PHARMACEUTICAL MICROBIOLOGY and IMMUNOLOGY

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OBJECTIVES

Variation in bacteria

- Phenotypic variations
- Genotypic variations

Variation in Bacteria

Bacterial Variation: Any change in the genotype of a bacterium or its phenotype is known as variation

- **Genotypic variation** can occur as a result of changes in the genes by way of mutation, loss or acquisition of new genetic elements. These variations are heritable

Variation in Bacteria

- **Phenotypic variations** are seen temporarily when bacteria are grown under certain environmental conditions. These variations are not heritable

Variant: As a result of variation, new features gained bacterium is called as variant

Variation in Bacteria

Variations in bacteria can be classified into two groups:

- **Non-heritable variations:** A variation in the phenotype of bacteria, in which the genetic constitution remains unchanged is a non-heritable variation
- Such variations are seen due to a change in environmental conditions and they are neither permanent nor heritable

Variation in Bacteria

- They may revert back to normal state when the conditions are restored

- **Heritable variations:** A variation in the genotype of bacteria can be defined as heritable variation
- It occurs as a result of the changes in the genes through mutation, loss or acquisition of new genetic elements
- These types of variations may affect the next generation

Non-heritable Variations

Morphological Variations

- Colony
- Flagella
- Spore
- Capsule
- Fimbriae
- Structure

Cultural Variations

Physiological and Biochemical Variations

- Staining property
- Pigment production
- Enzymatic
- Attenuation

Morphological Variations

- Variations in size and shape of the bacilli can be seen during late lag phase or stationary phase of the bacterial growth

- Capsule production by an microorganism depends upon the nutrients or nature of the medium

- Sporulation depends upon again on the environmental conditions

Colony Variation

- A **colony** is defined as a visible mass of microorganisms all originating **from a single mother cell**, therefore a colony constitutes a clone of bacteria all genetically alike

- Bacterial colonies can differ in the details of their appearance. A colony basically looks like a dot growing on the medium

Colony Variation

- Description of a colony’s morphology includes its shape, the margins or edges of the colony, its color and surface features
- Some colonies are round and smooth, others can have wavy edges and a wrinkled appearance

Colony Variation

- **Mucoid colony**: a colony showing viscous or sticky growth typical of an microorganism producing large quantities of a carbohydrate capsule

- **Smooth colony:** a bacterial colony with a glistening, rounded surface; this type of colony is usually associated with increased virulence with respect to rough colonies

Colony Variation

- **Rough colony:** a bacterial colony with a granular, flattened surface; this type of colony is usually associated with loss of virulence with respect to smooth colonies

Virulence: degree of pathogenicity of a microorganism

- Depending on the environmental conditions, it can alter to other one. These alterations are temporary
- When conditions become favorable again, it may revert back to its previous status

Capsule Variation

- Cells of most bacteria are surrounded by a gelatinous sheath called **capsule**

- Capsule is composed of **2% carbohydrate** and **98% water** (except *Bacillus anthracis*, the capsule of *B. anthracis* composed of poly-d-glutamic acid-protein)

- It is not essential for the survival of bacteria

Capsule Variation

- Capsular material is **antigenic** and can be demonstrated by mixing it with a specific anticapsular serum
- It helps in the disease-causing ability of some bacteria
- Capsule protects the bacteria against phagocytosis

Capsule Variation

- Capsulated bacteria may lose their capsule formation ability under unfavorable conditions
- If a pathogen bacterium losts its capsule formation ability, its virulence will decrease (it may lose its disease causing capacity and may become avirulent)
- It must be cultured in enriched media (with substances such as blood, serum) to gain capsule formation ability again

Flagella Variation

- Bacterial motility is commonly provided by flagella, long (12 μm), helical-shaped structures that project from the surface of the cell

- Bacterial flagellum is made up of the protein flagellin. **Flagellin** is highly antigenic.

- Some have a single, polar flagellum, whereas others are flagellate over their entire surface (peritrichous).

Flagella Variation

- Under unfavorable conditions, bacteria may lose their flagella. When conditions become favorable, flagella will be formed again.

