M.Sc. FOOD SCIENCE & NUTRITION
LAB MANUAL
2nd Semester

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Food Science & Nutrition

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PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for PG Food Science & Nutrition Second Semester. Hope this edition will help you during practical. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers , and non-teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

Laboratory Practice Safety Rules

1. Use safety glass when dealing with fire and chemical.
2. Should use front cover clothes during biochemistry practical.
3. Always use hand wash after dissection and any type of chemical use.
4. Carefully handle needles , forceps, microscope and any other dissecting instrument.

INDEX

FSN – 295 **FOOD MICROBIOLOGY LAB AND REVIEW**

1. Identification of microorganism – yeast, mould, algae.

Slide Identification of Saccharomyces sp. (Yeast)

Systematic position:

Class: *Ascomycetes*

Order: *Endomyce*

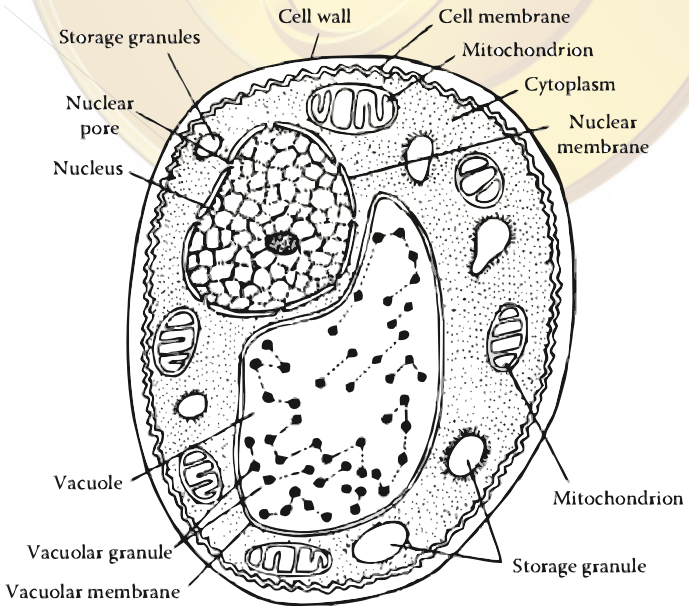
Family: *Saccharomycetaceae*

Genus: *Saccharomyces*

Species: *Cerevisiae*

Identification characteristic:

1. It is commonly used in bread-making. Hence, called brewer's or baker's yeast as well as wine yeast and distiller's yeast.
2. It is unicellular edible fungus, its thallus is nonmycellial.
3. It consists of single minute oral or spherical cell. It's size ranges from 2-8µm, in diameter and length 3-5µm.
4. Cells are hyaline in colour-consisting of protoplast covered by a cell wall.
5. A sexual reproduction take place by binary fission and budding, sexual reproduction is commenced through plasmogamy, karyogamy and meosis.
6. Compressed yeast is used as the source of vitamins while fresh yeast cells are execellent source of scp and vitamin-B complex.



Result: Hence, the specimen is ***Saccharomyces cerevisiae***

Slide Identification of Aspergillus sp. (Fungi)

Systematic position:

Class: *Deuteromycetes*

Order: *Moniliates*

Family: *Moniliaceae*

Genus: *Aspergillus*

Identification characteristic:

1. The conidial apparatus developed into, stalks and heads from foot cells (thick walled hyphal cells), producing condiophores at long axis.
2. Conidiophores septate or non-septate broadening into elliptical hemisphere or globe fertile vesicle.
3. Vesicle bear phialides is one serics (uniseriate), or two serics (biseriate).
4. Phialides clustered interminal groups or radiating from entire surface.
5. Conidia (Conidia bearing cells) elliptical, globes, smooth walled, rough or spinulose walls produced in chains.
6. They play a significant role in production of amylase, diastose, citric acid and also in food poisoning by mycotoxins.

