ARKAN
ACADEMY

MICROBIOLOGY 1

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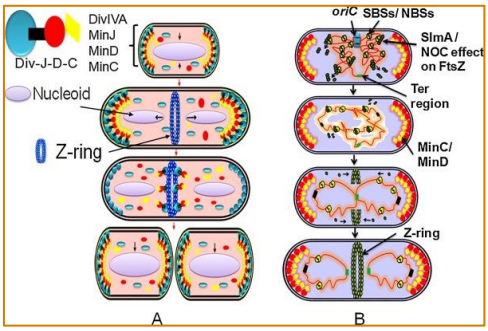
2025

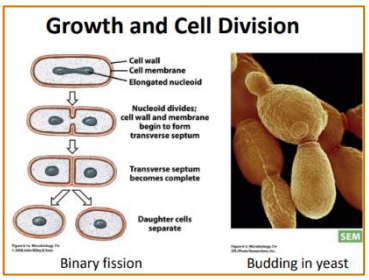
Study smarter, not harder!

Growth and Culturing of Bacteria

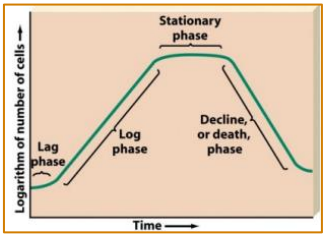
- **Growth and Cell Division**
  - ***Microbial growth*** is defined as the increase in the number of cells, *rather* than in terms of cell size
    - ✓ Nevertheless, the 'mother cell' usually *doubles in size* and duplicates its contents before it *divides* into two 'daughter cells'
  - ***Cell division*** in bacteria usually occurs by binary fission or sometimes by budding
  - ***In binary fission***:
    - ✓ The cell duplicates its components
    - ✓ A transverse septum grows in the middle of the cell
    - ✓ The septum divides it into two independent daughter cells.



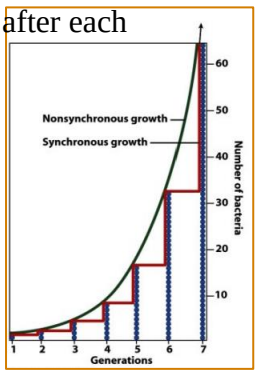
  - ***Continuously dividing cells***:
    - ✓ DNA synthesis is continuous
    - ✓ The bacterial chromosome replicates shortly before the cell divides.
    - ✓ The chromosome is attached to the cell membrane
    - ✓ The cell membrane grows and separates the replicated chromosomes
  - In some species, *incomplete separation* of cells occurs
    - ✓ which results in the formation of special cell arrangements, i.e. tetrads, sarcinae, sterptococci, etc
  - ***Budding in Yeast and Some Bacteria***:
    - ✓ In yeast and a few bacteria cell division occurs by **budding**,
    - ✓ A smaller new cell develops from the surface of an existing cell and then separates from the parent cell
  - Budding ***vs*** binary fission
    - ✓ Both are asexual forms of reproduction where two genetically identical cells 'clones' are produced
    - ✓ *Binary fission*: the parent cell is divided into two equally sized new cells
    - ✓ *Budding*: produces a small new cell in addition to the existing parent cell
      - The new cell is smaller than the parent cell.



- **Phases of Growth**
  - When bacteria are introduced (inoculated) into a fresh nutrient medium, they show **four** major phases of growth:



    1. Lag phase
    2. Log (exponential) phase
    3. Stationary phase
    4. Decline (death) phase
    - ✓ These phases form the standard bacterial growth curve
      1. *Lag phase*
         - Cells don't increase in number, but are metabolically active
         - Metabolic Activity:
           - Cells are *increasing* in size
           - Cells *incorporating* various molecules from the medium
           - Cells *synthesizing* enzymes and producing large quantities of ATP (energy)
         - Duration of Lag Phase:
           - Length of lag phase depends on :
             I. The *characteristics* of the bacterial species
             II. The *conditions* in the growth media (both the old medium and the new one)
         - Some species adapt to the new medium in 1-2hrs, others take several days
      2. *Log* (exponential) *phase*
         - Exponential Growth:
           - Once bacteria are adapted to the new medium, growth (**increase** in number) occurs at exponential (logarithmic rate)
           - This is represented by straight line if plotted on log y-axis
         - In log phase
           - Organisms divide at their most rapid rate
           - This division occurs a regular, genetically determined interval called the **generation time**
         - Generation time
           - for most bacteria is between 20 min to 20 hrs; typically less than 1 hr
           - The population of m.o. doubles in each generation time
         - Nonsynchronous growth'
           - Is a bacterial cells don't all divide exactly together, each cell divides at different times during the generation time.
           - This results in a **smooth curve**.
         - Synchronous growth:
           - Is a hypothetical situation where all cells divide exactly together after each generation time
           - This would result in a **stair-step curve**.



- In a flask or a tube, log phase is limited in time because;
  - As the number of cells increases:
    A. *Nutrients* and *O2* are used up
    B. *Waste* materials accumulate
    C. Living *space* is limited.
  - This will decrease the ability of cells to produce ATP and **growth rate decreases**.
- As the log phase levels off, it is followed by a stationary phase
  - This occurs unless:
    - Fresh *medium* is added or
    - Organisms are *transferred* to another fresh medium
- Maintaining Log Growth:
  - Log bacterial growth can be maintained by using a device called '**chemostat**' which has a growth chamber where:
    - Fresh medium is continuously added (from an attached reservoir) as old medium is withdrawn.