- *Listeria monocytogenes* is motile at room temperature (20-25 ºC), but it is immobile at body temperature (37 ºC). It can not synthesize flagella at body temperature

Fimbriae Variation

- Fimbriae are found on many bacteria
- They are shorter and straighter than flagella and more numerous
- Fimbriae do not function in motility. They play a role in attachment to surfaces. Some bacteria attach to hosts by fimbriae and successful colonization of many surfaces is totally dependent upon the ability to make fimbriae

Fimbriae Variation

- Under unfavorable conditions, bacteria may lose their fimbriae

- Some aerobic bacteria form a thin layer at the surface of broth culture. This layer consists of many aerobic bacteria that adhere to the surface with their fimbriae

- Thus, fimbriae allow the aerobic bacteria to remain on the broth, from which they take nutrients, while they congregate near the air. These bacteria may lose their fimbriae under anaerobic conditions

Spore Variation

- Some bacteria produce a thick protective wall around themselves under unfavorable conditions (such as changes in temperature or lack of nutrients). These structures are known as **Spores**, which can survive for many years

- When conditions become favorable again, the wall breaks and spore turns to vegetative form

Spore Variation

- *Bacillus anthracis* spores are found in soil, animal carcasses and feces.

- When anthrax spores get inside the body, they germinate to form the virulent (disease-causing) bacteria
  - Entering broken skin and germinating there to cause cutaneous anthrax;
  - being inhaled and germinating in the lungs to cause inhalation anthrax;
  - being eaten and germinating in the gastrointestinal tract to cause gastrointestinal anthrax

Spore Variation

- *Bacillus anthracis* does not form spores inside the human body

vegetative form → in body

spore form → outside the body

Structure Variations

- **Involution form:** In old cultures or under unfavorable conditions, abnormally shaped bacterial cells occur. We define these bacteria as involution forms

- When bacteria are treated with enzymes that hydrolyze the cell wall (e.g. lysozyme) or antibiotics that interfere with biosynthesis of peptidoglycan (penicillin), wall-less bacteria are often produced.

Structure Variations

- **Lysozyme** is an enzyme found in tears, saliva and nasal secretions

- **Protoplasts** are derived from Gram-positive bacteria. Their cell wall is completely removed by treating with lysozyme

- **Spheroplast** are derived from Gram-negative bacteria

Structure Variations

- Gram negative bacteria are refractory to lysozyme, because large protein molecules can not penetrate the LPS (lipopolysaccharide) layer

- Spheroplasts are produced by treating with first EDTA (ethylenediaminetetraacetic acid) and then lysozyme. Some residual but non-functional cell wall material retain in the spheroplasts

Structure Variations

- If protoplasts/spheroplasts are able to grow and divide, they are called **L-forms**
- When conditions become favorable again, they may return to their previous statuses

Cultural Variations

- **Culture:** Microorganisms that grow and multiply in or on a culture medium
- **Pure Culture**, consists of only a single type of microorganism
- The composition of the culture media and environmental conditions affect the growth of microorganisms

Cultural Variations

- Reproduction characteristics of microorganisms in liquid/solid media may change due to environmental conditions

- For example; *S. aureus* grow homogeneously in the liquid medium. If the surface tension increases, it start to grow at the top

- When conditions become favorable again, it may return to its previous status

Physiological and Biochemical Variations

Staining Property

- Bacterial staining is the process of coloring of colorless bacterial structural components using stains (dyes)

- Cell staining is important in the diagnosis of microorganisms because bacteria can be identified by the color differentiation of stains (dyes)

Staining Property

- Microscopic examination of stained bacterial cells allow examination of the size, shape and arrangement of organelles, as well as external appendages such as flagella

- Individual variation in the cell wall constituents among different groups of bacteria will consequently produce variations in colors during microscopic examination

Staining Property

- Fresh bacterial cultures must be used in staining methods. New cultures have the best form of peptidoglycan in their cell wall.
- In old cultures, structure of the cell wall is disrupted. As a result of this, bacteria may stain incorrectly
- For example; Gram positive bacteria can appear Gram negative if the culture used to prepare the smear is old

Pigment Production

- Some bacteria can produce pigments of different colors

- Under unfavorable conditions pigmented bacteria may lose their pigment production abilities

- For example; *Serratia marcescens* produces red pigments at a lower temperature (25 °C). It can not produce red pigment at 37 °C.