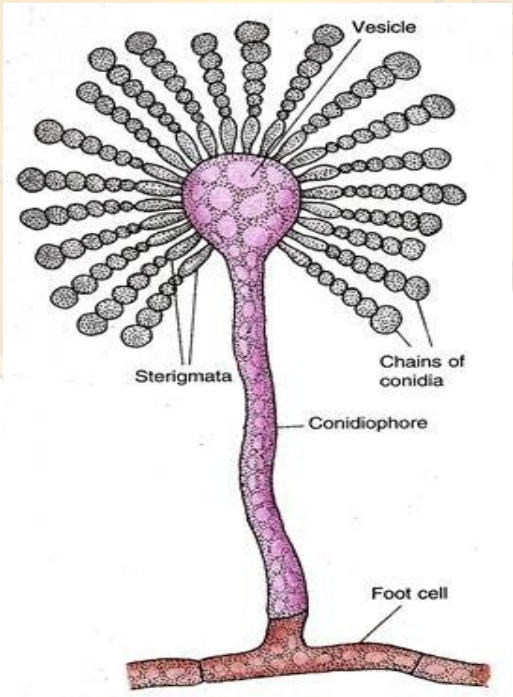


Fig. 10.3. *Aspergillus.* A mature conidiophore bearing sterigmata and chains of conidia.

Result: Hence, the specimen is ***Aspergillus* sp.**

Slide Identification of Penicillium sp. (Fungi)

Systematic position:

Class: *Deuteromycetes*

Order: *Moniliates*

Family: *Moniliaceae*

Genus: *penecillium*

Identification characteristic:

1. Colonies are of various colours, central raised or smooth, may be zoned with age, radially furrowed.
2. Hyphae branched and septate producing branched or unbranched conidiophores each with one, two or more vertical of philiades (conidia bearing cells) and metulae.
3. Conidia borne is chains typically forming brush like head, not enclosed in slime, well differentiated foot cells not present.
4. Conidia globes, orate or elliptical with smooth or rough surface.
5. Some species produce clestothecia with smooth or rough surface.
6. The species produce the penicillin are *penicillium rotates*, *penicillium chrysogenum etc.*
7. *Penicillium lilacinum* is a destructive parasite of nematodes causing disease in plants.

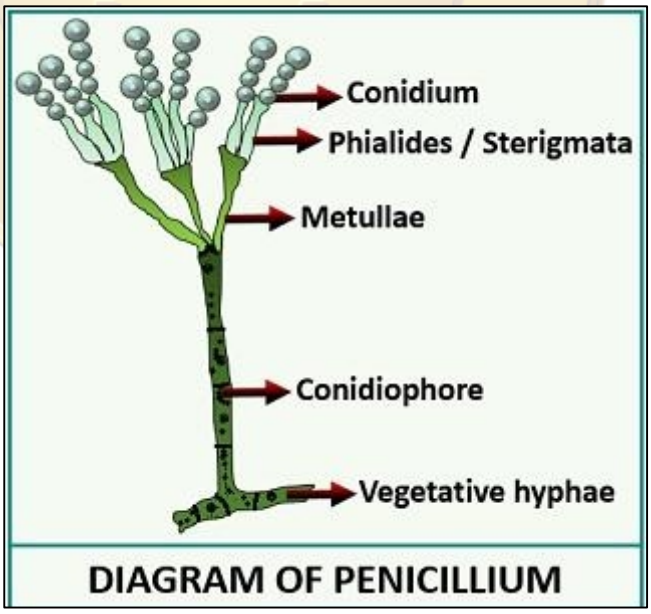


DIAGRAM OF PENICILLIUM

Result: Hence, the specimen is ***Penicillium spp.***

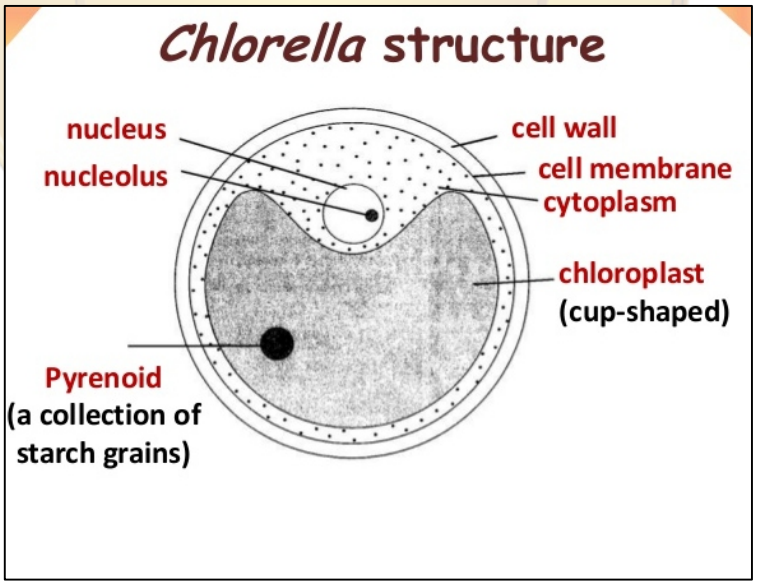
Slide identification of Chlorella sp. (Algae)