3. Stationary phase

- When cell division decreases to a rate equal to that of cell death, the number of cells remains constant,
- This appears as horizontal straight line on the bacterial growth curve
- Conditions in the Stationary Phase:
  - The medium contains *limited* amount of nutrients
  - The medium may contain *toxic* quantities of waste materials
  - O2 is *limited* to aerobic organisms
  - *Damaging* pH changes may occur in the medium.

4. Decline (death) phase

- The medium becomes *less supportive* of cell division, so cells lose their ability to divide and eventually die.
- The number of *live cells* decreases at a *logarithmic rate*.
- The duration of this phase is *highly variable* similar to the logarithmic phase
- The duration depends on *genetic* characteristics of the organism.
- Some bacteria contain few bacteria that remain *alive* after months or years.

➢ **Growth in colonies**

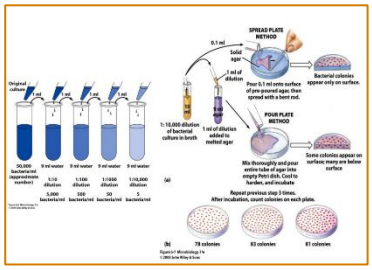
- ✓ When growing on a *solid medium*, a cell divides exponentially forming a small colony containing all the descendants of the original cell.
- ✓ The *colony* grows rapidly at its *edges* whereas cells nearer the centre grow more slowly & begin to die.
- ✓ All phases of growth occur simultaneously in a colony.
- ✓ Each single living bacterial cell will divide to form a colony i.e. each bacterial cell represents a colony forming unit (*CFU*).

- **Measuring Bacterial Growth** – Enumeration of Bacteria

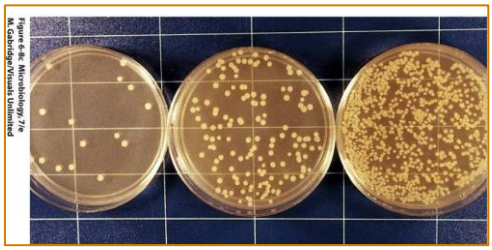
➢ It is measured by estimating the no. of cells that have arisen by binary fission during a growth phase. Expressed as number of viable (living) organism per *unit volume* (i.e. ml)

1. Serial dilution and standard plate count
   - ✓ *Principle: only living* bacterium will divide and form visible colony on agar plate.

- ✓ *Agar plate*: Petri dish containing nutrient medium solidified with agar.
- ✓ *Serial dilution:* series of dilutions e.g. 1/10 → 1/10 → 1/10 etc, then transfer 0.1ml to agar plate. The transfer is done either by
  a. Pour plate method or
  b. Spread plate method

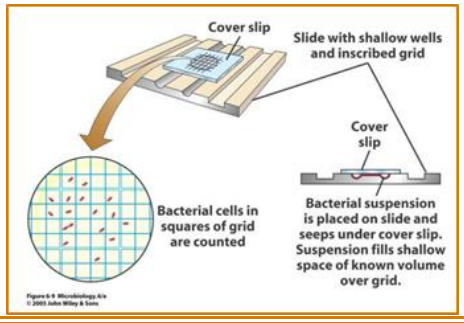


- ✓ Pour plate method: add 1ml diluted culture from serial dilutions to melted nutrient agar, mix, then pour in empty plate → agar cools down → solidified → incubated → colonies develop within medium and on medium surface
  - *Disadvantage*: damage to colonies exposed to heated agar, smaller colonies inside agar compared to those on surface.
- ✓ The spread plate method: 0.1ml sample is placed on the surface of cool solidified agar medium. The sample is spread evenly → incubate → colonies on surface.
- ✓ Countable no. of colonies /plate (30-300 CFU)
  - It is difficult to count more than 300 colonies on one plate whereas less than 30 is not statistically representative



- ✓ The colonies are counted by the aid of **colony counter** (magnifying lens+ special electrical marker).
- ✓ Actual *no. of colonies* = no. of colonies on plate x dilution factor
- ✓ The *concentration* of bacterial cells in the original suspension (culture) is calculated from the number of colonies and is expressed as cfu/ml
- ✓ To *improve accuracy*: shake tubes before sampling & make several plates from each dilution.
- ✓ *Weakness* of the process:
  - Doesn't count the cells that died by the time of plating & does not include m.o. that cannot grow on the utilized growth medium.

