

BACTERIOLOGICAL MEASUREMENT AND ITS PHYSICAL CHARACTERISTICS

PRESENTED BY :

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T14EE003

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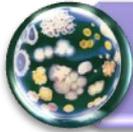
POWER AND ENERGY SYSTEMS

NIT MEGHALAYA

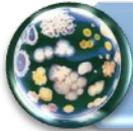
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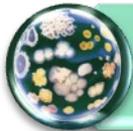
Introduction



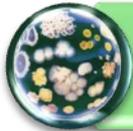
Binary fission



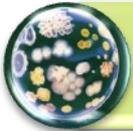
Other unusual forms



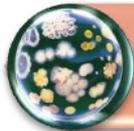
Bacterial growth curve



Measurement of bacterial growth



Conclusion



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Introduction

Bacterial growth is the asexual reproduction or division of one piece of bacteria into two daughter cells in a process **called binary fission**. Providing no mutational event occurs the resulting daughter cells are genetically identical to the original cell. Hence, "local doubling" of the bacterial population occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods.

Binary fission

Most bacteria rely on **binary fission** for propagation. Conceptually this is a simple process; a cell just needs to grow to twice its starting size and then split in two. But, to remain viable and competitive, a bacterium must divide at the right time, in the right place, and must provide each offspring with a complete copy of its essential genetic material.

BINARY FISSION:

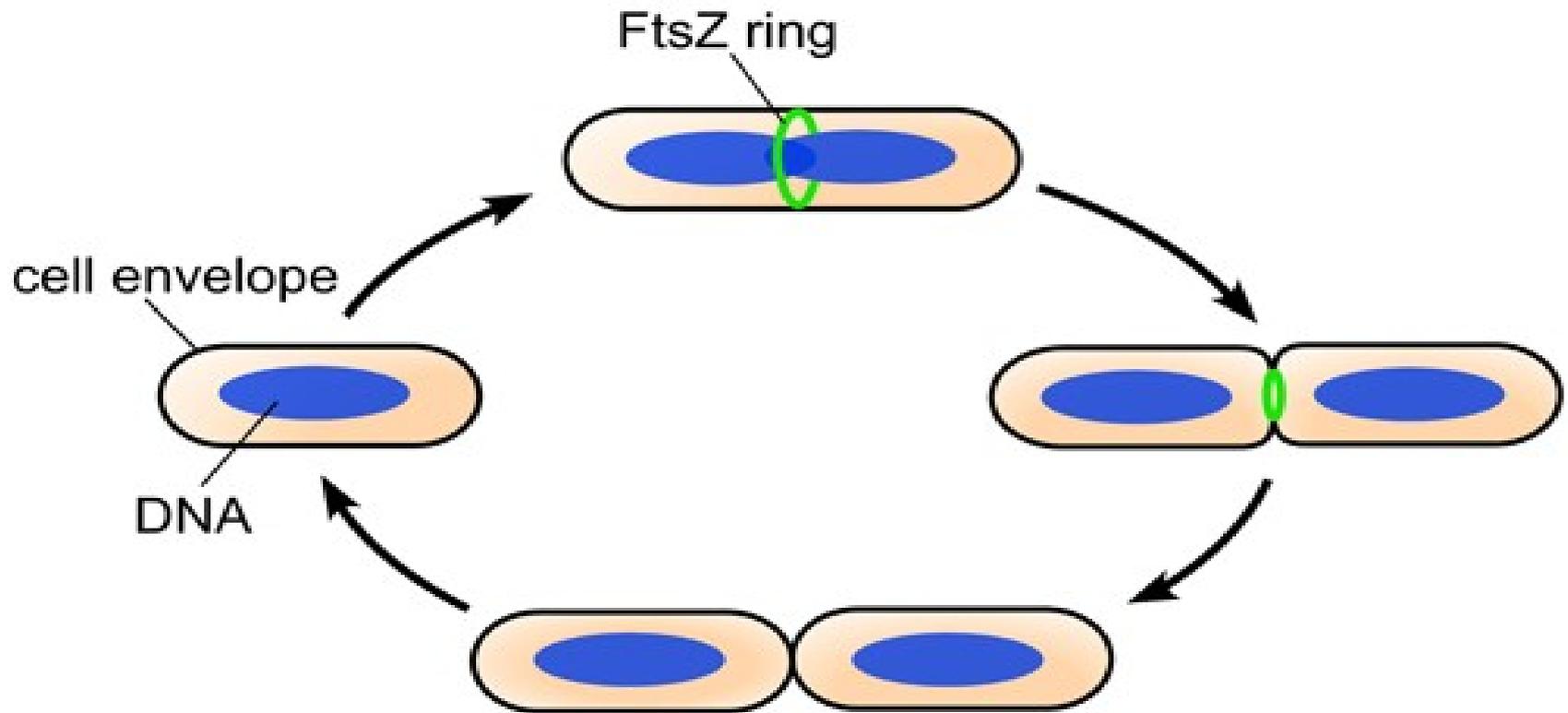


Fig1.Binary fission

Other Unusual forms :

- **Baeocyte production in the cyanobacterium *Stanieria***
- It starts out as a small, spherical cell approximately 1 to 2 μm in diameter. This cell is referred to as a baeocyte (which literally means "small cell"). The baeocyte begins to grow, eventually forming a vegetative cell up to 30 μm in diameter. As it grows, the cellular DNA is replicated over and over, and the cell produces a thick extracellular matrix. The vegetative cell eventually transitions into a reproductive phase where it undergoes a rapid succession of cytoplasmic fissions to produce dozens or even hundreds of baeocytes. The extracellular matrix eventually tears open, releasing the baeocytes. Other members of the Pleurocapsales (an Order of Cyanobacteria) use unusual patterns of division in their reproduction .

