Media prepration and sterilization- 6th Sem(M)

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Introduction :

- Culture media are available commercially as powder; they require only the addition of water. Nutrient medium is a general purpose preparation for culturing microorganisms which are not nutritionally fastidious.

- The media contains:

- 1.5 g/L “Lab-lemco” powder (a beef extract)
  1.5 g/L yeast extract
  5.0 g/L peptone (a nitrogen source)
  5.0 g/L sodium chloride
  15.0 g/L agar powder

Autoclaving

- Autoclaving is a process that use moist heat and pressure so that all parts of the material to be sterilized reach 121 degree celcius for 15 minutes. An autoclave is, in essence, a large pressure cooker; a chamber which may be sealed off against surrounding air.
- Materials for sterilization are placed in the chamber, the door is sealed, and pressurized steam is forced into the chamber. The incoming steam displaces cooler air through an exhaust valve; this valve closes when the cell cooler air has been vented.
- Steam is continually forced into the chamber until the pressure reaches 103 kPa above atmospheric pressure; at sea level, this pushes the temperature in the chamber to 121 degree celcius. The high pressure prevents solutions from boiling over at this temperature. Larger volumes require longer than 15 minutes to heat up to 121 degree celcius throughout. After sterilization, the steam pressure is slowly decreased to atmospheric pressure. The sterilized objects can then be removed.
- **Objective:**
- To prepare sterile nutrient agar for culturing microorganisms.
- **Material and reagents:**
- Commercial nutrient agar,Balance,Distilled water,Scott bottles,Measuring cylinder Beaker,Forcep,Universal bottles
- **Procedure**
- 1. Appropriate amount of broth (with agar) powder is weighed into Scott bottles and dissolve
- 2. The bottles are loosely recap and set aside for sterilization
- 3. All the media are sterilized at 121 degree celcius for 15 minutes
- 4. After autoclaving, the media is removed. The broth preparation is allowed to cool and the cap of each bottle is tightened.

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Discussion:

- There are several precaution steps we need to take when handling the experiment.
- **1) Balance**
- The appropriate amount of broth powder and agar powder is weighed using electronic analytical balance which has the precision of one hundredth of a gram, ±0.01 or one ten-thousandth of a gram, ±0.0001 g.
- The proper receiver for the material must be selected. The receiver's weight plus the weight to be measured must not exceed the maximum load for the balance. The size and shape of the receiver should permit it to fit into the space and on the balance pan without interfering with any operation. It is important that the receiver is clean and in dry condition. Common receivers are weighing bottles,weighing funnels,flasks,and weighing paper. The correct receiver depends upon the quantity and type of material (liquid,solid,or powder)to be weighed.
- Make sure the surrounding of the pan and the pan of the balance is clean. Place the receiver on the center of the pan of the balance and close the balance door. Then, press the appropriate tare key on the balance to set the signal from the strain gauge to zero so that the weight of the receiver is no longer indicated. Carefully add the powdered material using a spatula until the desired amount is added. Handle with care to avoid spilling.
- If solids are spilled, remove the receiver and sweep out all of the spilled material from the balance using a brush.The spilled material must be properly disposed.
- To be effective the autoclave must reach and maintain a temperature of 121-123 degree celcius for at least 30 minutes. This is achieved by using saturated steam under at least 15 psi of pressure.

- **2) Autoclaving process**
- Check the drain screen at the bottom of the chamber before using the autoclave.
- Clean out any debris for efficient heat transfer as steam must flush out of the autoclave chamber. If the drain screen is blocked with debris, a layer of air may form at the bottom of the autoclave and prevent proper operation.
- Make sure that the water level is higher than the material in the autoclave
- Make sure the water level should between range of low and high. If there are too low water level, water should be added
  in.
- Make sure the cap of the Scott bottles must not too tight to prevent breakage off the Scott bottles.
- Make sure the cap of the Scott bottles must not too loose to prevent the outflow of media inside the Scott bottles.