2. Direct microscopic count
   - ✓ A known volume of medium is introduced into specially calibrated etched glass slide called **counting chamber**.
   - ✓ Cells are then counted, *under* the *microscope*, in specific areas and their number per unit volume is calculated.
   - ✓ Disadvantages:
     - *Cannot* distinguish between living & dead cells
     - Requires *large* no. of cells
     - The bacterial *suspension* should be homogeneous



3. Most probable number (MPN) method
   - ✓ Used in case:
     a. The sample contains *too few* organisms to give reliable measure by plate count method e.g. food or water purity studies
     b. If m.o. *do not* grow on agar
   - ✓ Concept: as the dilution factor *increases*, a point is reached where some tubes contain only one m.o. while others have none
   - ✓ Procedures:
     a. A *series of dilutions* is made (10ml, 1ml, 0.1ml of a sample is added to growth media), for each volume prepare 5 tubes → incubate → observe for growth
        - *Growth indications*: turbidity, production of gas or change in colour of indicator (e.g. acid production).
     b. Count the *no. of tubes* showing growth then check MPN index or table which shows the count of m.o. in the actual culture at 95% confidence

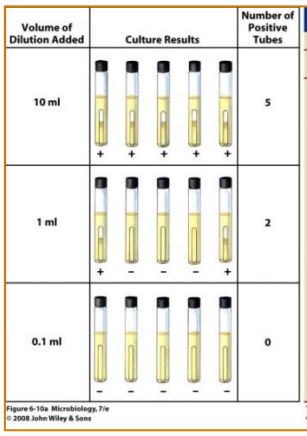


Figure 6-10a Microbiology, 7/e © 2008 John Wiley & Sons

Most Probable Number (/) Index for Combinations of Positive and Negative Results When Five Tubes Are Used per Dilution (Five Each of 10 ml, 1 ml, and 0.1 ml)

Number of Tubes with Positive Results							
10 ml	1 ml	0.1 ml	MPN Index/100 ml	10 ml	1 ml	0.1 ml	MPN Index/100 ml
0	0	0	<2	4	3	1	33
0	0	1	2	4	4	0	34
0	1	0	2	5	0	0	23
0	2	0	4	5	0	1	30
1	0	0	2	5	0	2	40
1	0	1	4	5	1	0	30
1	1	0	4	5	1	1	50
1	1	1	6	5	1	2	60
1	2	0	6	5	2	0	50
2	0	0	4	5	2	1	70
2	0	1	7	5	2	2	90
2	1	0	7	5	3	0	80
2	1	1	9	5	3	1	110
2	2	0	9	5	3	2	140
2	3	0	12	5	3	3	170
3	0	0	8	5	4	0	130
3	0	1	11	5	4	1	170
3	1	0	11	5	4	2	220
3	1	1	14	5	4	3	280
3	2	0	14	5	4	4	350
3	2	1	17	5	5	0	240
4	0	0	13	5	5	1	300
4	0	1	17	5	5	2	500
4	1	0	17	5	5	3	900
4	1	1	21	5	5	4	1600
4	1	2	26	5	5	5	≥1600
4	2	0	22				
4	2	1	26				
4	3	0	27				

Table 6-1 Microbiology, 7/e © 2008 John Wiley & Sons

4. Filtration method
   - ✓ A known *volume of fluid* (i.e. water or air) is *drawn* through a filter with pores smaller than bacteria (e.g. 0.45µm) → filter is placed on solid medium → incubate → count the no. of cells in each plate → calculate the number of cells per unit volume (e.g. 100 ml or 1 L)
5. Other methods
   - ✓ Simple observation:
     - *Gas production*: can be detected by capturing the gas in small inverted tubes
     - *Acid production*: by incorporating pH indicators
     - *Turbidity*
   - ✓ By measurements
     - Turbidity can be measured by *spectrophotometer* or colorimeter: important to monitor rate of growth without disturbing the culture
     - No. of cells can be determined by *dry weight measurement*

- **Factors Affecting Bacterial Growth** – Introduction
  - ➢ Microorganisms exist almost everywhere on earth because they:
    - ✓ Are *small* in size and easily dispersed
    - ✓ Occupy *little* space
    - ✓ Need only *small* quantities of nutrients
    - ✓ Are remarkably *diverse* in their nutritional requirements
    - ✓ Have *great* capacity for adapting to environmental changes

➢ However, the type of organisms and their growth rates are influenced by:

- ✓ **Physical factors** such as pH, temp, O2 concentration, moisture, hydrostatic pressure, osmotic pressure & radiation
- ✓ **Nutritional (biochemical) factors** which include the availability of C, N, S, P, trace elements & vitamins

A) Physical factors

1) pH

- ✓ Microorganisms usually have optimum pH for their growth
- ✓ According to their tolerance to acidity or alkalinity bacteria are *classified as*
  - Acidophiles (pH 0.1-5.4),
  - Neutrophiles (pH 5.4-8),
  - Alkaliphiles (pH 7-11.5).
- ✓ *Most* microorganism (especially pathogens) grow best near *neutral* pH, i.e. neutrophiles
- ✓ Microorganism usually don't grow well at pH values that are one or more unit above or below their optimum pH
  - Because significant changes in pH can lead to *denaturing* enzymes & other proteins and *interfere* with pumping ions at the cell membrane
- ✓ Organisms that tolerate extreme pH have impervious *cell walls* that *protect* their cell membrane from exposure to extreme pH in the medium and keeps the inside of the cell as neutral

2) Temperature

- ✓ Most bacterial species grow over 30 °C, but min & max vary
- ✓ Bacteria are *classified* to psychrophiles, mesophiles and thermophiles.
  - Within these groups bacteria are either *obligate* (must) or *facultative* (able to adjust & tolerate so can also live in other environments)

