (amplification of particular mRNA), so many mRNA molecules can be rapidly produced from a single copy of a gene. The characteristic elongation rates in prokaryotes and eukaryotes are about 10-100 nts/sec. In eukaryotes, however, nucleosomes act as major barriers to transcribing polymerases during transcription elongation. In these organisms, the pausing induced by nucleosomes can be regulated by transcription elongation factors such as TFIIS.

Elongation also involves a proofreading mechanism that can replace incorrectly incorporated bases. In eukaryotes, this may correspond with short pauses during transcription that allow appropriate RNA editing factors to bind. These pauses may be intrinsic to the RNA polymerase or due to chromatin structure.

Termination

Bacteria use two different strategies for transcription termination – Rho-independent termination and Rho-dependent termination. In Rho-independent transcription termination, RNA transcription stops when the newly synthesized RNA molecule forms a G-C-rich hairpin loop followed by a run of Us. When the hairpin forms, the mechanical stress breaks the weak rU-dA bonds, now filling the DNA–RNA hybrid. This pulls the poly-U transcript out of the active site of the RNA polymerase, terminating transcription. In the "Rho-dependent" type of termination, a protein factor called "Rho" destabilizes the interaction between the

template and the mRNA, thus releasing the newly synthesized mRNA from the elongation complex.

Transcription termination in eukaryotes is less well understood than in bacteria, but involves cleavage of the new transcript followed by template-independent addition of adenines at its new 3' end, in a process called polyadenylation.

Inhibitors

Transcription inhibitors can be used as antibiotics against, for example, pathogenic bacteria (antibacterials) and fungi (antifungals). An example of such an antibacterial is rifampicin, which inhibits bacterial transcription of DNA into mRNA by inhibiting DNA-dependent RNA polymerase by binding its beta-subunit, while 8-hydroxyquinoline is an antifungal transcription inhibitor. The effects of histone methylation may also work to inhibit the action of transcription.

Lecture 5:

Transformation

Transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane(s). For transformation to take place, the recipient bacteria must be in a state of competence, which might occur in nature as a time-limited response to environmental conditions such as starvation and cell density, and may also be induced in a laboratory.

Transformation is one of three processes for horizontal gene transfer, in which exogenous genetic material passes from one bacterium to another, the other two being conjugation (transfer of genetic material between two bacterial cells in direct contact) and transduction (injection of foreign DNA by a bacteriophage virus into the host bacterium). In transformation, the genetic material passes through the intervening medium, and uptake is completely dependent on the recipient bacterium.

As of 2014 about 80 species of bacteria were known to be capable of transformation, about evenly divided between Gram-positive and Gram-negative bacteria; the number might be an overestimate since several of the reports are supported by single papers.

"Transformation" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because

"transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the process is usually called "transfection".

Transformation is one of three forms of horizontal gene transfer that occur in nature among bacteria, in which DNA encoding for a trait passes from one bacterium to another and is integrated into the recipient genome by homologous recombination; the other two are transduction, carried out by means of a bacteriophage, and conjugation, in which a gene is passed through direct contact between bacteria. In transformation, the genetic material passes through the intervening medium, and uptake is completely dependent on the recipient bacterium.

Competence refers to a temporary state of being able to take up exogenous DNA from the environment; it may be induced in a laboratory.

It appears to be an ancient process inherited from a common prokaryotic ancestor that is a beneficial adaptation for promoting recombinational repair of DNA damage, especially damage acquired under stressful conditions. Natural genetic transformation appears to be an adaptation for repair of DNA damage that also generates genetic diversity.

Transformation has been studied in medically important Gram-negative bacteria species such as *Helicobacter pylori*, *Legionella pneumophila*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Haemophilus influenzae* and *Vibrio cholerae*. It has also been studied in Gram-negative species found in soil such as *Pseudomonas stutzeri*, *Acinetobacter baylyi*, and Gram-negative plant pathogens such as *Ralstonia solanacearum* and *Xylella fastidiosa*. Transformation among Gram-positive bacteria has been studied in medically important species such as *Streptococcus pneumoniae*, *Streptococcus mutans*, *Staphylococcus aureus* and *Streptococcus sanguinis* and in Gram-positive soil bacterium *Bacillus subtilis*. It has also been reported in at least 30 species of *Proteobacteria* distributed in the classes alpha, beta, gamma and epsilon. The best studied *Proteobacteria* with respect to transformation are the medically important human pathogens *Neisseria gonorrhoeae* (class beta), *Haemophilus influenzae* (class gamma) and *Helicobacter pylori* (class epsilon).

"Transformation" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the process is usually called "transfection".