Enzymatic Variations

- Bacterial enzymes are bacterial proteins
- Catalyst to accelerates a reaction (catalysts are compounds that accelerate the progress of a reaction)
- Enzymes are specific to their substrates
- Many bacteria use digestive enzymes to break down nutrients in their surroundings

Enzymatic Variations

- Some enzymes are synthesized continuously without being dependent on the content of the medium (**structural enzymes**)

- Some of them are formed only in the presence of its substrate or a substance resembling its substrate (**inducible enzymes**)

Enzymatic Variations

- For example; *E. coli* synthesizes the enzyme **beta-galactosidase** required for lactose fermentation when grown in a medium containing lactose

- In the absence of lactose, this enzyme is not synthesized

Enzymatic Variations

- Therefore, E. coli ferments glucose faster than lactose. Enzymes required for glucose fermentation are always present in the E. coli. It can act as soon as possible

- However, the synthesis of enzymes for lactose fermentation take place in a period of at least 30 minutes

Attenuation

- Decrease or loss of virulence
- An attenuated (weakened) microorganism would have lost the ability to cause serious illness, but still able to trigger an immune response
- These microorganisms may cause a mild or subclinical form of the illness
- Attenuation is used in vaccine production

Attenuation

- For example; Pasteur attenuated the *Bacillus anthracis* by growing it at high temperatures (42-43 °C)

- Its toxic properties and sporogenic functions disappeared owing to this process. Here he created a new type bacteria characterized by the loss of virulence and ability to form spores

1881 - Louis Pasteur created the first vaccine for anthrax

Heritable Variations

Mutation

- **Mutation** is a heritable change in DNA sequence that can lead to a change in phenotype

- If a gene that encodes for a specific protein is mutated, it may result in a change in the sequence of amino acids comprising the protein. The activity of the protein may be altered

- It can occur randomly

Mutation

- Mutations are caused by alteration in the nucleotide sequence at some point of DNA which can occur due to addition, deletion, substitution of one or more bases

- Mutations will be passed on to the next generation

- **Mutants** are variants in which one or more bases in their DNA are altered; which are heritable and irreversible

Mutation

- **Spontaneous mutation:** A mutation that arises naturally and not as a result of exposure to mutagens

- **Mutations may be harmful** (if they change the codes for protein synthesis in such a way that a particular protein will not be able to conduct its routine function)

Mutation

- **Mutations may be beneficial** (mutations in the bacterial DNA that alter the target of the antibiotic allow the bacteria to survive)

- **Mutations may have no effect on the microorganism** (most mutations are neither harmful nor beneficial)

Types of mutation

Substitution: Change of a single base

- One base is substituted in the DNA for another base
- Incorrect base pairing results from the change of a single nucleotide base
- Substitutions usually result in '**point mutations**'

Types of mutation

There are two types of **point mutations**:

- **Transition mutations**
- **Transversion mutations**

Transition mutations occur when a pyrimidine base (i.e., thymine [T] or cytosine [C]) substitutes for another pyrimidine base or when a purine base (i.e., adenine [A] or guanine [G]) substitutes for another purine base

T ↔ C A↔G

Types of mutation

Transversion mutations occur when a purine base substitutes for a pyrimidine base or when a pyrimidine base substitutes for a purine base (e.g. adenine to cytosine)

A→C-T T→A-G

Missense Mutation

A C G C C T A A A C G A DNA

U G C G G A U U U G C U mRNA

cys gly phe ala Protein

Substitution mutation T--->A at position #1 in DNA changes the mRNA codon to code for the amino acid serine in place of cysteine. This is a **missense mutation**

T C G C C T A A A C G A DNA

A G C G G A U U U G C U mRNA

ser gly phe ala Protein

Nonsense Mutation

A C G C C T A A A C G A DNA

U G C G G A U U U G C U mRNA

cys gly phe ala Protein

Substitution mutation in the third base of DNA G--->T generates terminator "stop" codon which terminates the translation of the protein. A shortened or "truncated" protein may be produced. This is called a **nonsense mutation**.

A C T C C T A A A C G A DNA

U G A G G A U U U G C U mRNA

stop --------------------------> no translation

Silent Mutation

A C G C C T A A A C G A DNA

U G C G G A U U U G C U mRNA

cys gly phe ala Protein

Substitution mutation in the sixth base of DNA T--->C causes degenerate codon for glycine so there is no change in the primary structure of the protein. This is called a **silent mutation**.

