Microbiology Lectures

Sheet #2

**Q- What is the difference between gram positive and gram negative ?**

gram + bacteria stains purple and gram – bacteria staines pink.

**Q- Why do they stain differently ?**

We take 2 cell one gram + and the second gram - , put each one of them on a separate slide and then stain both of them with Crystal Violet ( purple Dye ) both bacteria will take up the stain and appear purple in color.

After this we decolorize them with acid or alcohol . Alcohol takes out the stain from inside of the cell and due to the thickness of peptidoglycan in gram positive the purple granule inside the bacteria can't get out . But in gram negative the purple granules get out so it appears white or not stained .

Now we bring another stain which is (The safranin – pink stain ) and We stain them again , gram negative will become pink and gram positive will still look purple .

* Both bacteria stain in different colores due to the thickness of peptidoglycan, and it's not related to the fact that the bacteria is pathogenic or non-pathogenic or the shape of bacteria .
* Gram positive consists of many layers of peptidoglycan .
* Gram negative consists of 1-2 layers of peptidoglycan .

**cell wall (slide # 28)**

* Peptidoglycan is a polymer of amino acid and sugars.
* The sugars are 2 kind :-

1. N-acetyl glucosamine (NAG)
2. N-acetylmuramic acid (NAM)

* Sugars are connected to each other to form chains and the chain of amino acid is connected to NAM , So that help to make the layers of peptidoglycan .
* Amino acids are in the D form because it's more resistant to break down .

**\*\*\*slide # 29 and 30**

As you can see in the figure

1. NAM connected to NAG
2. NAM connected to peptide (4-5 amino acids )
3. Peptide chain cross link again with pentaglycine

* Peptidoglycan is a characteristic of bacteria cell (not in mammalian cell) and gives the rigidity to keep the shape of bacteria .
* In gram negative cell, due to the low number of peptidoglycan layers, they compensate the loss of rigidity by having an outer membrane .
* Only gram negative cell has an outer membrane .

**\*\*\*cell wall – gram positive bacteria :-**

1. Peptidoglycan
2. Teichoic acid and lipoteichoic acid
3. Polysaccharide

**\*\*\*cell wall – gram negative bacteria :-**

1. Peptidoglycan (1-2 layers )
2. Lipoprotein
3. Periplasmic space :space between inner and outer membrane and actually contain peptidoglycan layers
4. Outer membrane

**\*\*\*slide #33**

In the bottom of the figure inner membrane (cytoplasmic membrane )appears and above it there is a space (periplasmic space )and in it is their peptidoglycan layers (1-2) .The most outer structure is the outer membrane which is similar to inner membrane , consist of lipid bilayer ,the inner most leaflet made of phosphlipide and the outer leaflet made of lipopolysaccharide (LPS).

**\*\*\*LPS made of :-**

1. Lipid A (inner most part ) ( Yellow square)
2. Core polysaccharide
3. O-polysaccharide

* Lipid A is more or less the same in most bacteria .
* Core polysaccharide varies from species to species .
* The most variability is in the O-polysaccharide , so in the same species we have different strains or ( O-polysaccharide)
* O-polysaccharide are the most antigenic, so it's easily able to induce the antibody .
* O-polysaccharide can be present or absent. If its absent the appearance of colonies is rough and if its present the appearance of colonies is smooth
* smooth colonies are more pathogenic .
* O-polysaccharide and core polysaccharide are used for classification but lipid A is the most important part of pathogenesis of gram negative bacteria .
* LPS is the pathogenesity factor and this pathogenesity particularly resides in lipid A .

**Lipopolysaccharide (LPS)**

Another name is endotoxin ,which mean toxin in bacteria and also we have exotoxin which is secreted by bacteria in to the environment and cause diseases.

For example ,tetanus disease ( مرض الكُزاز) is due to exotoxin ,which is tetanus toxin ,that cause damage to nerve and produce the symptoms of this disease .

There are a Varity of exotoxin ,but when we talk about endotoxin there is one endotoxin (LPS) which is associated with gram negative bacteria only .And usually endotoxin is released upon the death of bacteria , but living bacteria secrete very little of endotoxin .

