Practical Course syllabus:

Course: Microbial physiology (MBIO 331) – 1st semester 1440/41.

Practical instructor: Mohamed Salah El-Din Hodhod.

Class time & Location: Tuesday 8-9:50am, Lab 1B/19.

Week	Topic
1	Syllabus & Introduction
2	Isolation & identification of microbes from soil samples
3	Metabolism
4	Test chemical factors affecting microbial growth 1-Carbon source
5	2-Nitrogen source
6	Test physical factors affecting microbial growth 1-pH
7	2-Temprature
8	3-Salt concentration
9	4- Aerobic and anaerobic conditions
10	Testing antibiotics against bacterial cultures
11	Testing antibiotics against fungal cultures
12	Revision
13	Final practical exam

1- Introduction

Giving a brief introduction to the microbial physiology practical course along with the syllabus content and objectives.

2- Isolation and identification of microbes from soil samples

Objectives

The student will be able to distinguish between different patterns of growth to various bacterial and fungal species isolated.

Materials

- soil
- plates
- Ethanol
- tubes

Practical

make a serial dilution from 1g of soil. Spread onto plates from each dilution.

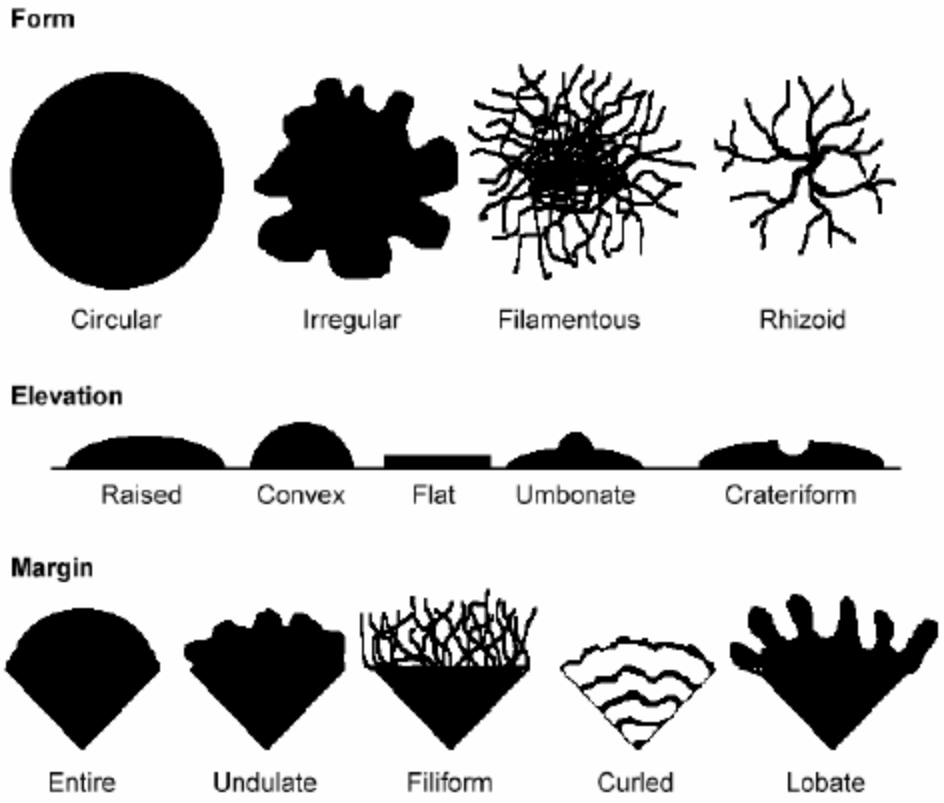
Thereafter, incubate at 28°C for one week. Evaluation of data on the next week.

Results

On a given medium, a colony's shape, color, consistency, surface appearance and size - for a given incubation time - are often characteristic, and these are often of use in the identification of particular bacterial strains. The full description of a colony can be very detailed. Thus e.g. the elevation of a colony may be flat, low convex, domed unbonate etc., its edge maybe entire [circular or unbroken], crenate [scalloped], lobed or fimbriate; its texture may be butyrous friable or mucoid; its surface may be matt or glossy; it may be whitish or pigmented or it may contain a dye taken up from the medium, or it may release water soluble pigment into the medium. The colonies of certain bacteria e.g. *Bacillus* can migrate across the surface of a culture plate, the tract of such movement is often marked by lines of bacterial growth which arise from the cells left behind by the migration colony. An interesting

feature of certain bacterial colonies is the so-called smooth-rough variation. In many types of bacteria, some type of S-R variation is responsible for a change in the cell-surface composition, which occurs spontaneously during in vitro or in vivo growth. S-R variation was first recorded in enterobacteria, in which smooth [glossy] colony may be formed on primary isolation, and rough [dull] colonies may develop on subcultures.

Expected results



3- Metabolism

Microbial metabolism

the Greek *metabole*, meaning change.