Natural competence and transformation

Naturally competent bacteria carry sets of genes that provide the protein machinery to bring DNA across the cell membrane(s). The transport of the exogenous DNA into the cells may require proteins that are involved in the assembly of type IV pili and type II secretion system, as well as DNA translocase complex at the cytoplasmic membrane.

Due to the differences in structure of the cell envelope between Gram-positive and Gram-negative bacteria, there are some differences in the mechanisms of DNA uptake in these cells, however most of them share common features that involve related proteins. The DNA first binds to the surface of the competent cells on a DNA receptor, and passes through the cytoplasmic membrane via DNA translocase. Only single-stranded DNA may pass through, the other strand being degraded by nucleases in the process. The translocated single-stranded DNA may then be integrated into the bacterial chromosomes by a RecA-dependent process. In Gram-negative cells, due to the presence of an extra membrane, the DNA requires the presence of a channel formed by secretins on the outer membrane. Pilin may be required for competence, but its role is uncertain. The uptake of DNA is generally non-sequence specific, although in some species the presence of specific DNA uptake sequences may facilitate efficient DNA uptake.

Natural transformation

Natural transformation is a bacterial adaptation for DNA transfer that depends on the expression of numerous bacterial genes whose products appear to be responsible for this process. In general, transformation is a complex, energy-requiring developmental process. In order for a bacterium to bind, take up and recombine exogenous DNA into its chromosome, it must become competent, that is, enter a special physiological state. Competence development in *Bacillus subtilis* requires expression of about 40 genes. The DNA integrated into the host chromosome is usually (but with rare exceptions) derived from another bacterium of the same species, and is thus homologous to the resident chromosome.

In *B. subtilis* the length of the transferred DNA is greater than 1271 kb (more than 1 million bases). The length transferred is likely double stranded DNA and is often more than a third of the total chromosome length of 4215 kb. It appears that about 7-9% of the recipient cells take up an entire chromosome.

The capacity for natural transformation appears to occur in a number of prokaryotes, and thus far 67 prokaryotic species (in seven different phyla) are known to undergo this process.

Competence for transformation is typically induced by high cell density and/or nutritional limitation, conditions associated with the stationary phase of bacterial

growth. Transformation in *Haemophilus influenzae* occurs most efficiently at the end of exponential growth as bacterial growth approaches stationary phase. Transformation in *Streptococcus mutans*, as well as in many other streptococci, occurs at high cell density and is associated with biofilm formation, Competence in *B. subtilis* is induced toward the end of logarithmic growth, especially under conditions of amino acid limitation.

By releasing intact host and plasmid DNA, certain bacteriophages are thought to contribute to transformation

Transformation, as an adaptation for DNA repair

Logarithmically growing bacteria differ from stationary phase bacteria with respect to the number of genome copies present in the cell, and this has implications for the capability to carry out an important DNA repair process. During logarithmic growth, two or more copies of any particular region of the chromosome may be present in a bacterial cell, as cell division is not precisely matched with chromosome replication. The process of homologous recombinational repair (HRR) is a key DNA repair process that is especially effective for repairing double-strand damages, such as double-strand breaks. This process depends on a second homologous chromosome in addition to the damaged chromosome. During logarithmic growth, a DNA damage in one chromosome may be repaired by HRR using sequence information from the other homologous chromosome. Once cells approach stationary phase, however, they typically have just one copy of the chromosome, and HRR requires input of homologous template from outside the cell by transformation.

To test whether the adaptive function of transformation is repair of DNA damages, a series of experiments were carried out using *B. subtilis* irradiated by UV light as the damaging agent. The results of these experiments indicated that transforming DNA acts to repair potentially lethal DNA damages introduced by UV light in the recipient DNA. The particular process responsible for repair was likely HRR. Transformation in bacteria can be viewed as a primitive sexual process, since it involves interaction of homologous DNA from two individuals to form recombinant DNA that is passed on to succeeding generations. Bacterial transformation in prokaryotes may have been the ancestral process that gave rise to meiotic sexual reproduction in eukaryotes (see Evolution of sexual reproduction; Meiosis.)

Lecture 6:

Transduction

Transduction is the process by which foreign DNA is introduced into a cell by a virus or viral vector. An example is the viral transfer of DNA from one bacterium to another. Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA (which occurs in conjugation), and it is DNase resistant (transformation is susceptible to DNase). Transduction is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome (both bacterial and mammalian cells).

When viruses, including bacteriophages (viruses that infect bacteria), infect bacterial cells, their normal mode of reproduction is to harness the replicational, transcriptional, and translationmachinery of the host bacterial cell to make numerous virions, or complete viral particles, including the viral DNA or RNA and the protein coat.