Systematic position:

Division- *Chlophyta*
Class- *Chlophyceae*
Family- *Chlorellaceae*
Order- *Chloro*
Genus- *Chlorella*

Identification characteristic:

1. Plant body is unicellular non-motile, may be solitary or aggregated.
2. Structurally cells are very small (2-12µm), may be spherical, sub-spherical or ellipsoidal.
3. Each cell is bounded by a thin, trace andcellulosic wall.
4. Each cell contain within a bell-shaped or cup-shaped particle and chloplast is present with or without a pyramid.
5. There is a transport cavity, i.e colourless central cytoplasm toward one side of the chloplast in which single nucleus, mitochondria and the golgi bodies are located.
6. Photosynthetic thylakoids are present but lack grane like structures.
7. The cells are devoid of flagella.



Result: Hence, the specimen is ***chlorella spp.***

Slide identification of spirulina sp. (Algae)

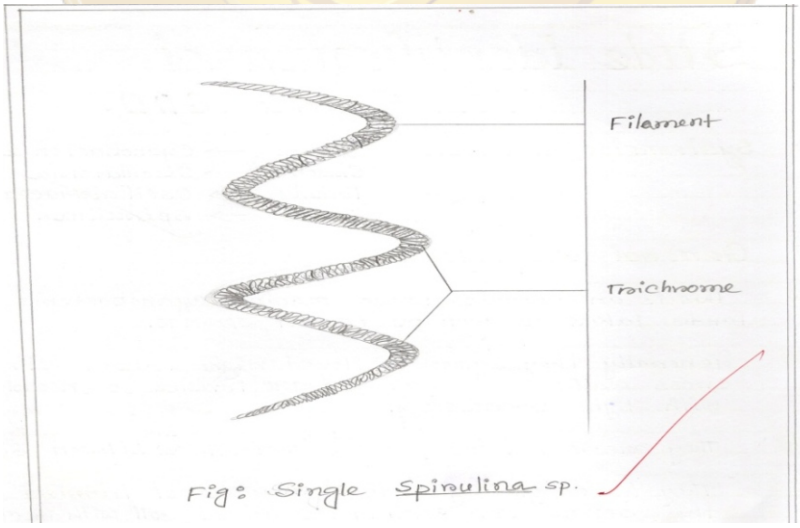
Systematic position: **Group**- *Cynobacteria*
Order - *Oscillatoria*
Family- *Oscillatoriaceae*
Genus- *spirullina*

General characteristics:

1. This is an aerobic, fresh marine cynobacteria in lands, lakes as well as in hot springs.
2. Generally they grow in closed right handed helix. The cross-walls are thin and are invisible or nearly so with light microscope.
3. They are self pH adjusters that grow between 8.5 to 11.
4. They have gliding motility consists of turning of the scraw for continuous helical coil within cross walls.
5. They are significant due to their industrial importance in the form of rich protein value.
6. Their colour is variable from blue green tored and percentage of G+C content is 54.

Identification characteristics:

1. Organization is thallus lik, may be solitary cell or colony which is filamentous.
2. Repeated cell division in a single plane and in single direction forms a chain or thread known as trichema.
3. The cell of trichema are held together either by separation walls or by a common gelatinous sheath around it.
4. Cells are more as less permanently spirally coiled, nucleus is not well defined.



Result: As the green filamentous unicellular, natural, but no hetrocyst was observed. Hence, the specimen is ***Spirullina*** *spp.*

2. Simple staining, gram staining and hanging drop preparation.

A. Simple Staining

Principle: Simple staining involved applying a single basic dye to impart colour to the bacterial cell. Basic dyes are positively charged and work well with bacteria because the bacterial cells bear a slight negative charge. Further basic dyes are attached to the acidic part of the cell such as techoic acid. In general there are two main stain types. Positively charged stains have a positive chromophore. The second type, negatively charged stains, has a chromophore that carries a negative charge. Positively charged stains are excellent in binding negatively charged structures such as bacterial cell walls and, if they can enter the cell, many macromolecular structures such as DNA and proteins.