Metabolism - the sum of all biochemical reactions required for energy generation AND the use of energy to synthesize cell material from small molecules in the environment.

Metabolism

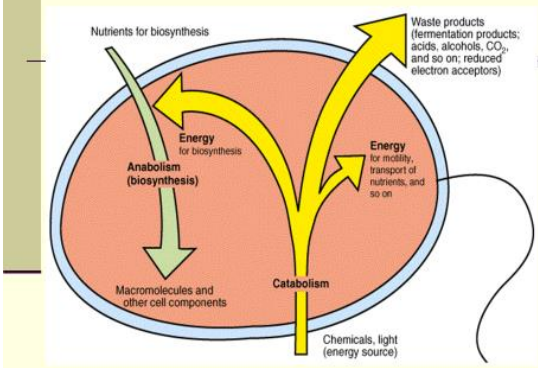
Two components:

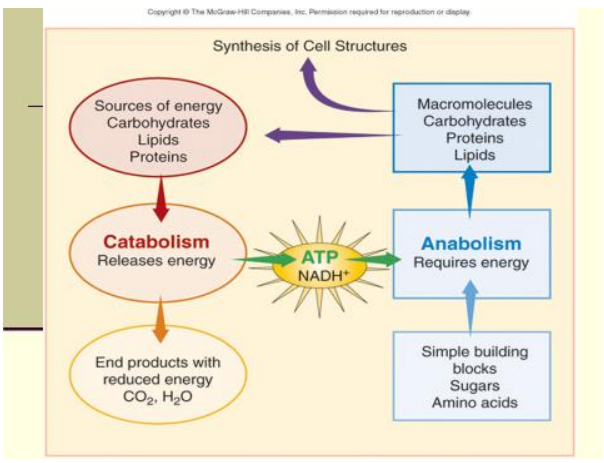
- **Anabolism** - biosynthesis
  - building complex molecules from simple ones
  - requires **ENERGY** (ATP)
- **Catabolism** - degradation
  - breaking down complex molecules into simple ones
  - generates **ENERGY** (ATP)

- Catabolic reactions or sequences produce energy as **ATP adenosine triphosphate** , which can be utilized in anabolic reactions to build cell material from nutrients in the environment.

Why do we must know the metabolism of bacteria?

Because we want to know how to **inhibit or stop** bacteria growth and want to control their metabolism.





4- Test chemical factors affecting microbial growth

1-Carbon source

Objectives

The student will be able to differentiate between different patterns of growth due to the effect of carbon source.

Materials

- Glucose
- Fructose
- Arabinose
- Ethanol
- tubes

Practical

Pipette 0.5 ml bacterial suspension aseptically into test tubes containing nutrient broth of different carbon sources (Glucose, Fructose and Arabinose). Followed by incubation at 28°C for a week.

Results

Observe the turbidity of each broth and compare it with other tubes.

Expected results

Bacteria will grow better on Glucose as simplest source of carbon followed by Fructose, then Arabinose.

5- Test chemical factors affecting microbial growth

2-Nitrogen source

Objectives

The student will be able to differentiate between different patterns of growth due to the effect of Nitrogen source.

Materials

- Peptone
- Tryptone
- Yeast extract
- Ethanol
- tubes

Practical

Pipette 0.5 ml bacterial suspension aseptically into test tubes containing nutrient broth of different Nitrogen sources (Peptone, Tryptone and Yeast extract). Followed by incubation at 28°C for a week.

Results

Observe the turbidity of each broth and compare it with other tubes.

Expected results

Bacteria will grow better on Pyptone as simplest source of carbon followed by Tryptone, then Yeast extract.

6- Test physical factors affecting microbial growth

1-PH

Objectives

The student will be able to differentiate between different patterns of growth due to the effect of PH.

Materials

- pH=3 solution
- pH=7 solution
- pH=9 solution
- Ethanol
- tubes

Practical

Pipette 0.5 ml bacterial suspension aseptically into test tubes containing nutrient broth of different pH values (pH=3, pH=7, pH=9). Estimate the pH tolerance of each strain by examining the growth rate of the bacteria following incubation at 28°C for a week.

Results

Observe the turbidity of each broth and compare it with a blank test tube that has not been inoculated with bacteria.

7- Test physical factors affecting microbial growth

2-Temperature

Objectives

The student will be able to differentiate between different patterns of growth due to the effect of Temperature.

Materials

- Ethanol
- tubes

Practical

Transfer the different strains of bacteria (from suspension or agar slants) onto fresh nutrient agar slants and incubate each strain at 4°C, 28°C and 50°C.

Results

Observe the rate of bacterial growth on the medium after a week-long incubation.

Expected results

Bacteria will grow better on at 28°C followed by 50°C then 4°C.

8- Test physical factors affecting microbial growth

3-NaCl

Objectives

The student will be able to differentiate between different patterns of growth due to the effect of different salt concentrations.