A. Psychrophiles (cold loving): optimal 15-20 °C, some live at 0C

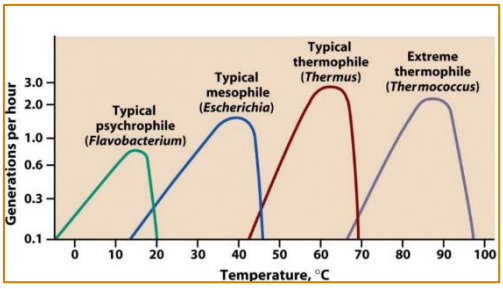
- *Obligate* psychrophiles: cannot grow over 20 °C
- *Facultative* psychrophiles: optimum below 20 °C but also can grow above 20 °C
- Psychrophile usually live in cold water & soil but not in humans

B. Mesophiles: optimum 25-40 °C,

- Includes most *bacteria* & *human* pathogens
- Most human pathogens live best at 37 °C

C. Thermophiles (heat loving): optimum 50-60 °C

- Few tolerate temp up to 110 °C in boiling hot springs
- *Obligate* thermophiles: can grow only at temp above 37 °C
- *Facultative* thermophiles: live at both below & above 37 °C
- *Extreme* thermophiles: require very high temp (80°C to 105°C)



- ✓ What determines the *temperature* at which microorganism survive is the Temperature at which its *enzymes* function
- ✓ Minimum growth Temperature: *lowest* Temperature at which cells divide
- ✓ Maximum growth Temperature: *highest* Temperature at which cells divide
- ✓ Optimum growth Temperature: Temperature at which cells divide *most rapidly* (have shortest generation time)
- ✓ Enzyme activity usually doubles for every 10 °C rise in temp until *high Temperature denatures* the enzyme
- ✓ Temperature controls growth of microorganism and hence spoilage of materials
  - e.g. food refrigeration or freezing
- ✓ Bacteria can survive extremes of cold but not extremes of heat
  - Because enzymes are denatured by extremes of heat but not by extremes of cold

3) Oxygen

- ✓ In general, bacteria can be divided into *aerobes* (require O2 to grow) and *anaerobes* (don't require O2 to grow)
  - Obligate aerobes: must have *free O2* for aerobic respiration (e.g. *Mycobacterium tuberculosis*)
  - Microaerophiles: grow best in presence of *little O2* (e.g. *Helicobacter pylori*). Some are also capnophiles which grow best in presence of *high conc. of CO2* (e.g. *Campylobacter spp*.)
  - Obligate anaerobes: produce energy by anaerobic respiration or *fermentation* and they are killed by free O2 (e.g. *Clostridium spp.* such as *C. botulinum* and *C. tetani*)
  - Facultative anaerobes: ordinarily carry on aerobic metabolism when O2 is present but *shift* to anaerobic metabolism when O2 is absent (e.g. *E. coli* and *Staphylococcus spp*.)
  - Aerotolerant anaerobes: can survive in presence of O2 but do *not use it* in their metabolism (e.g. *Propionibacterium acnes*)

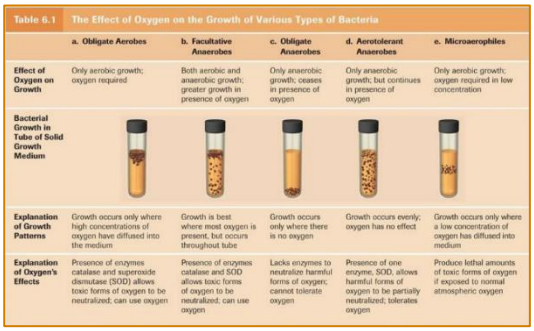


Table 6.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required	Both aerobic and anaerobic growth; greater growth in presence of oxygen	Only anaerobic growth; ceases in presence of oxygen	Only aerobic growth, but continues in presence of oxygen	Only aerobic growth; oxygen required in low concentration
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium	Growth is best where most oxygen is present, but occurs throughout tube	Growth occurs only where there is no oxygen	Growth occurs evenly; oxygen has no effect	Growth occurs only where a low concentration of oxygen has diffused into medium
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen

- ✓ In presence of free oxygen, a toxic form of oxygen called superoxide (O2 -) can be produced in living cells by some oxidative enzymes
  - O2 - is a highly reactive oxygen species (ROS) that can damage the cells causing their death
  - Superoxide can be metabolized into molecular oxygen and hydrogen peroxide (H2O2) by an enzyme called *superoxide dismutase* (SOD)
    - ❖ Although it is weaker than O2 - , *hydrogen peroxide* is still considered as a *ROS* and may cause cellular damage
- ✓ Hydrogen peroxide can also be metabolized into molecular oxygen and water by an enzyme called *catalase* (Cat)
  - Obligate aerobes & most facultative anaerobes *have* both SOD and Cat
  - Obligate anaerobes *have neither* SOD nor Cat
  - Aerotolerant & some facultative anaerobes *have* SOD *but not* Cat

4) Moisture

- ✓ *Presence* of enough *available water* is important for the growth of bacteria
- ✓ Most vegetative bacteria can live only *few hours* without moisture while spores can exist in dormant state in *dry environment*
- ✓ Halophilic bacteria can tolerate lower water levels