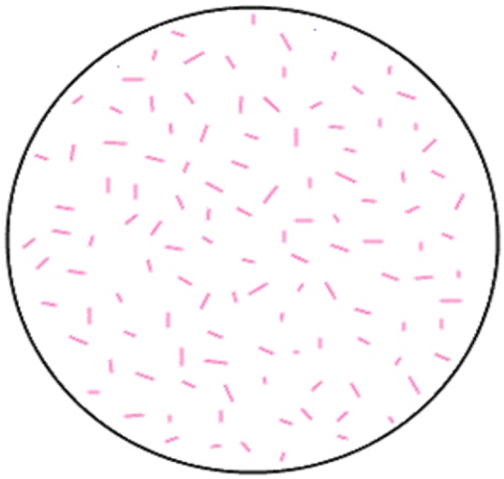
Example of the basic dye includes methylene blue, crystal violet and safranin.

Materials: Glass slide, bacterial culture, cotton, crystal violet, microscope, tray of disinfectant, inoculating loop bunsen- burner

Procedure:

- A clean glass slide was obtained.
- The smear was prepared by placing a drop of culture by using sterile inoculating loop.
- The smear was allowed to air dry and then heat fixed by using Bunsen-burner.
- The smear was covered with several drops of crystal violet and incubated for 30 sec-1 min.
- The slide was gently washed with drops of tap water.
- The slide was air dried and observed under oil immerson microscope (100x).

Observation and Result:

Draw a Representative field:	
Cell Morphology: **Shape:** **Arrangement:** **Cell Colour:**	 **Rod Shape** **Single** **Violet**

Comment: According to the above result, the supplied bacterial sample was rod shaped and arranged in single. (Please change accordingly with your result).

B. Gram Staining

Principle: Differential staining requires the use of at least three chemical reagents that are applied sequentially to a heat fixed smear. The first reagent is called the primary stain. Its function into impart its colour to all cells. In order to establish a colour contrast the second reagent is the discolouring agent. Based on the chemical composition of cellular components the decolourising agent may or may not remove the primary stain from the entire cells or only from certain cell structures. The final reagent, the counter stain has a contrasting than that of the primary stain.

Following decolourisation, if the primary stain is not washed out, the counter stain can't be observed and the cells or their components will retain the colour of the primary stain. If the primary stain is removed, it accepts the contrasting colour of counter-stain. In this way cell type or their structures can be distinguished from each other on the basis of the stain that cells retained.

Purposes: To become familiar with-

i) The chemical and theoritical basis for differential staining procedures.

ii) The chemical basis of gram-stain.

iii) Performance of the procedure for differentiating between the two principle group of bacteria-

a. Gram positive (+ve) bacteria.

b. Gram negetive (-ve) bacteria.

Materials:

- **Culture:** Twenty four hours old culture
- **Reagents:** Crystal violet- Primary stain
  Gram Iodine- Mordant
  Decolourising agent- 70% ethyl alcohol
  Counter Stain- Safranine
- **Equipment:** Bunsen burner, inoculating loop, staining tray, glass slide, lens paper and microscope.

Procedure:

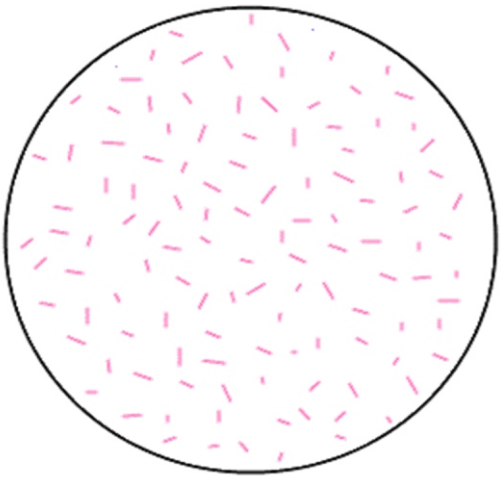
- A clean glass slide was obtained.
- The smear was prepared by placing a drop of culture by using sterile inoculating loop.
- The smear was allowed to air dry and then heat fixed by using Bunsen-burner.
- The smear was covered with several drops of crystal violet and incubated for 30 seconds to 1 minute.
- The slide was gently washed with drops of tap water.
- The smear was then flooded with the Gram's iodine and incubated for one minute.
- The slide was gently washed with drops of tap water.
- The slide was then decolourized with 90% ethyl alcohol.
- The slide was air dried followed by counter staining with safranine for 45 seconds.
- The slide was gently washed with drops of tap water.
- The slide was air dried and observed under oil immerson microscope (100x).

Caution: Don't over decolourise, add reagent drop by drop until alcohol runs almost clear, showing a blue colouration.

Gently wash it with tap water is when done, when the next step is to apply gently or counter stain gently with safranin for 45sec.

Bolt dry with bibulous paper and examine under oil immersion microscope.