- Autoclave doors must be firmly locked into place before running the autoclave.
- Do not stack or store combustible material next to an autoclave (cardboard, plastic, volatile or flammable liquids).
- Always use heat resistant gloves when removing materials after sterilization.
- Avoid touching the inner chamber surfaces after sterilization.
- **3) Agar**
- There are a few types of general nutrient agar plates.
- Luria Bertani (LB) agar is a common nutrient agar for the general routine growth of bacteria and is not preferentially suited toward a particular microbe type.
- Miller's LB agar is a variety of LB containing different proportions of the same components.
- Trypticase Soy agar (TSA) is another general purpose medium made with casein and soybean meal and is used as initial growth medium to observe bacterial morphology or increase bacterial growth for analysis or storage.
- Phenylethyl alcohol agar (PEA) is selective for species of Staphylococcus and inhibits Gram-negative bacteria.
- Brain Heart Infusion (BHI) agar is a general purpose medium suitable for the cultivation of a wide variety of organism types, including bacteria, yeasts and moulds. The BHI agar derives its nutrients from the brain heart infusion, peptone and dextrose components. The peptones and infusion are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Dextrose is the carbohydrate source that microorganisms utilize by fermentation action. The medium is buffered through the use of disodium phosphate

- **Other precautions:**
- When preparing commercial media, we must read the label and instruction on the container before use.
- In the progress of experiment, use distilled water to clean all the apparatus.
- Measuring cylinder is used to measure the volume of distilled water required accurately.
- Stir the mixture continuously to ensure that the nutrient powder dissolves completely.
- **Conclusion:**
- Different types of agar are needed for the cultivation of different types of microorganisms. Agar of the same composition with the commercial agar can be made by following the correct procedures. Preparation and sterilization of culture media should be done with great care to avoid contamination of unwanted microorganisms. We had learnt the preparation and sterilization of culture media via autoclaving process and the precaution steps that we need to take into consideration when handling this experiment.

Lecture 9: Microbial Growth Kinetics

Introduction-Studying growth of a microorganism is the basis of biotechnological exploitation of microflora for production of desired product. Optimization of growth of microorganism in a particular media is desirable due to economical and availability of particular growth constituent in a region. Despite this, some microorganisms have specific requirement and they grow in a particular growth media. Common media for growth of different microorganism, yeast and animal cells is discussed in future lecture. In today's lecture we will discuss bacterial cell division, methods of measuring growth, different phase in bacterial growth and growth kinectics.

Modes of Bacterial Cell Division-

1. Binary division-binary division is the most common mode of cell division in bacteria (Figure 9.1). In this mode of cell division, a single bacteria cell grows transversely with the synthesis of chromosomal DNA. A transverse septum appears in the middle of the cell body that divides the bacterial cell into the two with a distribution of chromosomal DNA, ribosome and other cellular machinery.