THE *STANIERIA* LIFE CYCLE:

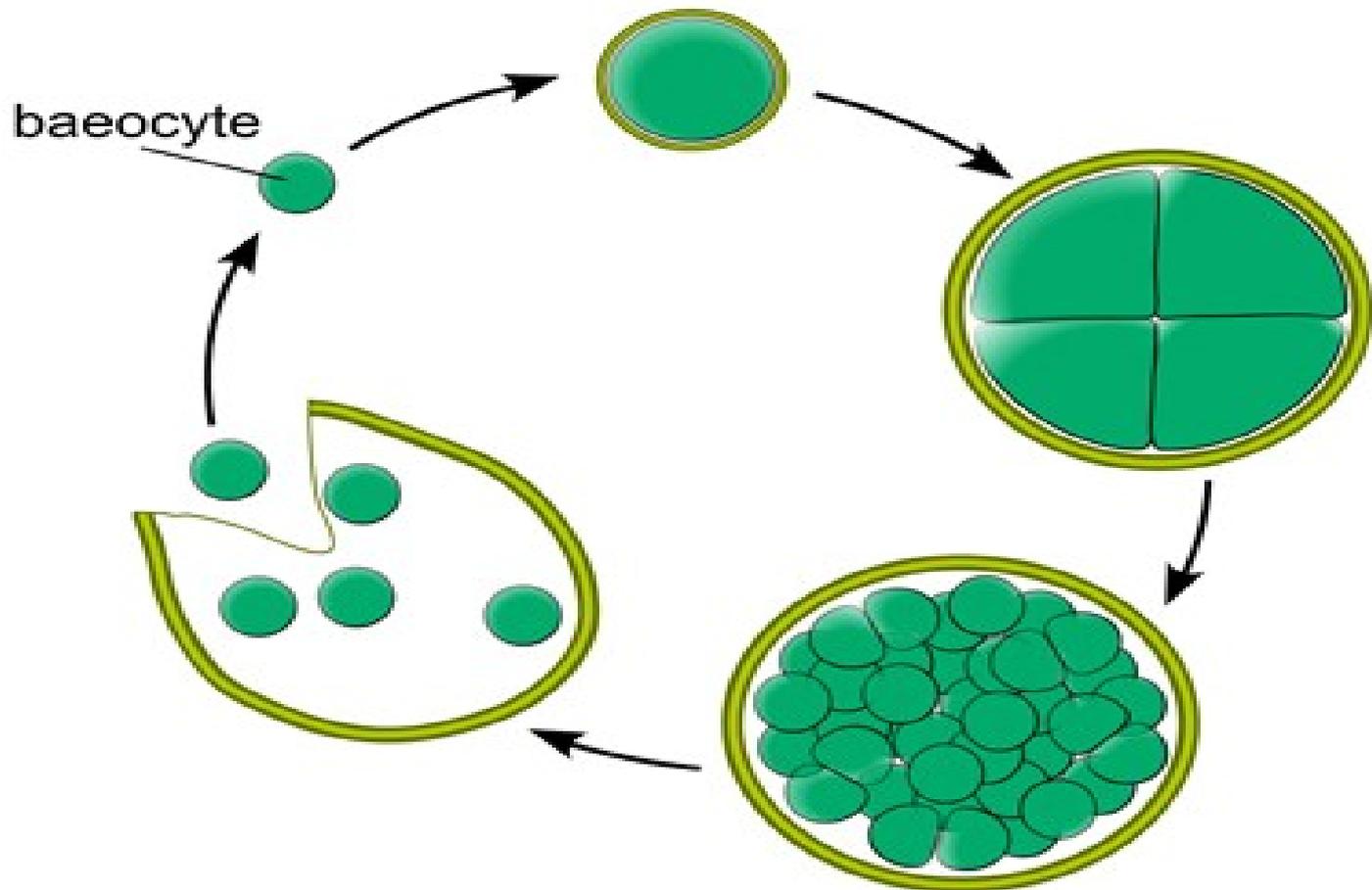


Fig 2. Baeocyte production

- **Budding in bacteria**
- Budding has been observed in some members of the Planctomycetes, Cyanobacteria, Firmicutes (a.k.a. the Low G+C Gram-Positive Bacteria) and the prosthecate Proteobacteria. Although budding has been extensively studied in the eukaryotic yeast *Saccharomyces cerevisiae*, the molecular mechanisms of bud formation in bacteria are not known.