Observation and Result:

Draw a Representative field:	
Cell Morphology:	
Shape:	**Rod Shape**
Arrangement:	**Single**
Cell Colour:	**Violet**
Gram reaction:	**Gram Positive**

Comment: Hence the supplied bacterial sample was Gram Positive rod shaped bacteria.

Hence it is Gram Positive Bacteria.

C. Observation of Motility in Bacteria by Hanging Drop Method:

Hanging drop preoaration is aspecial type of wet mount (in which a drop of medium containing the organisms is placed on a microscope slide), often is usedin dark illumination to observe the motility of bacteria.

In this method a drop of culture isplaced on acoverslip that is encircled with petrolium jelly (or any other sticky material). The coverslip and drop are then inverted over the well of a depression slide. The drop hangs from the coverslip and the petrolium jelly forms a seal that prevents evaporation. This preparation given good views of microbial motility.

Materials required:

1. Parafin wax
2. Loop
3. Cover slip
4. Microscope
5. Bunsen burner
6. Young broth culture of motile bacteria
7. Glass slides (glass slide with depression) or normal glass slide with adhesive or parasfin ring

Procedure:

- Take a clean glass slide and apply paraffin ring, adhesive tape ring to make circular concavity (this step is not needed if a glass slide with depression is available).
- Hold a clean coverslip by its edges and carefully apply vaseline on its corners using a tooth pick.
- Place a loop full of the broth culture to be tasted in the centre of the prepared coverslip.
- Turn the prepared glass slide or concavity slide upside down (concavity down) over the drop on the coverslip to the slide around the cavity.
- Turn the slide over so the coverslip is an top and the drop can be observed hanging from the coverslip over the concavity.
- Place the preparation in the microscope slide holder and align it using the naked eye so on edge of the drops is under the low power objectives.
- Turn the objective to its lowest position using the coarse adjustment and close the diaphragm.
- Look through the eyepiece and raise the objectives slowly using the coarse adjustment knob until the edge of the drop is observed as an irregular line coursing the field.
- Move the slide to make that line (the edge of the drop) pass through the centre of the field.
- Without raising or lowering the tube, swing high dry objective into position (be sure the high dry objective is clean).
- Observe the slide through the eyepiece and adjust the fine adjustment until the edge of the drop can be seen as a thick usually dark fine.
- Focus the edge of the drop carefully and look at each slide of that line for very small objects that are the bacteria. The cells will look either like dark or slightly grey. Very small rods are spheres. Remember the high dry objective magnifies a little loss than half as much as the oil immersion objective.
- Adjust the slide using the diaphragm lever to maximize the visibility of the cells.
- Observe the cells nothing there morphology and grouping and determine whether true motility can be observe.
- Brownian movement should be visible on slides of all the organism, but there should also show the true motility.
- Wash the depressive slide and after soaking in Lysol buckets or discard the prepared glass slide.

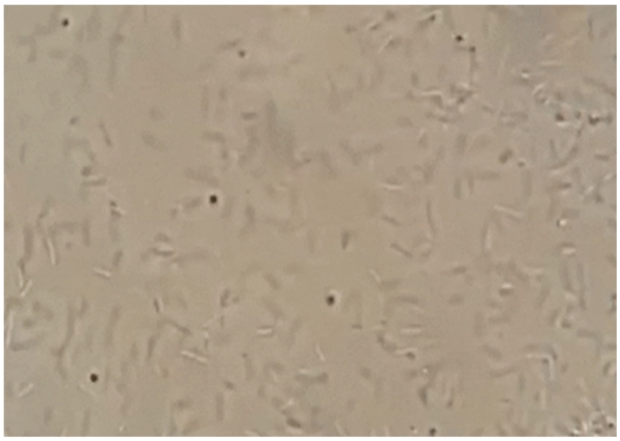


Fig: Organisms are motile in nature

Observation: Observe the slide under the microscope. First focus it under low power objectives and then place a drop of oil immersion on the coverslip. The motility is observed under oil immersion lense of the compound microscope.

Result: From the result it was observed that the supplied organisms are motile in nature.

3. Identification of microorganisms in curd:

Introduction: Basically curd is a milk product, produce due to fermentation i.e microbiological activity in milk. The bacteria i.e ***Lactobacillus*** produce lactic acid in milk and causes souring and milk is ultimately converted into curd. In addition to ***Lactobacillus*** sp. curd also contains the *Streptococcus* and yeast.

Curd organism can be studied by gram staining procedure which is double staining procedure discovered by Hans Christian Gram.