Lytic and lysogenic (temperate) cycles (bacteria)

Transduction happens through either the lytic cycle or the lysogenic cycle. If the lysogenic cycle is adopted, the phage chromosome is integrated (by covalent bonds) into the bacterial chromosome, where it can stay dormant for thousands of generations. If the lysogen is induced (by UV light for example), the phage genome is excised from the bacterial chromosome and initiates the lytic cycle, which culminates in lysis of the cell and the release of phage particles. The lytic cycle leads to the production of new phage particles which are released by lysis of the host.

Transduction as a method for transferring genetic material

Transduction by bacteriophages

The packaging of bacteriophage DNA has low fidelity and small pieces of bacterial DNA, together with the bacteriophage genome, may become packaged into the bacteriophage genome. At the same time, some phage genes are left behind in the bacterial chromosome.

There are generally three types of recombination events that can lead to this incorporation of bacterial DNA into the viral DNA, leading to two modes of genetic recombination.

Generalized transduction[Generalized transduction is the process by which any bacterial DNA may be transferred to another bacterium via a bacteriophage. It is a rare event; a very small percentage of phage particles happen to carry a donor bacterium's DNA, on the order of 1 phage in 10,000. In essence, this is the packaging of bacterial DNA into a viral envelope.

If bacteriophages undertake the lytic cycle of infection upon entering a bacterium, the virus will take control of the cell's machinery for use in replicating its own viral DNA. If by chance bacterial chromosomal DNA is inserted into the viral capsid which is usually used to encapsulate the viral DNA, the mistake will lead to *generalized transduction*.

If the virus replicates using 'headful packaging', it attempts to fill the nucleocapsid with genetic material. If the viral genome results in spare capacity, viral packaging mechanisms may incorporate bacterial genetic material into the new virion.

The new virus capsule now loaded with part bacterial DNA continues to infect another bacterial cell. This bacterial material may become recombined into another bacterium upon infection.

When the new DNA is inserted into this recipient cell it can fall to one of three fates

1. The DNA will be absorbed by the cell and be recycled for spare parts.
2. If the DNA was originally a plasmid, it will re-circularize inside the new cell and become a plasmid again.
3. If the new DNA matches with a homologous region of the recipient cell's chromosome, it will exchange DNA material similar to the actions in bacterial recombination.

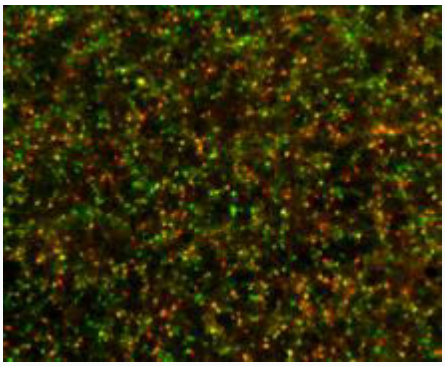
Specialized transduction

Specialized transduction is the process by which a *restricted* set of bacterial genes is transferred to another bacterium. The genes that get transferred (donor genes) depend on where the phage genome is located on the chromosome. Specialized transduction occurs when the prophage excises imprecisely from the chromosome so that bacterial genes lying adjacent to the prophage are included in the excised DNA. The excised DNA is then packaged into a new virus particle, which then delivers the DNA to a new bacterium, where the donor genes can be inserted into the recipient chromosome or remain in the cytoplasm, depending on the nature of the bacteriophage.

When the partially encapsulated phage material infects another cell and becomes a "prophage" (is covalently bonded into the infected cell's chromosome), the partially coded prophage DNA is called a "heterogenote".

An example of specialized transduction is λ phage in *Escherichia coli*.

Mammalian cell transduction with viral vectors



Rat nerve cells express red and green fluorescent proteins after viral transduction with two artificial adeno-associated viruses.

Transduction with viral vectors can be used to insert or modify genes in mammalian cells. It is often used as a tool in basic research and is actively researched as a potential means for gene therapy. In these cases, a plasmid is constructed in which the genes to be transferred are flanked by viral sequences that are used by viral proteins to recognize and package the viral genome into viral particles. This plasmid is inserted (usually by transfection) into a producer cell together with other plasmids (DNA constructs) that carry the viral genes required for formation of infectious virions. In these producer cells, the viral proteins expressed by these packaging constructs bind the sequences on the DNA/RNA (depending on the type of viral vector) to be transferred and insert it into viral particles. For safety, none of the plasmids used contains all the sequences required for virus formation, so that simultaneous transfection of multiple plasmids is required to get infectious virions. Moreover, only the plasmid carrying the sequences to be transferred contains signals that allow the genetic materials to be packaged in virions, so that none of the genes encoding viral proteins are packaged. Viruses collected from these cells are then applied to the cells to be altered. The initial stages of these infections mimic infection with natural viruses and lead to expression of the genes transferred and (in the case of lentivirus/retrovirus vectors) insertion of the DNA to be transferred into the cellular genome. However, since the transferred genetic material does not encode

any of the viral genes, these infections do not generate new viruses (the viruses are "replication-deficient").