**BUDDING IN A
PLANCTOMYCES
SPECIES:**

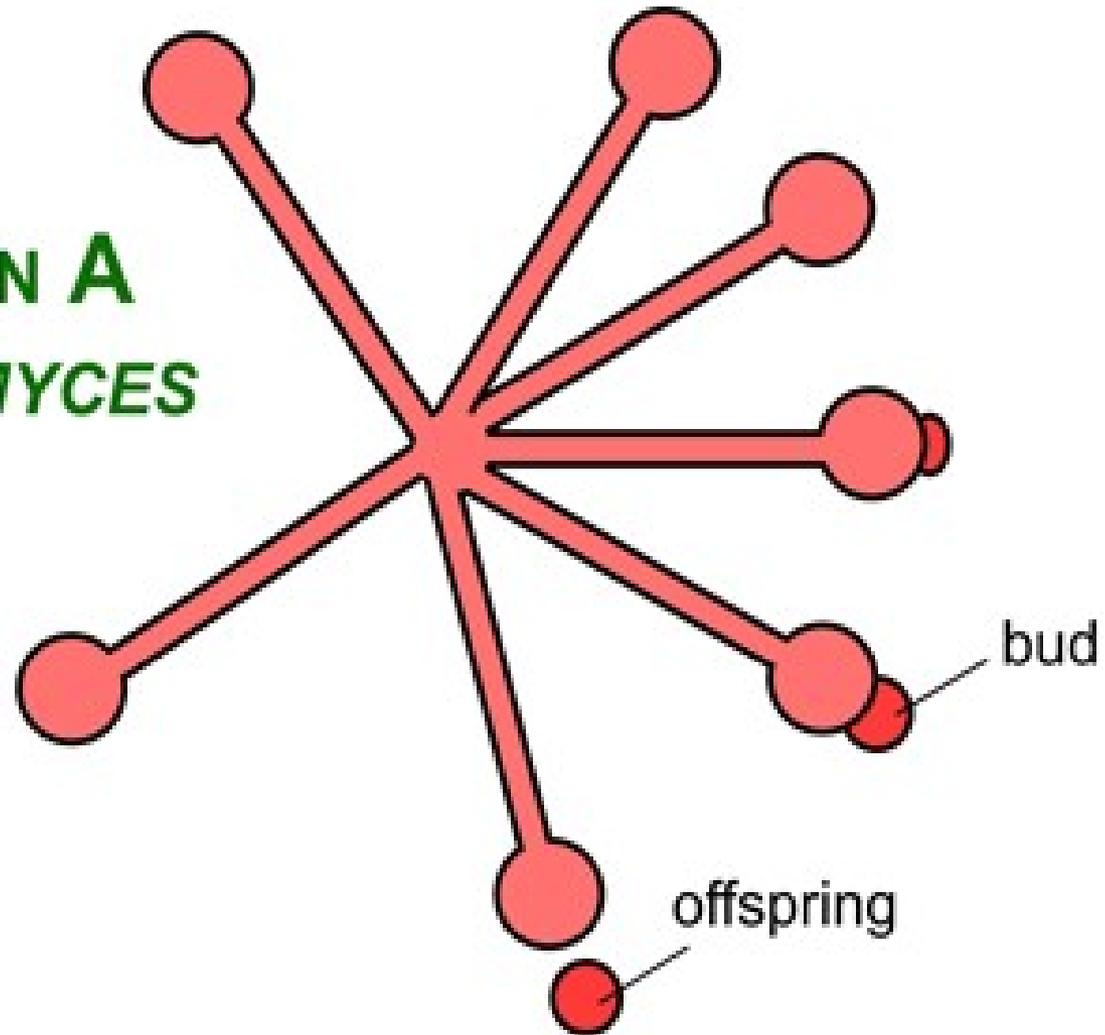


Fig 3. Budding

- Intracellular offspring production by some Firmicutes
- *Epulopiscium* spp., *Metabacterium polyspora* and the Segmented Filamentous Bacteria (SFB) form multiple intracellular offspring. For some of these bacteria, this process appears to be the only way to reproduce. Intracellular offspring development in these bacteria shares characteristics with endospore formation in *Bacillus subtilis*.

THE *EPULOPISCIUM*
LIFE CYCLE:

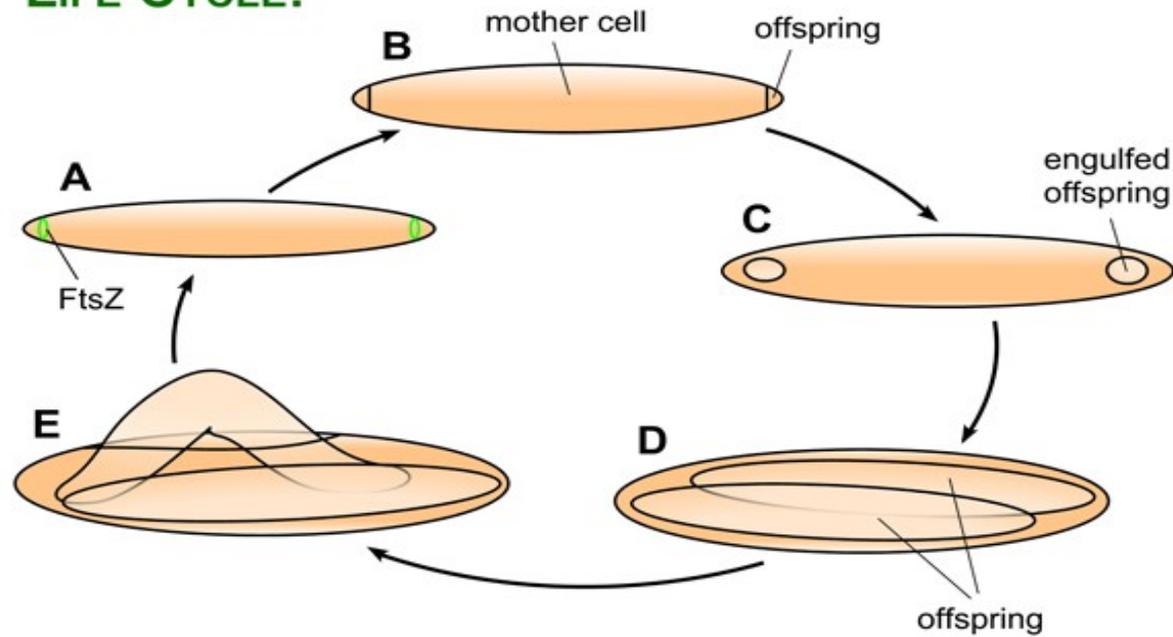


Fig 4. Intracellular offspring production

Bacterial growth curve

In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc. or $2^0, 2^1, 2^2, 2^3, \dots, 2^n$ (where n = the number of generations). This is called exponential growth. In reality, exponential growth is only part of the bacterial life cycle, and not representative of the normal pattern of growth of bacteria in Nature.

When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of time, plotting the data will yield a typical bacterial growth curve.

STAGES OF BACTERIAL GROWTH

- **Four characteristic phases of the growth cycle are recognized as :**
- Lag phase
- Exponential(log) phase
- Stationary phase
- Death phase