Materials:

- **Sample:** Curd
- **Reagents:** Crystal violet- Primary stain
  Gram Iodine- Mordant
  Decolourising agent- 70% ethyl alcohol
  Counter Stain- Safranine
- **Equipment:** Bunsen burner, inoculating loop, staining tray, glass slide, lens paper and microscope.

Procedure:

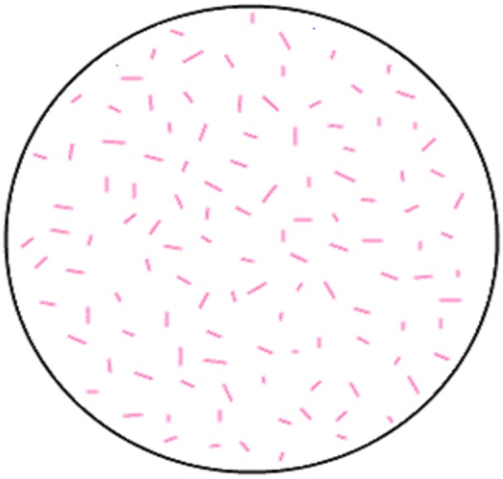
i. A clean glass slide was obtained.
ii. The smear was prepared by placing a drop of curd whey using sterile inoculating loop.
iii. The smear was allowed to air dry and then heat fixed by using Bunsen-burner.
iv. The smear was covered with several drops of crystal violet and incubated for 30 seconds to 1 minute.
v. The slide was gently washed with drops of tap water.
vi. The smear was then flooded with the Gram's iodine and incubated for one minute.
vii. The slide was gently washed with drops of tap water.
viii. The slide was then decolourized with 90% ethyl alcohol.
ix. The slide was air dried followed by counter staining with safranine for 45 seconds.
x. The slide was gently washed with drops of tap water.
xi. The slide was air dried and observed under oil immersion microscope (100x).

Caution: Don't over decolorize. Add reagent drop by drop until alcohol run almost clear, showing only a blue coloration.

Gently wash it with tap water, when previous procedure are done, then the next step is apply gently or counter stain gently with safranin for 45sec.

Blot dry with bibulous paper and examined under oil immersion microscope.

Observation and Result:

Draw a Representative field:	
Cell Morphology: **Shape:** **Arrangement:** **Cell Colour:** **Gram Reaction:**	 **Rod Shape** **Single** **Violet** **Gram-positive**

Comment: According to the above result, the curd sample contains Gram positive bacteria because it retains the crystal violet stain colour.

Hence, it is a gram positive bacteria.

4. Identification of Mould in Bread

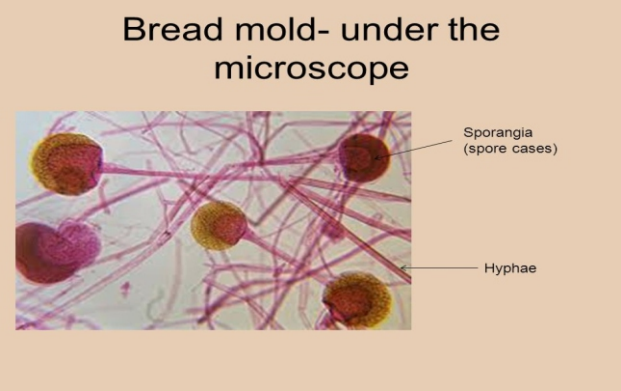
Principle: The mould propagules either are hyaline (colour-less) or of different colours. The hyaline mycelia/spores/conidia etc and their cytoplasm can be stained by using lactophenol and cotton blue. Cotton blue stain cytoplasm and result in a light blue background. Lactophenol as cleaning agent where as phenol a fungicides. For the routine work of mould identification the commonly used plus cotton blue.

Requirement:

a. Young mould culture
b. Mouting fluid that is lactophenol plus cotton
c. Glass slide
d. Cover slip
e. Needle
f. Ethanol (95%)
g. Bunsen Burner
h. Microscope

Procedure:

- Mould young culture (5-7 day old) of mould growing on culture media.
- Put a drop of mountain fluid in the centre of a glass slide.
- Transfer a portion of mycelia mat from mould colony into drop of mounting fluid with the help of flamed and cooled needle.
- With the help of two needle gently spread the mould propagules so that the mycelia should be mixed with stain.
- Repeat the process for all mould colonies.