5) Hydrostatic pressure

- ✓ Pressure exerted by standing water is in proportion to its depth
  - *10 m* increase in depth *doubles* atmospheric pressure
- ✓ Barophiles: bacteria that *live* at high pressure & *die* at atmospheric pressure
  - Pressure is needed to maintain their enzymes & membrane in the proper 3D- configuration

6) Osmotic pressure

- ✓ Hyperosmotic (hypertonic) environment usually causes the cells to shrink (*plasmolysis*)
- ✓ Hypoosmotic environment causes the cells to gain water & become highly turgid (*distended*)
  - but usually protected from bursting by cell wall
- ✓ *High* osmotic pressure (high salt conc.) water *loss* can inhibit growth or kill the bacterial cells that's why high concentrations of salt or sugar can be used to preserve food
- ✓ Halophiles: Salt-loving organisms which require moderate to high concentration of salt (sodium chloride) to grow well

7) Radiation

- ✓ *Gamma* rays & *UV* usually cause *mutational* changes in DNA & kill the m.o.
- ✓ Some m.o. have *pigments* that *protect* them from radiation while others have enzymes that repair certain DNA damage

B) Nutritional Factors

- ✓ Nutrients needed by microorganism include *carbon*, *nitrogen*, *sulphur*, *phosphorous*, *certain elements & vitamins*
- ✓ Few microorganism are fastidious,
  - Have special nutritional needs that can be difficult to meet in the lab.
  - Some fastidious pathogens *grow* well in human body but are *difficult* to grow on nutrient medium

1) Carbon sources

- ✓ Carbon containing compounds are used by bacteria as:
  - *Energy* source
  - *Building* blocks to synthesize cell components
- ✓ Heterotrophs: *obtain C* & energy from *organic* compounds e.g. glucose
- ✓ Autotrophs: *obtain C* from *CO2*.
  - Derive energy by oxidation of inorganic compounds, or from light (photoautotroph)
- ✓ Photoautotrophs: *convert CO2* to glucose & other organic molecules

2) Nitrogen sources

- ✓ All organisms need N2 to *synthesize* enzymes, other proteins & nucleic acids
- ✓ Some microorganism obtain N2 from *inorganic* sources and few m.o. even obtain *energy* by metabolizing inorganic N-containing compounds
- ✓ Many microorganism reduce nitrate (NO3-) to amino gp. (-NH2) and then use it to make amino acids
- ✓ Some microorganism can synthesize *all* 20 a.a. found in proteins while others must have *few* a.a. provided in the medium
- ✓ Purines (adenine and guanine) & pyrimidines (cytosine, thymine and uracil) are used to build DNA, RNA

3) Sulfur and Phosphorous (S & P)

- ✓ They are important *cell components*
- ✓ Sulfer is obtained from inorganic sulfate salts & from S-containing a.a.
  - Sulfer is used for the *synthesis* of coenzymes, proteins & other cell components
- ✓ Phosphorous is mainly obtained from inorganic phosphate ions (PO4 3-)
  - Used to *synthesize* ATP, phospholipids & nucleic acids

4) Trace elements

- ✓ Microorganisms need a variety of trace elements; (minute amount) of minerals such as copper, iron, zinc and cobalt
- ✓ Trace elements usually serve as *cofactors* in *enzymatic* rxns
- ✓ Cobalt is required by microorganism that *synthesize vit B12*
- ✓ Na, Cl are required by *all* microorganism
- ✓ K, Zn, Mg & Mn are used to *activate* certain *enzymes*
- ✓ Ca is required by *G+ve* bacteria for the *synthesis* of cell walls & by spore-forming bacteria for spore synthesis.

5) Vitamins

- ✓ Are organic substances that an organism require in small amounts & that are used as *coenzymes*
  - e.g. vit B12, folic acid and vit K
- ✓ Many microorganism make their own vits., others need it in nutrients
- ✓ Human *pathogens* often *require* variety of vitamins; thus grow well in host but in the lab they require complex medium that contains all nutrients they obtain from host
- ✓ *Microbes* in intestines *make* some of B vits & vit K

6) Nutritional complexity

- ✓ It is the no. of nutrients an organism must obtain to grow
- ✓ Nutritional *complexity* is determined by the *kind* & *no.* of organism's enzymes
  - *Absence* of certain enzyme means that the organism *cannot synthesize* specific substance & this substance must be obtained from nutrients; i.e. nutritional complexity reflects deficiency in biosynthetic enzymes
  - So microorganism with many enzymes → need *simple* nutrients
  - While microorganism with fewer enzymes → need *complex* nutritional requirements

✓ **Locations of enzymes**

- Most microorganism have endoenzymes: i.e. used within cells for *metabolism* & *transportation* of molecules
- Many bacteria & fungi have exoenzymes: i.e. released through the cell membrane
- Exoenzymes are divided into
  - ❖ Extracellular enzymes: produced by *G+ve* rods & act in the *medium* around the organism
  - ❖ Periplasmic enzymes: produced by *G-ve* & act in *periplasmic* space
- Most exoenzymes are *hydrolases* (they add water as they split molecules);
  - ❖ e.g. carbohydrases, amylases, proteases, lactases, lipases, sucrases