Materials

- Ethanol
- NaCl
- tubes

Practical

Inoculate bacterial suspension into culture broth of different salt concentration values (0%, 5%, 10%). The germicidal effect should be evaluated following incubation at 28°C for a week.

Results

Estimate the osmo-tolerance of each strain by examining the growth rate of the bacteria following incubation at 28°C for a week (observe the turbidity of each broth and compare it with a blank test tube that has not been inoculated with bacteria).

9- Test physical factors affecting microbial growth

4-Aerobic and Anaerobic conditions

Objectives

The student will be able to differentiate between different patterns of growth due to the effect of oxygen availability.

Materials

- Ethanol
- loop
- tubes
- Aerobic and anaerobic bacterial cultures

Practical

Inoculate bacterial suspension into culture broth. Incubate the tubes under both aerobic and anaerobic conditions at 28°C for a week.

Results

Estimate the bacterial strain tendency to grow under different conditions of oxygen availability by examining the growth rate of the bacteria following incubation at 28°C for a week (observe the turbidity of each broth and compare it with a blank test tube that has not been inoculated with bacteria).

10-Testing antibiotics against bacterial cultures

Objectives

The student will be able to differentiate between different patterns of growth due to the antibiotic resistance of some bacteria.

Materials

- Ethanol
- Plant extract
- tubes

Practical

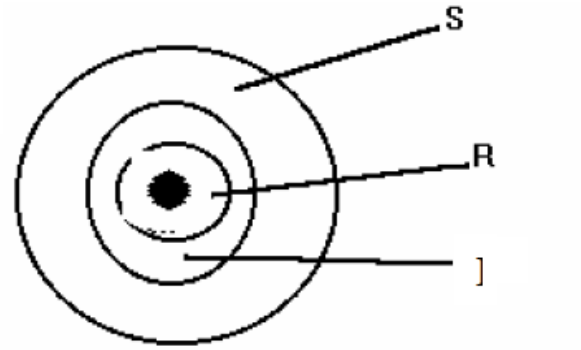
In this method a culture of known concentration is spreaded on an appropriate medium (Mueller-Hinton medium). Filter paper discs containing predetermined concentrations of an antimicrobial are placed on the seeded agar plate, with equal spacing between discs (Generally 4 discs / Petri dish). During incubation, the agent diffuses out of the disc, creating a concentration gradient that decreases according to distance away from the disc.

Results

After the incubation the organisms, sensitivity is measured on the basis of the size of the zone of inhibition (no growth) around each disc. This measure is compared to values on a standard, and it indicates whether the microorganism is resistant, intermediate or sensitive to the agar. Sensitive means that the organism is inhibited by clinically attained concentrations of the antimicrobial; resistant means that the

organisms is not inhibited; intermediate meant that special considerations are to be followed if the antibiotic is to be used.

Expected results



R : resistant zone of inhibition with a diameter equal to or less than inner circle.
I : intermediate zone of inhibition with a diameter greater than R.
S : sensitive zone of inhibition with a diameter greater than outer circle .

11-Testing antibiotics against fungal cultures

Objectives

The student will be able to differentiate between different patterns of growth due to the antibiotic resistance of some fungi.

Materials

- Ethanol
- Plant extract
- tubes

Practical

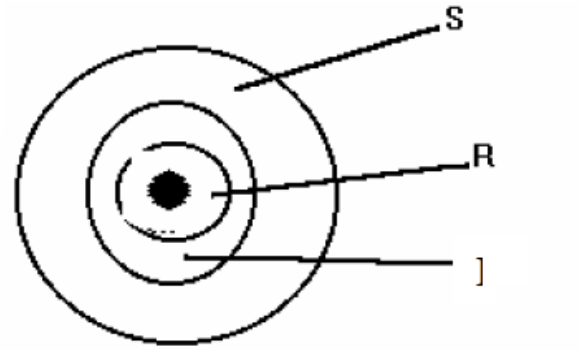
In this method a culture of known concentration is spreaded on an appropriate medium (PDA). Filter paper discs containing predetermined concentrations of an antimicrobial are placed on the seeded agar plate, with equal spacing between discs (Generally 4 discs / Petri dish). During incubation, the agent diffuses out of the disc, creating a concentration gradient that decreases according to distance away from the disc.

Results

After the incubation the organisms, sensitivity is measured on the basis of the size of the zone of inhibition (no growth) around each disc. This measure is compared to values on a standard, and it indicates whether the microorganism is resistant, intermediate or sensitive to the agar. Sensitive means that the organism is inhibited by clinically attained concentrations of the antimicrobial; resistant means that the

organisms is not inhibited; intermediate meant that special considerations are to be followed if the antibiotic is to be used.

Expected results



R : resistant zone of inhibition with a diameter equal to or less than inner circle.
I : intermediate zone of inhibition with a diameter greater than R.
S : sensitive zone of inhibition with a diameter greater than outer circle .

12-Revision

Revise all the practical sessions that have been taken throughout the practical course.