A C G C C C A A A C G A DNA

U G C G G G U U U G C U mRNA

cys gly phe ala Protein

Frameshift mutations

Deletion: Loss of a base

Insertion: Addition of a base

Frameshift mutations

- **Deletions** are mutations in which a section of DNA is lost or deleted
- **Insertions** are mutations in which extra base pairs are inserted into a new place in the DNA

Frameshift mutations

- **Frameshift mutation:** A genetic mutation caused by a deletion or insertion in a DNA sequence that shifts the way the sequence is read

- **Insertions** or **deletion** of one or more bases may result in the addition or deletion of one or more amino acids to the produced protein

Frameshift mutations

Insertion

I.	AAT / TCC / GGA / TGC / T…	normal DNA
	UUA / AGG / CCU / ACG / A…	mRNA
	leu arg pro thr	amino acids
II.	AAT / GTC / CGG / ATG / CT…	mutated DNA
	UUA / CAG / GCC / UAC / GA…	mRNA
	leu glu ala tyr	amino acids

Frameshift mutations

Deletion

Normal DNA: CGA – TGC – ATC

Alanine – Threonine – stop

Mutated DNA: CGA – TCA- TC

Alanine – Serine

Which mutation would have the least effect on microorganism?

- **Substitution** has the least effect because it changes only one amino acid or it may change no amino acid

- **Insertion** and **deletion** mutations have the most effect on a microorganism because they affect many amino acids and consequently the whole protein

Mutagenic Agents (Mutagens)

A **mutagen** is a physical or chemical agent that changes the genetic material, usually DNA of an microorganism

- **Chemical Mutagens**
- **Physical Mutagens**

Chemical Mutagens

- **Base Analogs** are chemicals that are structurally similar to the nucleotide bases
- Base Analogs can substitute for a normal nucleobase in nucleic acids. They are categorized in two separate groups.

    - **Purine analogs**
    - **Pyrimidine analogs**

Chemical Mutagens

- 2-aminopurine and 5-bromouracil are common examples of these chemical agents

  Thymine analog: 5-Bromouracil (5-BU)

  Adenine analog: 2-Aminopurine (2-AP)

- **Intercalating agents** like acridine orange, proflavine and ethidium bromide get inserted in between bases in the DNA and induce a frameshift mutation in the DNA

Mutagenic Agents (Mutagens)

- **Chemicals which alter structure and pairing properties of bases:**

- **hydroxylating agent** (add OH-group to C)
- **alkylating agent** such as EMS (ethylmethane sulfonate); chemical mutagens that react with bases and add methyl or ethyl groups

- **deaminating agents** such as nitrous acid; formed by digestion of nitrites (preservatives) in foods. It causes C to U, C to T, and A to hypoxanthine deaminations. Deamination by nitrous acid, causes transitions

Physical Mutagens

- **Ultraviolet radiations** of above 260 nm induce certain molecular lesions in the DNA known as pyrimidine dimers
- **Ionizing radiations** such as **X-rays** and **gamma rays** usually cause breakage in the DNA strand

UV irradiation	Causes formation of adjacent T-T dimers that distorts the DNA backbone, altering the binding properties of bases near the dimer
X-ray	Alters bases chemically, causes deletions and induces breaks in DNA chain

Mutant Types

- **Resistance Mutants:** As a result of mutation, bacteria can become resistant to various drugs, disinfectants, antibiotics etc
- **Nutritional Mutants:** As a result of mutation, bacteria need some nutrients to grow
- **Fermentation Mutants:** *E. coli* can ferment lactose but as a result of mutation bacteria may lose this property

Mutant Types

- **Pigmentation Mutants:** As a result of mutation, pigmented bacteria may lose their pigment production abilities

- **Antigenic Mutants:** As a result of mutation, antigenic structure of bacteria may change

Reversion

Reverse Mutations (reversions or back mutations) are defined as mutations that fully or partially restore the activity of a mutant gene

$$(G\text{-}C) \xrightarrow{M} (A\text{-}T) \xrightarrow{R} (G\text{-}C)$$