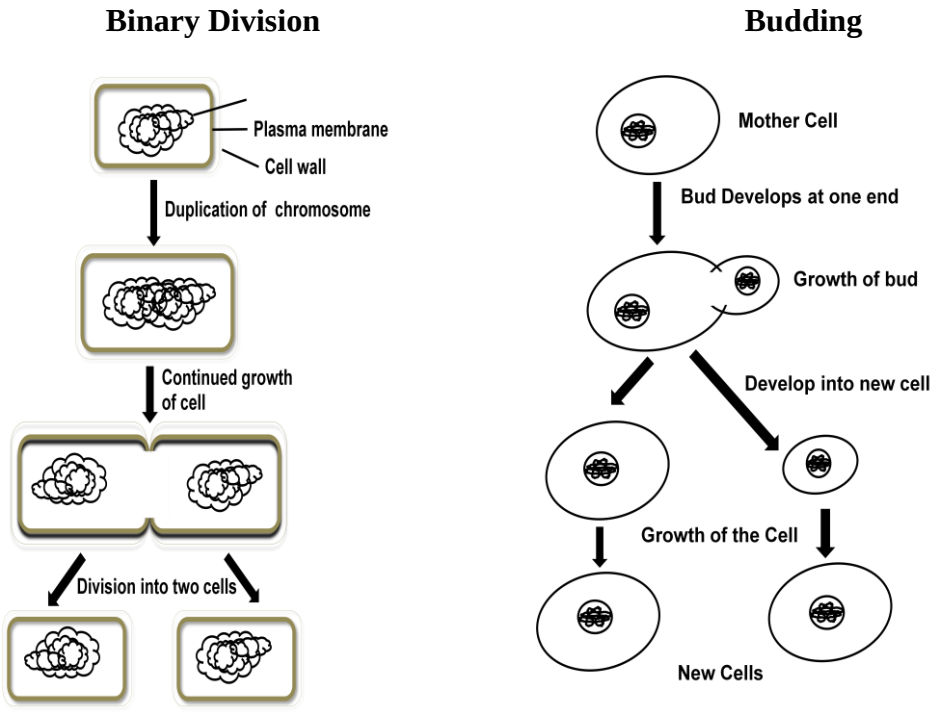

Figure 9.1: Different modes of cell division in bacteria.

2. Budding-In this mode of cell division, chromosomal DNA divides to form two copies. Sister chromosomal DNA moves to the one side of the cell and this portion of the cells protrude from main body to form bud. Eventually bud grows in size and get separated from main cell to develop a new cell.

3. Fragmentation-This mode of asexual division is more common in filamentous bacteria. In this mode, filament of the growing cell gets fragmented into small bacillary or coccoid cells, these cellular fragments eventually develop into new cell.

Measuring Bacterial growth- A number of methds have been developed to measure bacterial growth in liquid media and in solid support media. A few are discussed below:

Microscopic count-bacterial cells can be counted easily on a "petroff-hausser counting chamber" (Figure 9.2). The chamber has a ruling to make square (1/400 mm^2) of equivalent volume. A glass slide is placed (~1/50mm height) to make a chamber filled with bacterial cell suspension. Volume of each chamber is 1/20,000 mm^3. This chamber can be used to observe bacteria with phase contrast microscope. For example, if each chamber has 8 bacteria then there are 8x20,000,000 or $1.6x10^8$ bacteria/ml. A very high or low concentration of bacterial sample can not be counted accurately.

Plate count method-In this method, a defined amount of bacterial culture suspension is introduced onto solid support media to grow and give colonies. If number of colonies on solid media is too high, then serial dilution of original stock can be plated on solid media and number of colony can be counted with a colony counter. A manual colony counter has lamp at the bottom, a grid to divide the bacterial culture plate and a magnifying glass to visualize and count single colony. A plate with colony count of 30-300 can be used to determine the number of bacteria present in original stock.

Number of bacteria per ml= Number of colonies counted on plate X dilution of sample