Lecture 7:

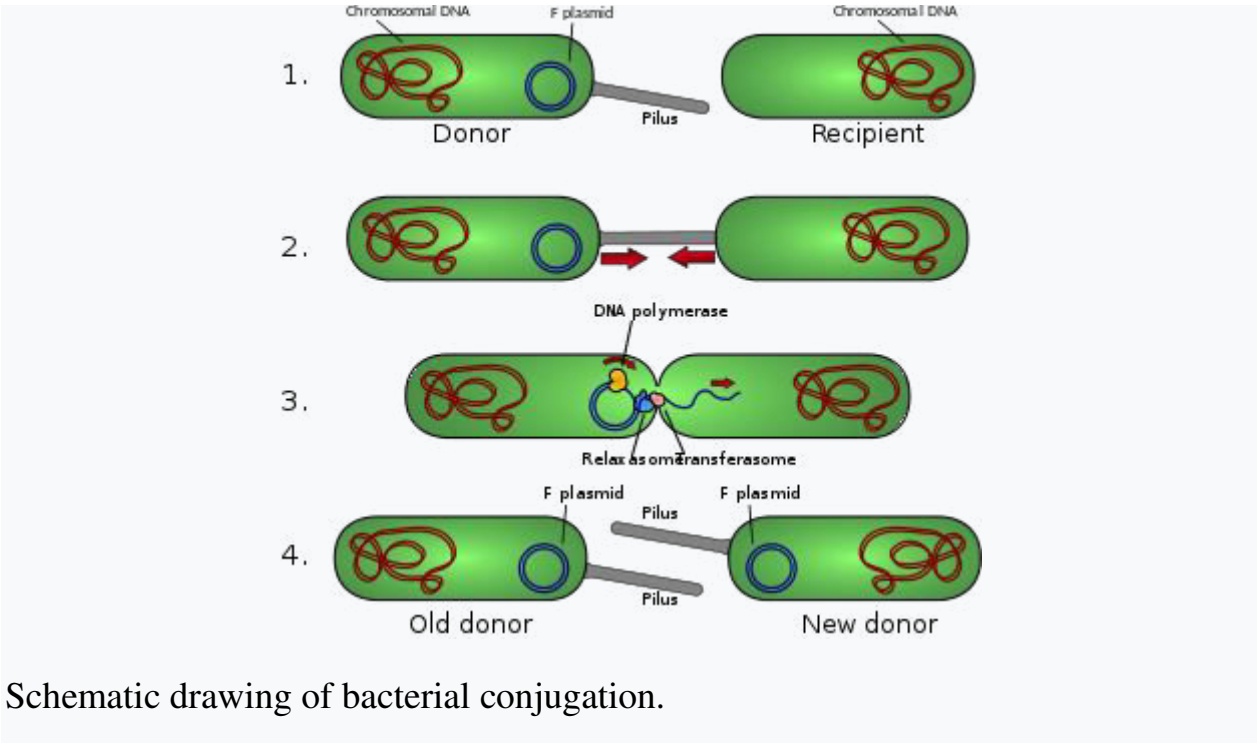
Conjugation

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. This takes place through pilus (Pili in plural).

It is a mechanism of horizontal gene transfer as are transformation and transduction although these two other mechanisms do not involve cell-to-cell contact.

Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating since it involves the exchange of genetic material. However, it is not sexual reproduction, since no exchange of gamete occurs, and indeed no generation of a new organism : instead an existing organism is transformed. During conjugation the *donor* cell provides a conjugative or mobilizable genetic element that is most often a plasmid or transposon. Most conjugative plasmids have systems ensuring that the *recipient* cell does not already contain a similar element.

The genetic information transferred is often beneficial to the recipient. Benefits may include antibiotic resistance, xenobiotic tolerance or the ability to use new metabolites.[Such beneficial plasmids may be considered bacterial endosymbionts. Other elements, however, may be viewed as bacterial parasites and conjugation as a mechanism evolved by them to allow for their spread.



Schematic drawing of bacterial conjugation.