Result: Observe the slide under low and high power objectives of a microscope and note the types conidia, conidiospore, hyphae and their arrangement. Identify mould following the microbiological literature. For preliminary identification of a few mould.

5. Bacteriological testing of milk:

Aim: To check the quality (microbiologically) of raw milk.

Principle: Milk is susceptible to degradation by many genera of microorganism because it contains carbohydrate, fat, minerals, and protein.

Periodical microbiological analysis of the raw milk and processed milk is carried out to check the spread of disease transmitted through milk. The tests commonly used are standard plate count, coliform test, reductase test.

The standard plate count is one of the routing procedures widely used to determine the number of microorganism in milk. It is an agar plate method for estimating population of bacteria.

Requirements:

a. **Glass goods:** Test tube, Pipettes, Petridish, Conical flasks, Glass Spreader
b. **Instrument:** Autoclave, Water bath, Thermometer, Balance, Hot air over, Spreader
b. **Sample:** Raw milk sample and/ Pasteurized milk
d. **Media:** MRS, Nutrient Agar Media

Procedure:

i) MRS agar, nutrient agar, and Macconkey agar media were prepared by dissolving the appropriate amount of media component with 100 ml distilled water followed by addition of 2 gm of agar in 100 ml media.
ii) The media were autoclaved at 121 ^{o}C for 15 min and the media was poured into the glass petriplates.
iii) The serial dilutions were prepared upto 10^{-5}.
iv) After that 0.1 ml of milk was spread on the solidified agar media.
v) The plates were incubated at 37 °C for 24 – 48 hours in inverted position.

Result:

Sample	Media	Dilution	No. of Colony	Average No. of CFU/ml	Total count (CFU/ml)
Raw milk	Nutrient Agar				
	Nutrient Agar				
Raw milk	MRS				
	MRS				
Raw milk	Macconkey agar				
	Macconkey agar				

Comment: From the above result, it can be concluded that the raw milk may not be suitable for consumption as it contains a vast number of bacteria (? CFU/ml) including *Escherichia coli* (? CFU/ml). It is recommended that the milk should be pasteurized before consumption.

6. Observation of culture characteristics and Preparation of culture media

Principle:

Nutrient agar is used as a general purpose medium for the growth of a wide variety of non-fastidious microorganisms. It consists of peptone, beef extract and agar. This relatively simple formulation provides the nutrients necessary for the replication of a large number of non-fastidious microorganisms. The characteristics of the components used in nutrient agar:

Beef extract is an aqueous extract of lean beef tissues. It contains water-soluble substances of animal tissue, which include carbohydrates, organic nitrogen compounds, water soluble vitamins, and salts.

Peptone is made by digesting proteinaceous materials e.g., meat, casein, gelatin, using acids or enzymes. Peptone is the principal source of organic nitrogen and may contain carbohydrates or vitamins. Depending up on the nature of protein and method of digestion, peptones differ in their constituents, differing in their ability to support the growth of bacteria.

Agar is a complex carbohydrate obtained from certain marine algae. It is used as a solidifying agent for media and does not have any nutritive value. Agar gels when the temperature of media reaches 45°C and melts when the temperature reaches 95 °C.

Materials:

Glass petri plates, conical flasks (250 ml), pipettes (1 ml and 10 ml), spirit lamp, 70% alcohol, fuel alcohol, non-absorbent cotton, tissue paper, distilled water

Media Composition: Nutrient broth 13.0 gm

Agar 20.0 gm

Distilled Water1000 ml

Final pH- 6.8 ± 0.2.

Procedure:

Media preparation:

Required amount (1.3 gm) of nutrient broth was dissolved into 100 ml of distilled water kept in a conical flask.

pH was adjusted to 6.8 ± 0.2.

Then 2.0 gm of agar was added to 100 ml of nutrient broth.

The media was sterilized at 15 lb pressure and 121 ^{o}C for 15 min by using autoclave.

After autoclaving, the media was cooled down to 50 ^{o}C and poured in to the autoclaved Petriplates.

Dilution preparation:

Nine milliliter of autoclaved distilled water was added to 6 test tubes.

One milliliter of water sample (Tap water) was mixed with 9 ml of distilled water and thus 10^{-1} dilution was prepared.

Then 1 ml of water from10^{-1} dilution was poured in another test tube containing 9 ml of autoclaved distilled water and thus 10^{-2} dilution was prepared.

Like this, upto 10^{-6} dilution was prepared.

Plating:

One hundred microliter of 10^{-4}, 10^{-5}, 10^{-6} dilutions were spread on the solidified nutrient agar plates.