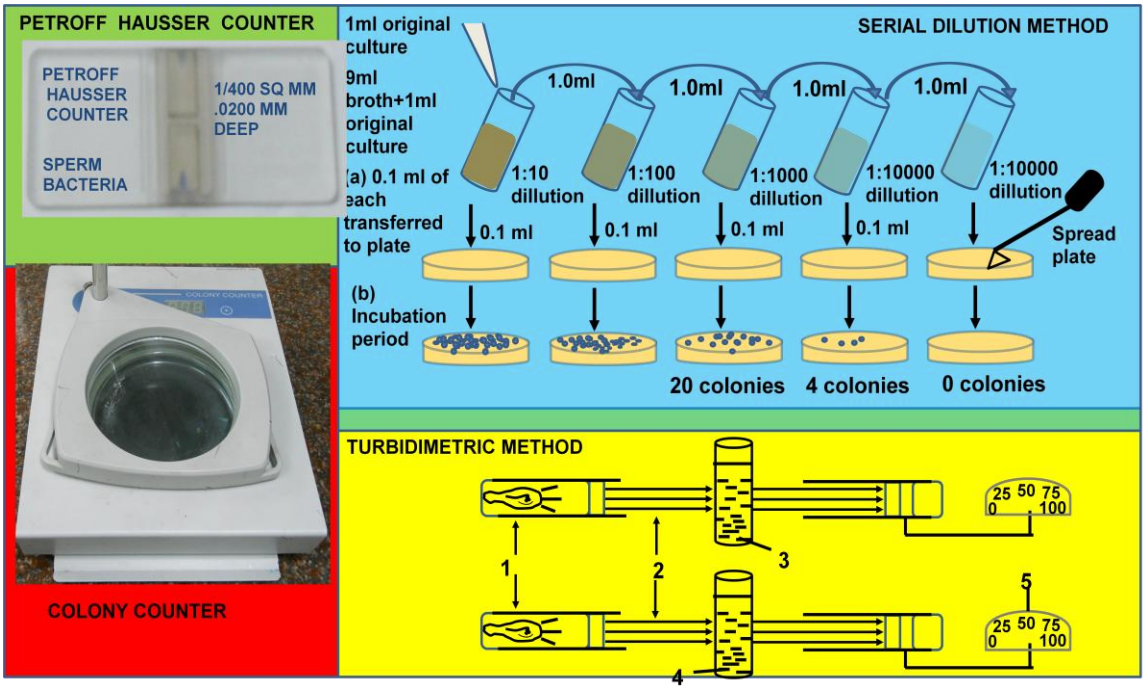

Figure 9.2: Different methods of bacterial counting.

Turbidimetric methods-This method is based on light scattering principles of particulate matter such as bacteria. A bacteria cell suspension is placed in test cuvette and corresponding media in reference cuvette. The optical density or absorbance of the bacterial suspension is used to measure the number of bacteria number. This method can not distinguish between live or dead bacteria as both form contribute to the turbidity.

Nitrogen content and Dry weight- A bacterial cell mass can be measured by direct measuremenof dry weight of culture or nitrogen content.

Growth cycle of bacteria- As discussed earlier, the most common method of bacteria division is binary fission and by this method, one bacteria cell gives two daughter cells. The time a bacteria takes to complete one division is called as generation time and it depends on bacteria species and media properties.

Hence, if we start from one bacteria, it divides after every generation time as follows-

Generation (n)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**n**
No. of bacteria	**1**	**2**	**4**	**8**	**16**	**32**	**64**	
No. of bacteria	**1**	**2^1**	**2^2**	**2^3**	**2^4**	**2^5**	**2^6**	**2^n**

Hence, After n generations, no of bacteria will be

$N=1 \times 2^n$……. Eq 9.1

But assume if number of bacteria at time 0 is N_o, then

$N=No \times 2^n$……..Eq 9.2

$\text{Log } N=\text{Log } N_o+n \log_{10} 2$……...Eq 9.3

$n= 3.3 (\text{Log}_{10} N-\text{Log}_{10} N_o)$…….Eq 9.4

Eq 9.2 can be used to determine number of bacteria, if initial number of bacteria and number of generation is known where as Eq 9.4 can directly been used to calculate number of generations.

Bacterial growth in a liquid media is given in Figure 9.3 and it has 4 distinct phases:

1. Lag Phase-The single cell inoculation into the liquid media doesn't start dividing as per its generation time. During this phase bacteria gets adjusted to the new media and grow in size instead of dividing into daughter cells. In this phase, bacteria synthesize the most crucial enzymes or co-enzyme present in traces and required for optimal growth and multiplication. In addition, cell is metabolically active and be busy in synthesizing large amount of protoplasm. At the end of this phase, each bacterial cell divides and enter into the next phase of active multiplication.