Conjugation diagram

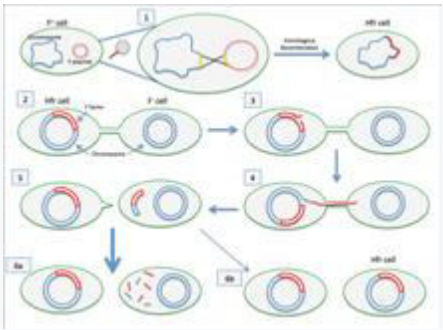
1. Donor cell produces pilus.
2. Pilus attaches to recipient cell and brings the two cells together.
3. The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell.
4. Both cells synthesize a complementary strand to produce a double stranded circular plasmid and also reproduce pili; both cells are now viable donor for the F-factor.

The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome by homologous recombination) with a length of about 100 kb. It carries its own origin of replication, the *oriV*, and an origin of transfer, or *oriT*. There can only be one copy of the F-plasmid in a given bacterium, either free or integrated, and bacteria that possess a copy are called *F-positive* or *F-plus* (denoted F^+). Cells that lack F plasmids are called *F-negative* or *F-minus*(F^-) and as such can function as recipient cells.

Among other genetic information, the F-plasmid carries a *tra* and *trb* locus, which together are about 33 kb long and consist of about 40 genes. The *tra* locus includes the *pilin* gene and regulatory genes, which together form pili on the cell surface. The locus also includes the genes for the proteins that attach themselves to the surface of F^- bacteria and initiate conjugation. Though there is some debate on the exact mechanism of conjugation it seems that the pili are not the structures through

which DNA exchange occurs. This has been shown in experiments where the pilus are allowed to make contact, but then are denatured with SDS and yet DNA transformation still proceeds. Several proteins coded for in the *tra* or *trb* locus seem to open a channel between the bacteria and it is thought that the traD enzyme, located at the base of the pilus, initiates membrane fusion.

When conjugation is initiated by a signal the **relaxase** enzyme creates a nick in one of the strands of the conjugative plasmid at the *oriT*. Relaxase may work alone or in a complex of over a dozen proteins known collectively as a **relaxosome**. In the F-plasmid system the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM and the integrated host factor IHF. The nicked strand, or *T-strand*, is then unwound from the unbroken strand and transferred to the recipient cell in a 5'-terminus to 3'-terminus direction. The remaining strand is replicated either independent of conjugative action (vegetative replication beginning at the *oriV*) or in concert with conjugation (conjugative replication similar to the rolling circle replication of lambda phage). Conjugative replication may require a second nick before successful transfer can occur. A recent report claims to have inhibited conjugation with chemicals that mimic an intermediate step of this second nicking event.



1.The insertion sequences (yellow) on both the F factor plasmid and the chromosome have similar sequences, allowing the F factor to insert itself into the genome of the cell. This is called homologous recombination and creates an Hfr (high frequency of recombination) cell. 2.The Hfr cell forms a pilus and attaches to a recipient F- cell. 3.A nick in one strand of the Hfr cell's chromosome is created. 4.DNA begins to be transferred from the Hfr cell to the recipient cell while the second strand of its chromosome is being replicated. 5.The pilus detaches from the recipient cell and retracts. The Hfr cell ideally wants to transfer its entire genome to the recipient cell. However, due to its large size and inability to keep in contact with the recipient cell, it is not able to do so. 6.a. The F- cell remains F- because the entire F factor sequence was not received. Since no homologous recombination occurred, the DNA that was transferred is degraded by enzymes. b.

In very rare cases, the F factor will be completely transferred and the F- cell will become an Hfr cell.

If the F-plasmid that is transferred has previously been integrated into the donor's genome (producing an Hfr strain ["High Frequency of Recombination"]) some of the donor's chromosomal DNA may also be transferred with the plasmid DNA. The amount of chromosomal DNA that is transferred depends on how long the two conjugating bacteria remain in contact. In common laboratory strains of *E. coli* the transfer of the entire bacterial chromosome takes about 100 minutes. The transferred DNA can then be integrated into the recipient genome via homologous recombination.

A cell culture that contains in its population cells with non-integrated F-plasmids usually also contains a few cells that have accidentally integrated their plasmids. It is these cells that are responsible for the low-frequency chromosomal gene transfers that occur in such cultures. Some strains of bacteria with an integrated F-plasmid can be isolated and grown in pure culture. Because such strains transfer chromosomal genes very efficiently they are called **Hfr** (**h**igh **f**requency of **r**ecombination). The *E. coli* genome was originally mapped by interrupted mating experiments in which various Hfr cells in the process of conjugation were sheared from recipients after less than 100 minutes (initially using a Waring blender). The genes that were transferred were then investigated.

