Principles of biotechnology

Lab2.

Microorganisms growth requirements and culture media.

A microorganism is defined as a living thing that is so small that must be viewed with a microscope or with electron microscope.There are five different categories of microorganisms—bacteria, algae, protozoa, fungi, and viruses. Microorganisms exist throughout the world, from Antarctica to your kitchen, from inside animals, like humans, to an expansive wilderness. An initial aim of all microbiologists is the reproducible growth of their microbial cultures. Microbial growth requires suitable environmental conditions, a source of energy, and nourishment. **These requirements can be divided into two categories, physical and chemical.**

1. **Chemical requirments.**

Chemical factors	Form usually found in nature	Chemical form commoly added media
carbon	CO_2, organic compounds	Organic; simple sugars e.g. glucose, acetate or pyruvate; extracts such as peptone, tryptone, yeast extract etc.
oxygen	Water (H2O), organic compounds	
hydrogen	Water (H2O) organic compounds	
nitrogen	NH_3, amino acids	Organic; amino acids,nitrogenous bases. Inorganic; NH_4CI
phosphorus	PO_4	KH_2PO_4, Na_2HPO_4
potassium	K+	KCI, K_2HPO_4
Magnesium	Mg^{2+}	$MgCI_2$, $MgSO_4$
Calcium	Ca^{2+}	$CaCI_2$
Sodium	Na^{+}	NaCI
Iron	Fe^{3+} organic iron complexes	$FeCI_3$
Trace elements	Usually present at very low concentrations	$ZnCI_2$, $CuCI_2$
Organic growth factors	Usually present at very low concentrations	Vitamins, amino acids, purines, pyrimidines

2. **Physical / Environmental requirements.**

2.1 Temperature.

Most microorganisms grow well at the normal temperatures favoured by man, higher plants and animals. However, certain bacteria grow at temperatures (extreme heat or cold) at which few higher organisms can survive. Depending on their preferred temperature range, bacteria are divided into three groups:

- Psychrophiles (cold-loving microorganisms): have an optimum growth temperature between 0°C and 15°C.
- Mesophiles (moderate-temperature-loving bacteria): have an optimum growth

temperature between 25°C and 45°C.

- Thermophiles (heat-loving microbes): have an optimum growth temperature between 50°C and 65°C.

2.2 PH

- **Neutrophils:** pH range near neutrality between pH 6.5 and 7.5.
- **Acidophils** (acid-loving): grow at pH values below 4 with some bacteria still active at a pH of 1.
- **Alkalinophils (**base-loving): prefer pH values of 9-10 and most cannot grow in solutions with a pH at or below neutral.

2.3 Osmotic Pressure

Microbes contain approximately 80-90% water and if placed in a solution with a higher solute concentration will lose water which causes shrinkage of the cell (plasmolysis). However, some bacteria have adapted so well to high salt concentrations that they actually require them for growth. These bacteria are called halophiles (salt-loving) and are found in salterns or in areas such as the Dead Sea.

Culture media

Culture media contains nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in a single culture medium. culture media can be distinguished on the basis of **composition, and consistency.**

1. **Classification of culture media based on consistency**
   1. **Solid medium**
      solid medium contains agar at a concentration of 1.5-2.0%. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks). Solid medium is useful for **isolating bacteria** or for determining the colony characteristics of the isolate.
   2. **Semisolid media**
      They are prepared with agar at concentrations of 0.5% or less. They have soft custard like consistency and are useful for the determination of bacterial motility.
   3. **Liquid (Broth) medium**
      These media contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies, and various other tests. e.g. sugar fermentation tests

2. **Classification of culture media based on composition**
   1. Synthetic or chemically defined medium
      A chemically defined medium is one prepared from purified ingredients and therefore whose exact composition is known.
   2. Non synthetic (Natural) or chemically undefined medium, like: Molasses, and Whey.
   3. Semisynthetic media the media of which chemical composition is partially known is as semisynthetic media, like: PDA and nutrient agar.

- **Preparation of nutrient broth and agar media**.

Laboratory supplies
Flask, 1 L
Graduated cylinder, 1 L
Spatula
Weigh boats
Beef extract
Peptone
Agar, powder
Test tube rack
Test tubes
Labeling tape, roll
Autoclave
Petri plates

PROCEDURE

1. Wipe down lab bench carefully with Disinfectant to help prevent contamination of your media.
2. Measure approximately 250 ml of distilled water (located in 60°C water bath) in a 1 L graduated cylinder and pour into a 1 L flask.
3. Weigh out 1.5 g beef extract and 2.5 g peptone and add into the flask.
4. Stir over gentle heat from a bunsen burner to dissolve completely.
5. Check the pH of the medium and adjust to pH 7.0, if necessary, using the HCl and/or NaOH.
6. Pour the mixture into the 1 L graduated cylinder and add warm water to the 500 ml mark. Pour back into the flask.
7. Using a 10 ml pipette, dispense 10 ml of the mixture into each test tube. Make 10 tubes and place in a test tube rack.
8. Add 6.0 g of agar to the flask and label it NA.
9. Heat to just boiling for 1-2 minutes while stirring constantly. The agar will not dissolve unless it is boiled; the solution will become completely clear when it has dissolved. Allow agar to cool until there is no danger of you being burned and then dispense into the tubes using a 10 ml pipette. Make ten 10 ml tubes.
10. Close the flask with a Styrofoam plug covered.
11. Autoclave the flask and the tubes for 15 minutes at 121 °C and 15 lb/in2 pressure.
12. After removing the media from the autoclave, allow the broth tubes to cool, and store for later use. Place the flask in the 48°C water bath. Quickly lay the tubes of NA on the slant racks on the center table so that the medium forms a long slant and a short butt, and allow them to cool and solidify. Do not allow the agar to reach the top of the tube. Allow them to cool completely before returning to the rack. Store for later use.
13. Lay your petri dishes on the bench. The cover should be on top. Light your bunsen burner, then remove the NA flask from the water bath. Carefully wipe the bottom dry to prevent the dripping water from contaminating the plates.
14. Remove the tapes and cotton plug from the flask. Carefully flame the neck of the flask, open the plate cover about half way and fill the plate about 1/2 full.
15. Flame the neck of the flask between each plate.

16. Allow plates to solidify completely, which will take 15 minutes. Then invert, label and incubate at 37 °C overnight to dry off excess moisture and check for contamination.
17. Clean all glassware and leave on paper towels beside sink.