✓ **Adaptation to limited nutrients**

- Microorganisms adapt to nutrient limitation by:
  - ❖ Synthesizing *increased* amounts of enzymes (upregulation) for uptake & metabolism of the limited nutrients
  - ❖ Synthesizing enzymes to use different nutrients
    - e.g. if low glucose → synthesize enzymes to use lactose (if present)
  - ❖ Adjust rate of growth & metabolism to fit the available nutrients
    - i.e. *slow* down & *no* energy is wasted on synthesizing products that are not used

- Sporulation

➢ Sporulation: is the formation of endospores which usually occurs in:

✓ *Bacillus*, *Clostridium* and a few other G+ve genera

➢ Endospores are formed *inside* the bacterial cell & generally produced in *stationary phase* in response to environmental, metabolic & cell signals;

✓ endospores are considered as *insurance* from *extinction*

✓ While fungal spores are produced in large no. & are a form of *reproduction*

➢ When nutrients (C, N) are limited → resistant endospores form inside mother cells

- (very few exceptions, endospores form even when nutrients are available)

➢ Although endospores are *metabolically inactive*, they can survive long periods of drought, resist killing by extreme temp, radiation and toxic chemicals

➢ They *cannot divide*; a parent cell produces only one endospore

✓ i.e. sporulation is protective or survival mechanism not means of reproduction

➢ Endospore formation

✓ **DNA** replicates & forms long compact axial nucleoid

- The 2 chromosomes formed by replication separate & move to different locations in the cell
- Endospore form either in the middle or at one end
- DNA where endospore is forming directs the process

✓ **RNA & proteins** gather around DNA to make the core (living part of endospore).

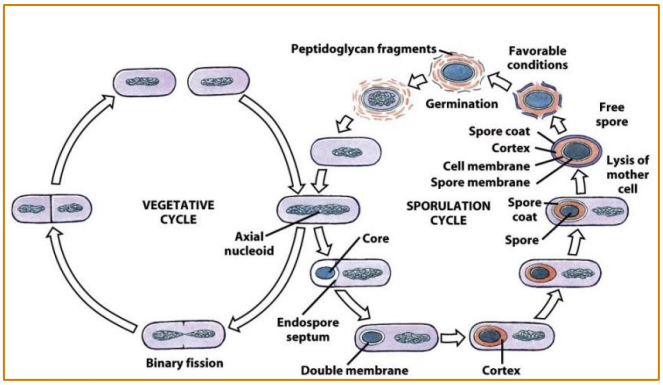
- The core has dipicolinic acid & Ca2+ ions (responsible for heat resistance by stabilizing protein structures)

✓ Endospore **septum** then grows around the core: double cell membrane but lacking a cell wall

✓ Both layers of this membrane release peptidoglycan into the space between them

- This forms a laminated layer called **cortex** which protects the core from osmotic pressure like that resulting from drying

- ✓ Spore coat of **keratin-like protein** is then layered around the cortex (to protect against chemicals)
- ✓ In some endospores, an **exosporium** (a lipid-protein membrane) is formed outside the coat (unknown function)
- ✓ Under lab. conditions, sporulation takes about 7 hours

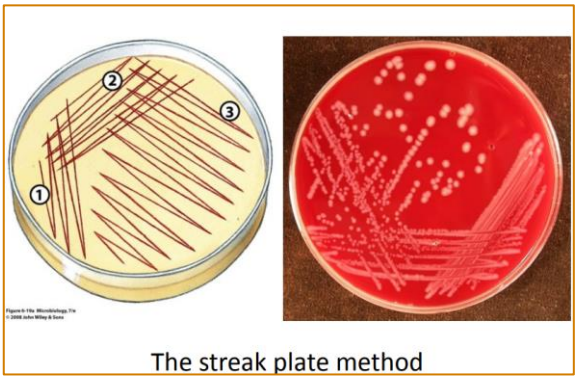


- ➢ If favourable conditions return, germination occurs;
  - ✓ i.e. the spore returns to its *vegetative state* & loses its resistance
- ➢ Germination stages
  - ✓ Germination is initiated by *activation* step which requires some traumatic agent (e.g. low pH, heat) to damage the coat, otherwise germination is slow
  - ✓ 'Germination proper' then *occurs*:
    - *Water penetrates* the damaged coat
    - *Cortex* (peptidoglycan) is *broken* down
    - The *living cell* (inside the core) *takes water* & loses its resistance to heat & staining
  - ✓ Outgrowth finally occurs; proteins & RNA are synthesized, then DNA synthesis occurs, the cell becomes vegetative & undergoes binary fission

- **Culturing Bacteria** – Introduction
  - ➢ To study bacteria, it is important to obtain pure culture
    - ✓ i.e. a culture that contains *only a single* species of organisms
  - ➢ Pure culture is necessary **to study** nutritional needs, growth characteristics, pathogenicity and antimicrobial susceptibility of individual spp.
  - ➢ Pure cultures are usually obtained using '*solid*' growth *media*
  - ➢ Agar is an ideal solidifying agent for microbiological media, why?
    - ✓ It *doesn't melt* below *95°C*, and after melting it solidifies at ~40°C (hysteresis)
    - ✓ *Inert substance*: only very few organisms can digest it