Since integration of the F-plasmid into the *E. coli* chromosome is a rare spontaneous occurrence, and since the numerous genes promoting DNA transfer are in the plasmid genome rather than in the bacterial genome, it has been argued that conjugative bacterial gene transfer, as it occurs in the E. coli Hfr system, is not an evolutionary adaptation of the bacterial host, nor is it likely ancestral to eukaryotic sex.

Lecture 8:

GM Food

Genetically modified foods (**GM foods**), also known as **genetically engineered foods** (GE**foods**), or bioengineered **foods** are **foods** produced from organisms that have had changes introduced into their DNA using the methods of genetic engineering.

Genetically modified foods are foods produced from organisms that have had changes introduced into their DNA using the methods of genetic engineering as opposed to traditional cross breeding.

Genetically engineered organisms are generated and tested in the laboratory for desired qualities. The most common modification is to add one or more genes to an organism's genome. Less commonly, genes are removed or their expression is increased or silenced or the number of copies of a gene is increased or decreased.

Advantages of GM food

1. Nutritional content can be improved.

2. Herbicides and pesticides are used less often.

Disadvantages of GM food

1. GMO crops may cause antibiotic resistance.

2. Genes go into different plant species.

3. Some genetically modified foods may present a carcinogen exposure risk.

MODEL QUESTIONS SET – A

Subject: Microbial Technology & Food Biotechnology

Subject code: FT-604

Stream: Food Technology (6th semester)

Time:-3 hours **Full Marks:-70**

GROUP A

1 . Multiple Choice Question (MCQ) (Answer any ten) 10X1=10

i)The disadvantage of bacteria as SCP is (a)its large size, (b)its undigestable cell wall, (c)its high content of nucleic acid, (d)none of these.

ii) In GM foods

a) Genotype is changed b) phenol type is changed c) both are changed d) none of these

iii) In liquid cored candies the used enzyme is

a) Pectinase b) protease c) invertase d) none of these

iv) The transfer of genetic information by direct cell to cell contact is known as bacterial a) transduction b) transcription transformation d) none of these

v) *S.carlbergensis* has the limitation *to* be used in bakery as it cannot tolerate

a) High temperature b) high p_H c) low pH d) none of these

vi) Oyster mushroom is nothing but

a) Aspergillus , b) Penicillium c) Saccharomyces d) none of these.

vii) Example of a lactic acid bacteria is a)*Lactobacillus*, b)*Leuconostoc*, c)both of these, d)none of these.

viii) Example of a coliform bacteria is a)*Lactobacillus*, b)*Leuconostoc*, c)both of these, d)none of these.

ix) Example of an aerobic spore former is

a)Bacillus subtilis, (b) Campylobacter jejuni, (c) Eschericha coli, (d) none of these.

x)*Spirulina* is a)mold, b)yeast, c)algae, d)bacteria.

GROUP B

Short Question (SQ) (answer any three) 5 X3=15

2.What are the differences between DNA&RNA? (5)

3.What are GM food? (5)

4.Why SCPs are so named? What are the advantages of yeast to be a SCP compared to other organisms?(5)

5.What is Point mutation? What is frame shift mutation? (2.5+2.5)

6.What do you mean by back mutation? How DNA damage can be repaired? (5)

7. What do you understand by 'Indicator Organism'? Give one example.

8. What is the coliform organism? Give example.

9. What is the difference between Selective and elective media?

10. How can you determine the viable bacteria count of a food sample?

11. Mention the rapid tests used in dairy industry. For the determination of metabolically active microorganisms.
12. What are the immunological methods used to determine the presence of aflatoxin?

13. What is ELISA?

14. What do you understand the by the term “Quality” and “Safety” of food for human use?
15. How can you maintain the quality of a food using microbiological criteria?
16. What is HACCP concept?
17. What is food hazard? Give some example.
18. What are the major causes of food born illness?
19. Give some example of fermented foods. What types of organisms are present in a fermented food?
20. Name a few lactic acid bacteria. What is homo and hetero fermentation?
21. With a flow diagram discuss the production of yoghurt from milk.
22. What is beer? What are hops?
23. What is wine? What is the organism used in wine production?
24. With a flow diagram discuss the production of ethanol from molasses.
25. Discuss the sulphite process for the microbial production of glycerol.

