PRACTICAL MICROBIAL PHYSIOLOGY

Prepared by
Assist.Prof. Nareen Qadr Faqi Abdullah and M.Sc. Neveen N. Rajab

First week: Course Program, Preparation of media for microbial isolation

Second week: Effect of Nutrition on microbial growth

Third week: Effect of oxygen on microbial growth: using different methods of aerobic and anaerobic incubation

Fourth week: Effect of temperature on microbial growth

Fifth week: Effect of pH on microbial growth

Sixth week: Effect of osmotic pressure on microbial growth

Seventh week: Practical examination

Eighth week: Effect of ultraviolet light on microbial growth

Ninth week: Microbial relationship

Tenth week: Production of antibiotic by Microorganism

Eleventh week: Effect of chemical agents on microbial growth

Twelfth week: Effect of plant extract on the growth of microorganisms

Thirteenth week: Minimal Inhibitory Concentration (MIC)

Fourteenth week: Practical examination

Safety Rules in the Microbiology laboratory:

1. Lab coats must be worn at all times in the lab
2. The working area disinfected with 5% detol…..etc.
3. All culture material should be properly labeled
4. Do not pipette by mouth to avoid accidental ingestion of cultures or chemicals, use pipettes with rubber bulbs or mechanical manipulators.

5. When flaming an inoculating loop or needle, always place the loop or needle near the base of the flame (to incinerate any m.o. on the loop).
6. After lab session wash your hands before leaving the lab.

Preparation of media for microbial isolation

Types of Media according to chemical structure

1. Natural media: Consist of Natural complex substances of unknown structure and concentration (for morphological study).
2. Semi synthetic media: Consist of 2 parts of known structure and concentration and other of unknown structure and concentration (for morphological study).
3. Synthetic media: Consist of known structure and concentration (for physiological study).

Types of Media according to texture

1. Solid media: These media contain solidified substance (%2 agar) or gelatin, agar is used more than gelatin, because agar is complex carbohydrate substances, and not lysis by microorganisms and solidified at 4°C while gelatin is protein substances, lysis by microorganisms and become liquid at 25°C (used for the study of structure and morphology of fungal culture).
2. Semi-solid media: Consist of 1% of agar that produces semi solid texture to the media (used for studying of zoospores).
3. Broth media: These media lack of agar (used for studying of nutrition and metabolism of microorganisms).

Preparation of media

1. Nutrient agar: Medium used for cultivating a wide variety of non fastidious microorganisms

Procedure: Mix 23gm of the medium in one liter of purified water until evenly dispersed, heat with repeated stirring to dissolve completely then distribute and autoclave at 121°C for 15 minutes.

2. Potato Dextrose Agar (PDA)

Chemical compound	**Quantity**
Dextrose	20 g.
Potato	200 g.
Agar	20 g.
Distill water (D.W.)	1 L.

Procedure:

1. Scrub the potatoes, clean then cut into small cubes, weight out 200 gm and place in 500 ml of D.W., and boil for 20 minutes until potato tissue become soft then filtered through 3-4 layers of fine gauze.

2. Add the sugar to another 500 ml of D.W., then add the agar gradually and boil, then mixed with potato extract.
3. The volume make-up to 1000ml by D.W. then distributes into 4 flasks and sterilized by autoclave at 121ºC, 1.5 bar for 15-20 minutes.

3. Sabouraud Dextrose Agar (SDA): For isolation and cultivation of dermatophytes

Chemical compound	**Quantity**
Peptone	10 g.
Dextrose	40 g.
Agar	20 g.
Cyclohexamide	0.5 g.
Chloramphenicol	0.05g.
D.W	1 L.
pH	6.5

Soak all ingredients in 100ml water and boil remaining water and boil to dissolve then autoclave it. It provides basic nutrition that will support the growth of any pathogenic fungi; also Chloramphenicol and Cycloheximide were added to the medium.

Note: Chloramphenicol (Anti bacterial) + Cycloheximide (Antifungal-Antisaprophyte).

4. Czapek (Dox) Agar

Chemical compound	**Quantity**
$NaNO_3$	2g.
KH_2PO_4	1g.
$MgSO_4.7H_2O$	0.5g.
KCl	0.5g.
$FeSO_4.7H_2O$	0.01g.
Sucrose	30g.
Agar	20g.
D.W	1L.

Soak the entire ingredient in small amount of water, bring the remaining water to boil and bring to the boil again, stirring continuously, then autoclave it.

5. Mueller-Hinton Agar

Chemical compound	**Quantity**
Beef Extract	2.0 g.
Acid Hydrolysis of Casein	17.5
Starch	1.5
Agar	17.0
D.W	1L

6. Water agar

Chemical compound	**Quantity**
Agar	20gm
Tap water	1L.

Sterilization of media: Sterilization is the kill and destruction of living microorganisms

Sterilization methods

1. Physical methods:-

a. heating:

i. Dry heat: Hot air oven used for sterilizing of glassware at 180ºC for 1 hour (150ºC for 1.5 hour, 200ºC for 0.5 hour).

ii. Wet heat: Moist heat under pressure use for sterilizing of culture media, autoclave used for this purpose at 121ºC for 17 minutes, 1.5 bar.

iii. Flame: Used for sterilization of needle, loop, cork hole, forceps by heating to redness.

b. Radiation: Both ultraviolet and ionized radiation used for sterilization by using hood.

c. Filtration: This technique used for separation of micro organisms from liquids, as Seitz filter, chamber land filter.

2. Chemical methods:

a. Disinfectants: Chemical agents used for surface sterilization as chloroform, formaldehyde, detol, mercuric chloride.

b. Gaseaous sterilization: This method is used for soil sterilization and usually used for vaporing of agricultural products, as methyl bromide, ethylene oxide.

References

- Johnson, T.R. and Case, C.L. (2007). Laboratory Experiments in Microbiology.
- Atlus, Ronald M., Lawrence C. Parks and Alfred E. Brown (1995) Laboratory Manual of Experimental Microbiology.
- Benson, A.E. (2005). Bensons microbiological Applications.