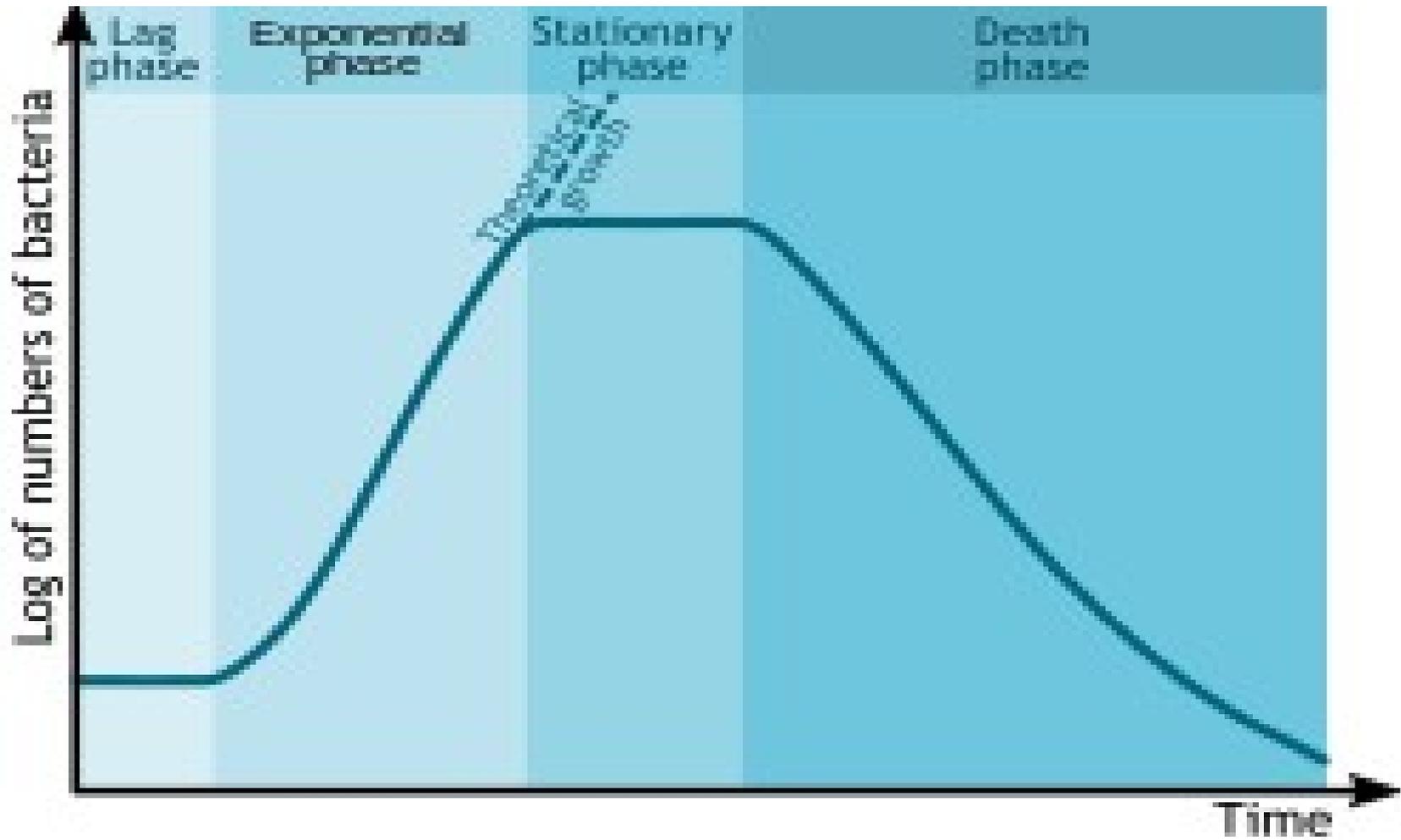


Fig 5. Bacterial growth curve

BACTERIAL GROWTH MEASUREMENT

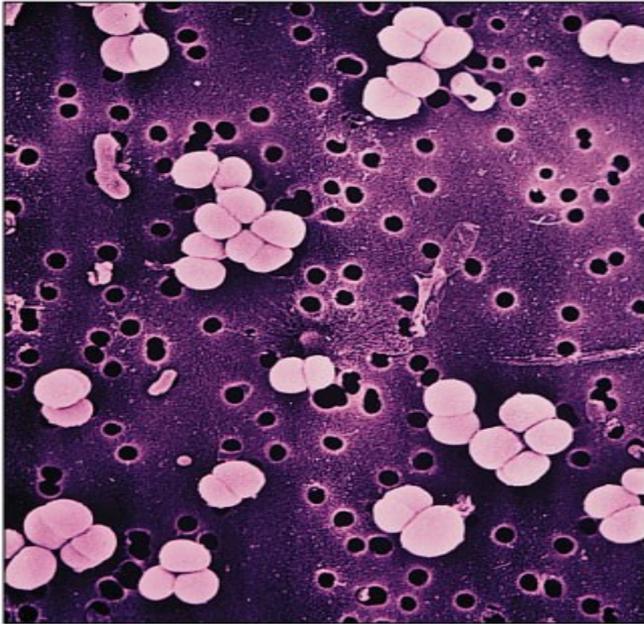
For unicellular organisms such as the bacteria, growth can be measured in terms of two different parameters:

i) changes in cell mass

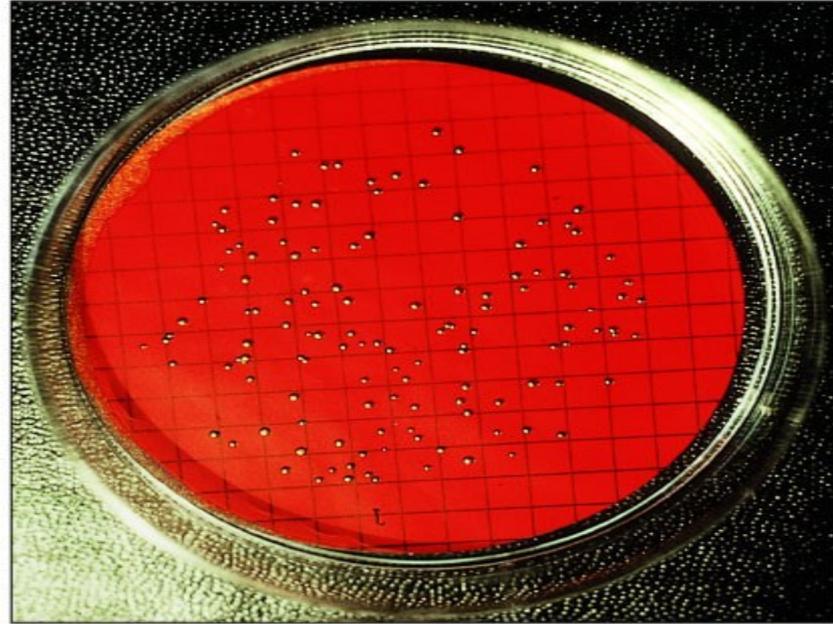
ii) changes in cell numbers.

- **Methods for Measurement of Cell Mass**

- Methods for measurement of the cell mass involve **both direct** and **indirect** techniques.
 1. **Direct physical measurement** of dry weight, wet weight, or volume of cells after centrifugation.
 2. **Direct chemical measurement** of some chemical component of the cells such as total N, total protein, or total DNA content.
 3. **Indirect measurement** of chemical activity such as rate of O₂ production or consumption, CO₂ production or consumption, etc.
 4. **Turbidity measurements** employ a variety of instruments to determine the amount of light scattered by a suspension of cells. Particulate objects such as bacteria scatter light in proportion to their numbers. The turbidity or optical density of a suspension of cells is directly related to cell mass or cell number, after construction and calibration of a standard curve. The method is simple and nondestructive, but the sensitivity is limited to about 10⁷ cells per ml for most bacteria.



(a) The bacteria in 100 ml of water were sieved out onto the surface of a membrane filter.