GROUP C

Long Question (SQ) (answer any three) 15 X3=45

26. Describe Baker’s yeast fermentation highlighting the nutritional sources, medium composition, pH and temperature.
(15)

27. .a) What are the characteristics of penicillin fermentation? What are the different stages of penicillin fermentation? What do you mean by precursors and which types of precursors are used for penicillin fermentation?
(2+3+3)

b) Where streptomycin is used? What are the limitations of streptomycin? How one can overcome these problems? (2+2+2)

28. What do you mean by RDT? What is plasmid ? Write short note on transduction ?
(3+3+9)

29. Describe Watson-Crick Model of DNA. What do you mean by mutation? Briefly write about different type of mutation.

(10+1+4)

30. What do you mean by mutagen? Name some mutagens. Describe briefly about bacterial transformation.

(1+3+11=15)

31. What are the advantages and disadvantages of mushrooms as a food? Describe about the procedure of mushroom production. Briefly discussing its requirements, composition and preparation of compost and conditions.Name two consumable and two toxic mushroom.

(2+2+9+2)

32. Describe Watson-Crick Model of DNA. What do you mean by mutation? Briefly write about different type of mutation.

(10+1+4=15)

33. What do you mean by mutagen? Name some mutagens. Describe briefly about bacterial transformation.

(1+3+11=15)

34. What do you mean by RDT? What is plasmid ?write short note on transduction ?

(3+3+9)

35. Write short notes on (any three)

use of hops in beer , Botulism , salmonellosis, Elisa test , Resazurin test .

36. What do you mean by spoilage? What are the different spoilage of processed foods & describe their controls. (5+5+5)

37. What are the intrinsic and extrinsic factors which effects the growth of microorganisms (7.5+7.5)

(7.5+7.5)

38. Define food intoxication and food infection? What are the major bacterial food infections? Why food borne fungal intoxications are a major concern for public health? (4+7+4)

39. How would you proceed for microbiological examination of sample of water? What is 'Indicator Organism'? How would you determine the presence of E.coli or coliform in a food sample? (15)

43. What are the major causes for food borne illness? Mention the microbial agents responsible for food borne illness. (15)

44. What is vinegar? Name the organism used in vinegar production. Discuss the quick vinegar process for vinegar production. (15)

45. What is malt? What is the purpose of malting of barley? With a flow diagram discuss the production of beer from barley malt. What is the role of hops in beer production? (15)

46. What are the raw materials for wine production? How red and white wines are produced from grape juice? What is rose wine? What is Champagne? (15)

47. Discuss the process for microbial production of lactic acid. How lactic acid is recovered from fermentation broth? Mention some uses of lactic acid. (15)

MODEL QUESTIONS SET – B

Subject: Microbial Technology & Food Biotechnology

Code: FT 604, Semester**: 6th**

Group-A

Multiple Choice Question (MCQ)

1. **Choose the correct alternatives of the following (Any Ten):-** **10X1=10**

i)For industrial use, invertase is usually derived from

a)yeast b)molds c)bacteria d) all

ii)Streptomycin is produced by

a)synthetic process b)semi synthetic process c)fermentative process d)none of these

iii)Examples of β-lactum antibiotics are

a)streptomycin b)penicillium c)tetracycline

iv)Penicillin-G is produced by

a)synthetic process b)semi synthetic process c)fermentative process d)none of these

v)Vit.B12 is a byproduct of

a)alcohol fermentation b)penicillium fermentation c)streptomycin fermentation d)none of these

vi)The host organism used for cloning

a)*Salmonella* b)*Escherichia* c)*lactobacillus* d)*Bacillus*

vii)Yeast cells are killed before consumption as SCP because

a)it produces diseases b) absorb vit.B c) both of these d) none of these

viii)Oyster mushroom is nothing but

a) *V.volvacea* b) *A.bisporus* c) *A.phalloids* d) none of these

ix)Button mushroom is nothing but

a) *V.volvacea* b) *A.bisporus* c) *A.phalloids* d)none of these

x)S. Carlbergensis is not suitable in bakery industry because

a) It is genetically unstable b) Undergo autolysis c) Can't tolerate high osmotic pressure d) all of these

xi)Bubionic plague is caused by

a) *S.griseus* b) *Y.pestis*c) *E.coli* d) None

xii)Example of mold is

a)*Pseudomonas* b) *Fusarium* c) *Alcaligenes* d) All of these

xiii)The disadvantage of bacteria as SCP is

(a)its large size, (b)its undigestable cell wall, (c)its high content of nucleic acid, (d)none of these.

xiv)In GM foods

a) Genotype is changed b) Phenotype is changed c) Both are changed d) None of these

xv)The transfer of genetic information by direct cell to cell contact is known as bacteria

a)transduction b) Transcription c) Transformation d) None of these

Group-B

Short Answer Questions (SQ)

Answer Any Three Question: - **3X5=15**

2 .What is active dry yeast? how can it be prepared? (5)

3. What are full forms of NTG & EMS? What do you mean by DNA damage repair? (5)

4. What do you mean by homo fermentative and hetero fermentative? (5)

5 .What are the difference between DNA and RNA? (5)