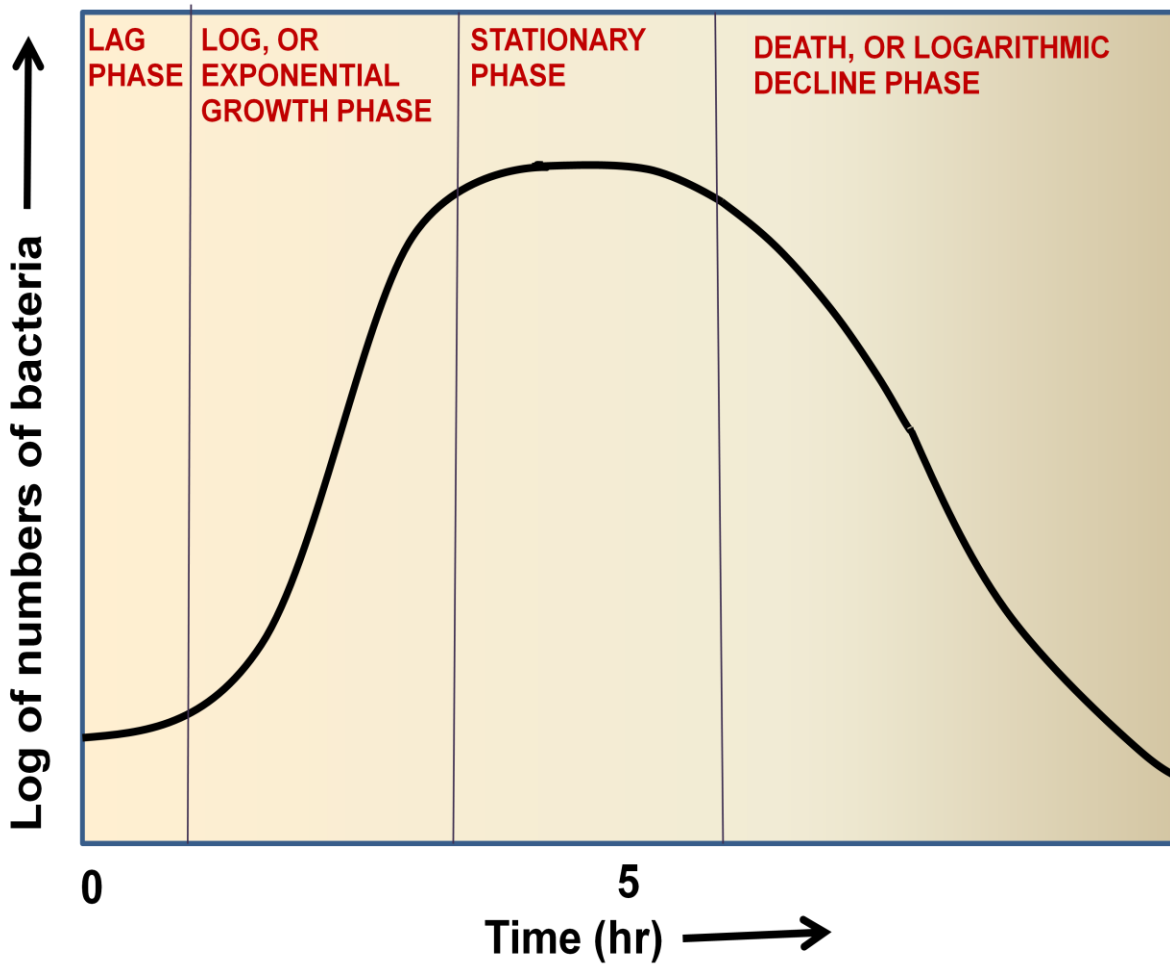

Figure 9.3: kinetics of Growth of bacteria.

2. Log Phase-In this phase, bacterial cell population is involved in active division and whole cell population is more or less homogenous in terms of chemical composition, physiology and metabolic activity. A plot of number of cell (in log scale) against time gives straight line. The growth of bacterial cell population is increasing at a constant rate and continues until substrate concentration is not limiting.

3. Stationary Phase-Once substrate is limiting, the logarithmic phase of growth begins to decline gradually with a constant number of cells to give a staright line. The population remains constant because number of divisions are equal to the number of death events. As substrate is limiting, death of old cell provides enough nutrient for remaining cells to grow and multiply to maintain the constant number.

4. Death Phase-When substrate is not sufficient from dying cells, death rate of bacteria superseed rate of growth and as a result number of bacteria declines sharply.

Quiz

Q1: In a culture of bacteria, a sample is taken at 10:00AM and contains 1000 cells per ml. A second sample at 8:00PM has 10,000 cells per ml. what is the generation time ?

Q2: A scientist wants 20,000/ml *E.Coli* cells for his molecular biology experiment, he has inoculated 1000 cells at around 8:00PM. The generation time of *E.coli* is 20 mins. Please tell when he should harvest the culture?

Q3: What is the significance of lag phase in bacterial growth curve?

Q4: Which mode of cell division is most common in bacteria?

Q5: The unit of measurement in the turbidimetric method of measuring bacteria growth ?