- **Culturing Bacteria** – Methods of Obtaining Pure Cultures
  - ➢ The streak plate method
    - ✓ Procedures:
      - Pick bacteria on *sterile* wire *loop*
      - *Move* the wire along the agar surface depositing streaks of bacteria on surface
      - Loop is flamed
      - *Pick* bacteria from the bacteria deposited on agar & streak new regions on agar
      - *Flame* & *repeat*…

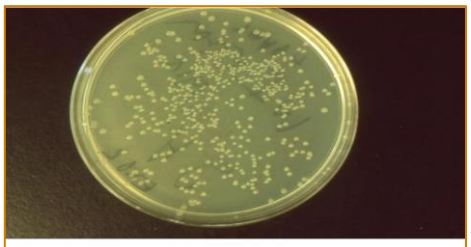
- ✓ Individual organisms are deposited in the region streaked last
  - i.e. *after* incubation, *isolated* colonies usually appear on agar surface in that region
  - *isolated* colonies, that represent an individual m.o., can then be *picked up* and *transferred* to fresh medium for further studying



The streak plate method

➢ The pour plate method

- ✓ Makes use of *serial dilutions* so that the final dilution contains about *1000 organism*
- ✓ *1ml* of this dilution is then placed in *9ml* of melted agar medium (at *45°C*) & the medium is quickly poured into a sterile plate
- ✓ The resulting plate will contain *small no. of bacteria* some of which will form isolated colonies on the agar
- ✓ Since some m.o. are embedded in agar medium, this method is useful for growing microaerophiles that cannot tolerate exposure to atmospheric levels of oxygen


The pour plate method

- **Culturing Bacteria** - Culture Media

➢ Growing bacteria in the lab requires knowledge of their nutritional needs & the ability to provide these substances in a medium

➢ Although many bacteria can be grown in the lab nowadays, some m.o., such as those causing *syphilis* & *leprosy*, still *cannot be cultured* in lab media but rather need cultures containing living human or animal cells

➢ **Types of media**

- ✓ Lab medium is a synthetic medium prepared from materials of *precise* or *reasonably* well-defined composition
- ✓ Defined synthetic medium: synthetic medium that contains *specific kind* & *amount* of chemical substances
- ✓ Complex medium (chemically nondefined medium): contains reasonably *familiar materials* but varies *slightly* in *chemical* composition from batch to batch
  - Complex media may contain peptone, blood or extracts from beef, yeasts, soybean, etc.
  - *Peptone*: a product of *enzymatic digestion* of proteins (from meat or fish) that provides small peptides that m.o. can use

- ✓ Both liquid nutrient broth & solid agar medium are used to culture bacteria

A Defined Synthetic Medium for Growing *Proteus vulgaris*			
Ingredient	**Amount**	**Ingredient**	**Amount**
Water	1 liter	K_2HPO_4	1 g
$MgSO_4 \cdot 7H_2O$	200 mg	$FeSO_4 \cdot 7H_2O$	10 mg
$CaCl_2$	10 mg	Glucose	5 g
NH_4Cl	1 g	Nicotinic acid	0.1 mg
Trace elements (Mn, Mo, Cu, Co, Zn as inorganic salts, known quantities of 0.02–0.5 mg each)			

A Complex Medium Suitable for Many Heterotrophic Organisms	
Nutrient Broth Ingredient	**Amount**
Water	1 liter
Peptone	5 g
Beef extract	3 g
NaCl	8 g
Solidified Medium	
Agar	15 g
Above ingredients in amounts specified	

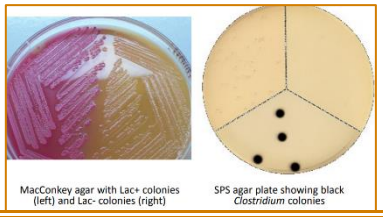
- ➢ **Commonly** used **media**:
  - ✓ Most routine lab culture media contain peptone, such media can be enriched by:
    - Yeast extract: *contains* a number of vitamins, coenzymes and nucleosides
    - Casein hydrolysate: *made from* milk protein and contains many a.a.
    - Blood (or serum): *contains* many nutrients needed by fastidious pathogens
      - ❖ *Blood* agar (usually sheep's blood) is also used to identify m.o. that cause *hemolysis*

- ➢ **Selective**, **differential** and **enrichment media**:

  These media are very important in diagnostic medicine

  - ✓ Selective medium: it encourages the *growth* of some m.o. but *suppresses* the growth of other
    - e.g. an antibiotic can be added to the growth medium so as only m.o. that are resistant to this antibiotic can grow
  - ✓ Differential medium (indicator media): has an *indicator* constituent that causes an observable change (colour change or pH change) in the medium when a biochemical reaction , that is characteristic to a certain m.o., occurs
    - This will allow to *distinguish* a certain type of m.o. (colony) from others growing on the same plate
    - E.g. *blood agar* can be used to distinguish hemolytic bacteria
  - ✓ Some media can be selective and differential at the same time, examples:

    1. MacConkey Agar:
       - It has crystal violet & bile salts which *inhibit* the growth of G+ve bacteria but *allows* the growth of G-ve ones → *selective*
       - It also has sugar lactose and pH indicator that turns colonies of lactose-fermenters (Lac+) into *red* colonies & the non-lactose fermenters (Lac-) into *colorless* colonies →*differential*
       - E.g. it can be used to differentiate between *E. coli* (Lac+) and *Salmonella* (Lac-)
    2. Sulfite Polymyxin Sulfadiazine (SPS) Agar:
       - Used for the detection of *Clostridium botulinum*
       - The two antibiotics *inhibit* the growth of most m.o. other than Clostridium spp→ *selective*
       - Sulfite is reduced by Clostridium botulinum to sulfide which a *black* iron sulfide precipitate → *differential*


MacConkey agar with Lac+ colonies (left) and Lac- colonies (right)

SPS agar plate showing black *Clostridium* colonies

3. Enrichment medium:

- It contains special nutrients that allow the growth of particular m.o. that might not otherwise be present in sufficient numbers to allow it to be isolated and identified
  - e.g. *Salmonella typhi* may be in very small no. in faecal samples, so it is cultured on a medium containing the trace element selenium which supports the growth of this m.o.
- Unlike selective medium, it doesn't suppress the growth of other m.o.
  - *Blood* agar and *chocolate* (heat-treated blood) agar are also considered as enrichment media and are frequently used to grow fastidious m.o.

- **Culturing Bacteria** – Controlling Oxygen Content of Media

➢ Obligate aerobes:
- ✓ Usually obtain *O2* from nutrient broth or on the surface of solidified agar, but some may need more.
- ✓ So O2 can *bubbled* through medium (with filters to prevent contamination) •

➢ Microaerophiles:
- ✓ A broth tube or agar plate can be incubated in a *jar* in which a *candle* is lit before the jar is sealed
- ✓ Burning candle uses *O2* & adds *CO2* , when the candle extinguishes → suitable conditions

➢ Obligate anaerobes:
- ✓ All molecular *O2* must be *removed*
- ✓ Addition of *oxygen-binding agents* like thioglycolate, cysteine (a.a) or sodium sulfide prevent O2 from exerting its toxic effects on anaerobes
- ✓ If the culture is in plates, *special jars* are used where special bags containing a chemical substance are placed to *remove* O2 & generate CO2
- ✓ Stab cultures: a culture of anaerobic bacteria can be made quickly by stabbing a straight wire coated with m.o. into a tube of agar-solidified medium

- **Culturing Bacteria** – Maintaining Cultures

➢ Stock culture:
- ✓ A pure culture prepared and *stored* for future usage.
- ✓ It is *not used* in the experiments by itself but rather it can be *used to prepare* subcultures (by inoculating fresh medium)

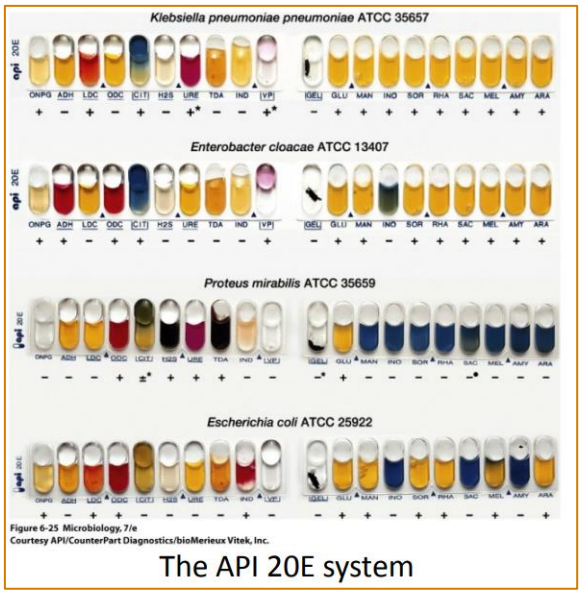
➢ Aseptic techniques:
- ✓ Taking precautions & measures to *prevent contamination* of and from the bacterial cultures

➢ Preserved culture:
- ✓ A culture in which organisms are *maintained* in *dormant state*
- ✓ e.g. lyophilization: freezing with vacuum to dry the culture, placed in vials which can be stored at room temp for long periods

➢ Reference culture (microbial standards):
- ✓ A preserved culture that maintains the m.o. with the characteristics as *originally defined* (these m.o. are usually well charecterized), examples:
  - ATCC: American Type Culture Collection (USA)
  - NCTC: National Collection of Type Cultures (UK)

- Culturing Bacteria – Methods of Performing Multiple Diagnostic Tests
  - Many kits use culture systems that contain a large no. of differential & selective media to identify different m.o.
    - ✓ e.g. Analytical Profile Index (**API**) and **Enterotube Multitest System**
    - ✓ Advantages:
      - Use *small* amount of media
      - Occupy *little* space
      - *Efficient* & reliable means of identifying infectious organisms
  - API kit:
    - ✓ Plastic tray with 20 microtubes containing different dehydrated media
    - ✓ The microtubes are *rehydrated* & *inoculated* with bacterial suspension from an isolated colony

      →*incubate* → write the *no*. → check the list for *identification*
  - Other tests depend on
    - ✓ Immunological properties of the m.o.



The API 20E system

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