6. What are the beneficial effects of mushroom consumption? What are its limitations?(2.5+2.5)

7. What is the other name of β-lactam antibiotics? What are the problems arise for its oral use? What are the solutions of these problems? (1+2+2)

8. State the effect of temperature, pH, aeration on Vit.B_{12} fermentation. Give the name of the organism.

9. What is Point mutation? What is frame shift mutation? (2.5+2.5)

10. What do you understand by 'Indicator Organism'? Give one example. (5)

11. What is the coli form organism? Give example.(5)

12. What is the difference between Selective and elective media?(5)

13. How can you determine the viable bacteria count of a food sample? (5)

14. Mention the rapid tests used in dairy industry for the determination of metabolically active microorganisms. (5)

15. What are the immunological methods used to determine the presence of aflatoxin? (5)

16. What is HACCP concept? (5)

17. What is food hazard? Give some example. (5)

18. What are the major causes of food borne illness? (5)

19. Give some example of fermented foods. What types of organisms are present in a fermented food? (2+3)

20. With a flow diagram discuss the production of yoghurt from milk.(5)

21. What is wine? What is the organism used in wine production? (4+1)

22. With a flow diagram discuss the production of ethanol from molasses.

23. Discuss the sulphite process for the microbial production of glycerol. (5)

Group-C

Long Answer Questions (LQ)

Answer any three of the following Questions :- **(3X15=45)**

24. .Describe DNA structure ?What are the differences of DNA & RNA? What are the functions of DNA & RNA? (6+5+4)

25 .What do you mean by mutation? Discuss about different type of mutations. What are the difference between GM crops and crops obtained by the traditional breeding process? What are the problems of GM Crops? (2+6+2+5)

26. a)Why microbial synthesis of vit.B_2 are advantageous in compare to chemical synthesis of the same? Name the organism. How the organism is subcultured? What is the C –source which are used for the fermentation? What are the fermentation condition?

b) In making of fructose why glucose isomerase is preffered compared to invertase?

c) Name the organisms used for cellulase and xylanase production?

(5+1+1+1+3+2+2)

27. Where streptomycin is used? What are the problems arise for its use? What are the solutions of these problems? What is the function of the precursor in streptomycin fermentation? Describe different phase of Streptomycin fermentation. What are the problems of this fermentation process? (2+2+2+1+6+2)

28. Where the tetracyclines are used? What are the side effects of tetracyclines treatment? Write the factors considering which the selection of microorganism is done. (5+3+7)

29. What are the important factors for selection of organisms in penicillin fermentation? Give the flow sheet of Penicillin production. What is precursor? What are the roles of precursor in penicillin production? What is the unit of penicillin? Discuss about the raw materials of fermentation medium. (2+2+2+2+1+6)

30. Why Baker's yeast is not produced from the byproduct of alcohol fermentation industry? Discuss about the fermentation parameters of Baker's yeast producton. What are the advantages of yeast cells for the use of SCP? Name one bacteria and one algae used as SCP. (2+8+3+2)

31. What is recombination of DNA? What is plasmid? What is episome? What is bacterial conjugation? Describe the test supporting it. (3+3+1+2+6)

32. What is transduction? Briefly describe it. What do you mean by spontaneous mutation and induced mutation? How DNA damage id repaired? (2+5+4+2+2)

33. Give the name of two toxic mushroom How mushroom can be cultivated? Name the different types of substrate which can be used as raw materials for SCP producton.Give one name of microorganisms of each class. How SCP can be purified from fermentation broth? (1+4+2+4+2+2)

34. What do you mean by spoilage? What are the different spoilage of processed foods & describe their controls. (5+5+5)

35. What are the intrinsic and extrinsic factors which effects the growth of microorganisms (7.5+7.5)

36. Define food intoxication and food infection? What are the major bacterial food infections? Why food borne fungal intoxications are a major concern for public health?
(4+7+4)

37. How would you proceed for microbiological examination of sample of water? What is 'Indicator Organism'? How would you determine the presence of *E.coli* or coliform in a food sample? (15)

38. .What are the major causes for food borne illness? Mention the microbial responsible for food borne illness.

(15)

39. What is vinegar? Name the organism used in vinegar production. Discuss the quick vinegar process for vinegar production.
(15)

40. What is malt? What is the purpose of malting of barley? With a flow diagram discuss the production of beer from barley malt. What is the role of hops in beer production? (15)

41. What are the raw materials for wine production? How red and white wines are produced from grape juice? What is rose wine? What is Champagne?
(15)

42. .Discuss the process for microbial production of lactic acid. How lactic acid is recovered from fermentation broth? Mention some uses of lactic acid.
(15)