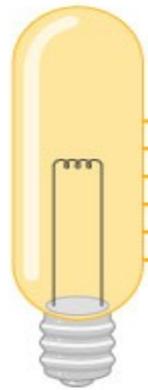


(b) Such a filter as shown in photo (a) with the bacteria much more widely spaced, was placed on a pad saturated with liquid Endo medium, which is selective for gram-negative bacteria. The individual bacteria grew into visible colonies. One hundred twenty-four colonies are visible, so we would record 124 bacteria per 100 ml of water sample.

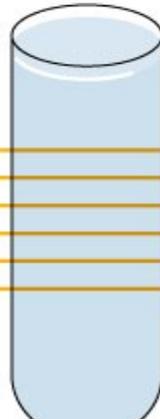
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Fig 6. Filtration

Light source

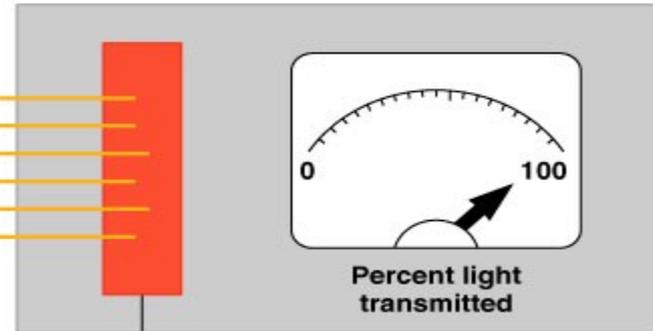


Direct light

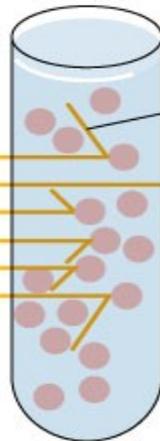
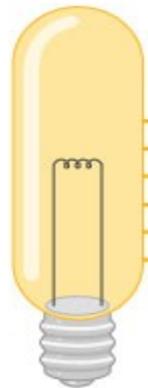


Blank

Spectrophotometer

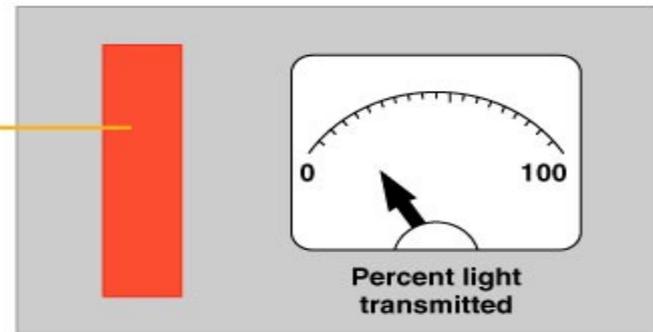


Light-sensitive detector



Bacterial suspension

Scattered light that does not reach detector



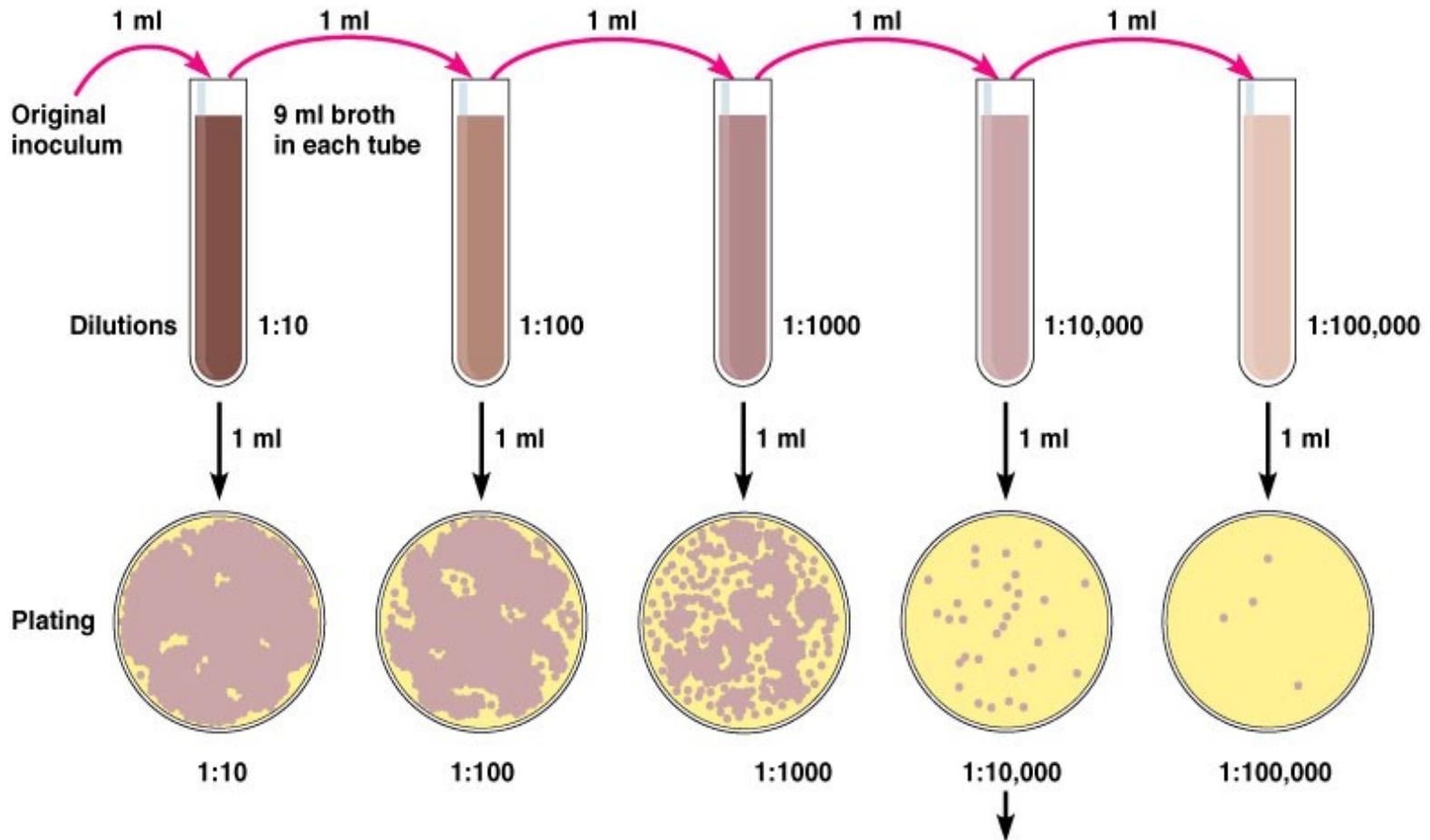
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Fig 7. Turbidity measurement