The plates were incubated at 37 ^{o}C for 24 h.

Result:

After 24 h, the colony appeared on the petri plates were enumerated and the colony characteristics were observed.

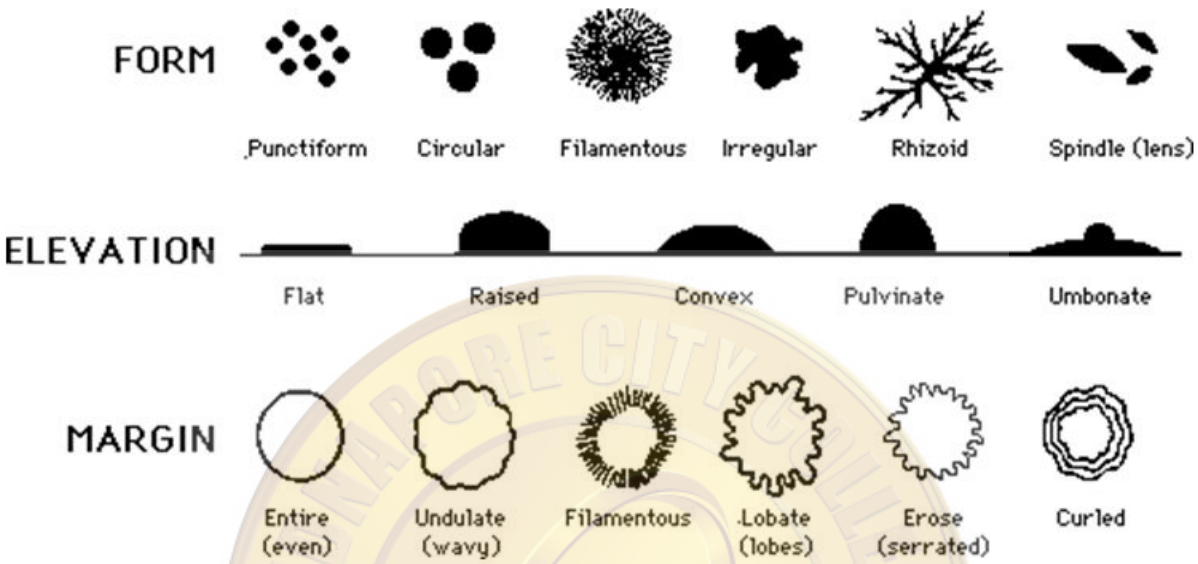
Table 1: Enumeration of bacteria from water sample:

Sample number	Dilution no.	Sample added (ml)	CFU numbers/ 0.1 ml	CFU numbers/ 1 ml	CFU No./ml in original sample	Average number of CFU No./ml in original sample

Table 2: Colony characteristics:

Colony number	Colour	Form	Elevation	Margin	Figure

Colony Morphology



REVIEW WORK:

An independent review work should be undertaken by student under the guidance of a teacher. A report should be submitted at the end of semester in a standard format. The review topic can be selected in consultation with the supervisor.
The student will be required to appear before examiners board and to deliver a seminar on the review work.

FSN – 296

THERAPEUTIC DIET PREPARATION

1. Therapeutic diet preparation for metabolic disease

a. Therapeutic diet chart preparation for Diabetes

b. Therapeutic diet chart preparation for Hypertension

c. Therapeutic diet chart preparation for Atherosclerosis

d. Therapeutic diet chart preparation for Nutritional anaemia

2. Therapeutic diet preparation for Gastro Intestinal Diseases

a. Therapeutic diet chart preparation for Diarrhoea

b. Therapeutic diet chart preparation for Dysentery

c. Therapeutic diet chart preparation for Flatulence

d. Therapeutic diet chart preparation for Jaundice

e. Therapeutic diet chart preparation for Hepatitis

f. Therapeutic diet chart preparation for Irritable bowel Syndrome, Inflammatory bowel disease

g. Therapeutic diet chart preparation for Constipation

h. Therapeutic diet chart preparation for Colitis

i. Therapeutic diet chart preparation for Ulcer.

3. Therapeutic diet preparation for rheumatic diseases

a. Therapeutic diet chart preparation for Arthritis.

b. Therapeutic diet chart preparation for Osteoarthritis**.**

4. Food sensitivity and cell proliferation related diseases

a. Therapeutic diet chart preparation for cancer and food allergy

5. Critical care condition

a. Therapeutic diet chart preparation for critical care conditions like Sepsis, trauma, burns, pre and post-surgical conditions