* Exotoxin can be produced by gram – and gram + equally.
* Another name of endotoxin is pyrogen
* Pyrogen means an agent that produces fever in human beings.

**\*\*\*LPS function**

1. **Toxics** : can kill mice ,pigs and humans because they produce toxic shock

\* Shock : is a condition in which there is a fever , drastic drop in blood pressure, intravascular coagulation ,etc. and then the patient dies .

\*there are different types of shock; anaphylactic shock ,cardiogenic shock and septic shock .

\*septic shock due to septicemia because of present of gram negative bacteria growing in the blood .

1. **Pyrogen** ,causes fever

Any preparation of normal saline or plasma that will be injected intravenously , should be scanned for the presence of LPS. because after sterilization the bacteria dies , but even after the bacteria dies there will still be LPS present in the preparation and you don’t want that . so you can test for that by injecting into rabbits and notice if the rabbits got fever then you expect that prepration contain LPS.

\*Sometimes the manufacturer uses the Amoebocytes (immune cell present in horse shoe crab ) also to test the preparation for containing of pyrogen. Amoebocytes lyse in presence of LPS

3. **Adjuvant** , stimulates immunity

LPS stimulate WBC in the body and they become over active which leads to septic shock .

**Cell Wall Summary**

* Unique to bacteria
* 20-40% of bacterial cell weight
* Determines shape of bacteria
* Prevents osmotic rupture

Target for some antibiotics (Penicillin)

**Cell Membrane**

* The plasma membrane encloses the cytoplasm of the cell and provides selective permeability for nutrients to enter.
* Phospholipid Bilayer
* Water can penetrate
* Flexible
* Not strong, ruptures easily
  + Osmotic Pressure created by cytoplasm

**Cytoplasmic Structures**

* DNA is a single long circular molecule of double-stranded DNA “bacterial chromosome”.
* Plasmids : extra bits of genetic materials , they are important because they can carry genes which give resistance to antibiotics and because they can transfer from one of bacteria to another by conjugation , so you can say it is pathogenisty factor
* Bacteria also contain **transposons** which are jumping genes (sequence of DNA) that have a capability to replicate and transfer to another place on the chromosome itself or they can jump from chromosome to plasmids and vice versa .
* **Ribosomes** in the bacteria are different of those in mammalian cell because they are of different sedimentation rate .
* No organelles

**Appendages of Bacteria**

1. ***Flagella***

-used for locomotion

* Swarming occurs with some bacteria
  + Spread across Petri Dish
  + Proteus species most evident
* Arrangement basis for classification
  + Monotrichous; 1 flagella
  + Lophotrichous; tuft at one end
  + Amphitrichous; both ends
  + Peritrichous; all around bacteria
* Bacteria use the flagella to move toward something (for the site where it can divide or cause diseases), or run away from something (WBC) .
* Flagella is pathogenisty factor
* The structure of flagella is made of protein known as flagellum attached to cytoplasmic membrane by anchored part known as basal body

1. ***Pilli***

-thinner and smaller than flagella

-present all over the bacteria

-two types :-

1. sex pilli or F-pilli (F= Fertility)

Used for exchange of genetic material by conjugation

2.fimbria

Used for adherence of bacteria to surface

**Endospores**

* When essential nutrients are depleted, certain gram positive bacteria (e.g., *Clostridium* and *Bacillus*), form “resting” cells called endospores.

* These endospores contain condensed nuclear material and protein and can survive extreme heat, lack of water, and exposure to toxic chemicals.
* When growth conditions are available, the cell will germinate into a dividing bacterium.
* Resistant structure
  + Heat, irradiation, cold
  + Boiling >1 hr
* Takes time and energy to make spores
* Location important in classification
  + Central Spores: in the middle of bacteria
  + Subterminal spores: btw central and terminal
  + Terminal spores: at the edge of bacteria
* Bacillus stearothermophilus –spores
  + Used for quality control of heat sterilization equipment
  + Not pathogenic bacteria
* Bacillus anthracis - spores
  + Used in biological warfare
* Endospores ; form inside bacteria (don’t confuse with spores present in fungi )
* The bacteria that make endospores is bacilli only.
* Endospores don’t stain and appear as empty space.

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