- **Methods for Measurement of Cell Numbers**
- **1. Direct microscopic counts** are possible using special slides known as counting chambers. Dead cells cannot be distinguished from living ones. Only dense suspensions can be counted ($>10^7$ cells per ml), but samples can be concentrated by centrifugation or filtration to increase sensitivity.
- A variation of the direct microscopic count has been used to observe and measure growth of bacteria in natural environments. In order to detect and prove that thermophilic bacteria were growing in boiling hot springs, T.D. Brock immersed microscope slides in the springs and withdrew them periodically for microscopic observation. The bacteria in the boiling water attached to the glass slides naturally and grew as microcolonies on the surface.

2. Electronic counting chambers count numbers and measure size distribution of cells. For cells the size of bacteria the suspending medium must be very clean. Such electronic devices are more often used to count eucaryotic cells such as blood cells.

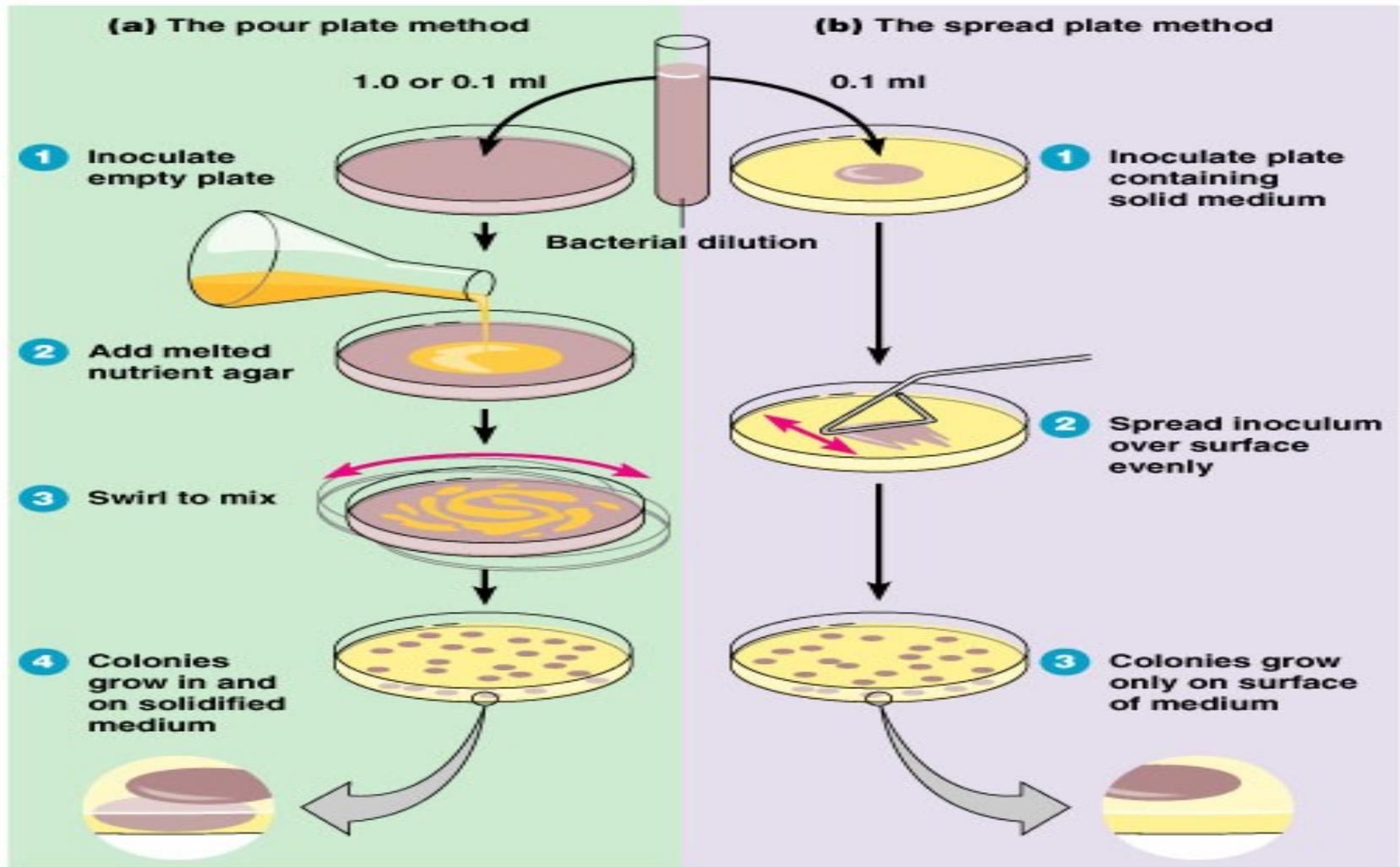
3. Indirect viable cell counts, also called plate counts, involve plating out (spreading) a sample of a culture on a nutrient agar surface. Each colony that can be counted is called a colony forming unit (cfu) and the number of cfu's is related to the viable number of bacteria in the sample.



Calculation: Number of colonies on plate \times reciprocal of dilution of sample = number of bacteria/ml
 (For example, if 32 colonies are on a plate of $1/10,000$ dilution, then the count is $32 \times 10,000 = 320,000/\text{ml}$ in sample.)

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Fig 8. Direct measurement of microbial growth



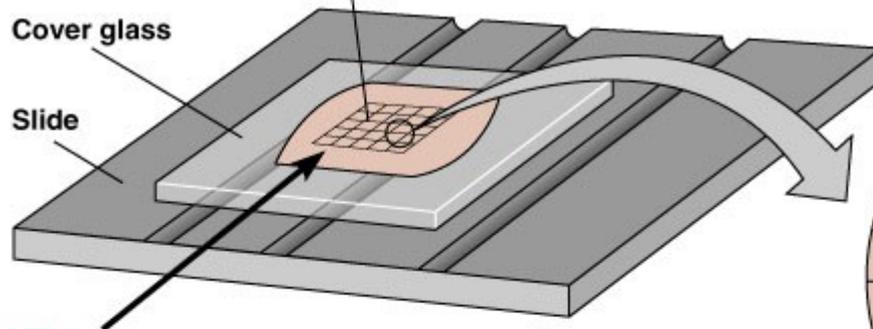
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Fig 9. Plate method

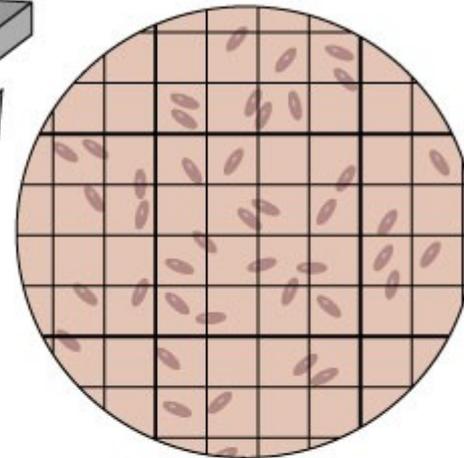
Grid with 25 large squares

Cover glass

Slide



- 1** Bacterial suspension is added here and fills the shallow volume over the squares by capillary action.



Bacterial suspension

Cover glass

Slide



- 2** Cross section of a cell counter. The depth under the cover glass is known, and the area of the squares is known, so the volume of the bacterial suspension over the squares can be calculated (depth \times area).

- 3** Microscopic count: All cells in several large squares are counted, and the numbers are averaged. The large square shown here has 14 bacterial cells.

- 4** The volume of fluid over the large square is $1/1,250,000$ of a milliliter. If it contains 14 cells, as shown here, then there are 14 times 1,250,000 (17,500,000) cells in a milliliter.

Advantages of the technique are its sensitivity (theoretically, a single cell can be detected), and it allows for inspection and positive identification of the organism counted.

Disadvantages are (1) only living cells develop colonies that are counted; (2) only dense solutions are needed.

CONCLUSION :

Bacterial cell division is studied in many research laboratories throughout the world. These investigations are uncovering the genetic mechanisms that regulate and drive bacterial cell division. Understanding the mechanics of this process is of great interest because it may allow for the design of new chemicals or novel antibiotics that specifically target and interfere with cell division in bacteria. The concentration of bacteria, viruses and other pathogens in the blood or in other bodily fluids can reveal information about the progress of an infectious disease and about the degree of success with which the immune system is dealing with the infection.

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Thank you!

