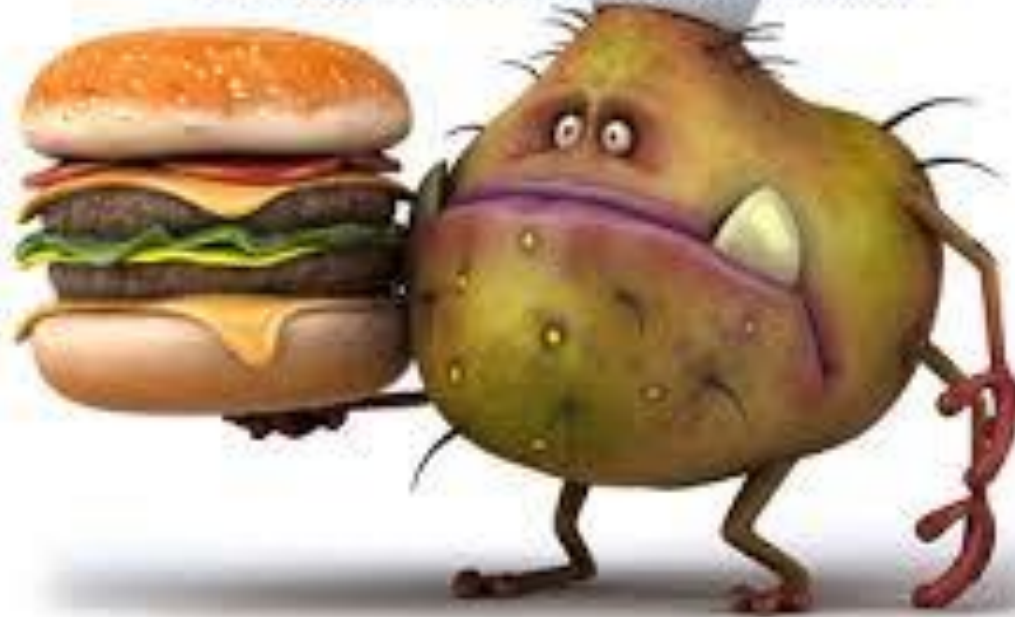


Microbial Nutrition And Metabolism



BACTERIAL NUTRITION

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- Microorganism require about 10 elements in large quantities for the synthesis of macro molecules these elements are called **macro elements**
- 95% of cell dry weight is made up of a few major elements :Carbon, oxygen, hydrogen, nitrogen, sulphur, phosphorus, potassium, calcium, magnesium and iron
- The nutrients which are required in small amounts is called **micro nutrients/trace elements**
- Eg. Zinc, Manganese, Molybdenum



NUTRITIONAL TYPES OF MICROORGANISM

- Based on the sources of **carbon, electron and energy** micro organism are placed in different nutritional types
- **A) Carbon sources**
 - a) **Autotrophs**: They use carbon dioxide as a sole source of carbon
 - b) **Heterotrophs** : They use reduced preformed organic molecules from other organism as carbon source
- **B) Energy source**
 - a) **Phototroph** : Light is used as source of energy



- b) **Chemotroph**: oxidation of organic / inorganic compound as a source of energy

C) **Electron source**

- a) **Litho trophs** : reduced inorganic molecules
- b) **Organotrophs** – organic molecules



MICROBIAL GROWTH

- Prokaryotes Reproduce by Binary fission
- Some prokaryotes reproduce by budding, fragmentation or other means



BACTERIAL GROWTH CURVE

- In growth curve, the growth of microorganism reproducing by binary fission is plotted as logarithm of number of viable cells versus the incubation time
- The growth curve has four distinct phases



Figure 6.6 Microbial Growth Curve in a Closed System.
The four phases of the growth curve are identified on the curve and discussed in the text.



LAG PHASE

- When micro organism are introduced into fresh culture, there is no increase in cell number occurs
- So this period is called lag period
- There is no increase in the number of cells
- During this period bacteria is adapting itself to the new environment and synthesise the essential cofactors, ribosome and ATP which are required for growth



EXPONENTIAL PHASE OR LOG PHASE

- In this phase microorganism are growing and dividing exponentially
- The rate of growth is constant in exponential phase
- The population is most uniform in terms of chemical and physiological properties during this phase



STATIONARY PHASE

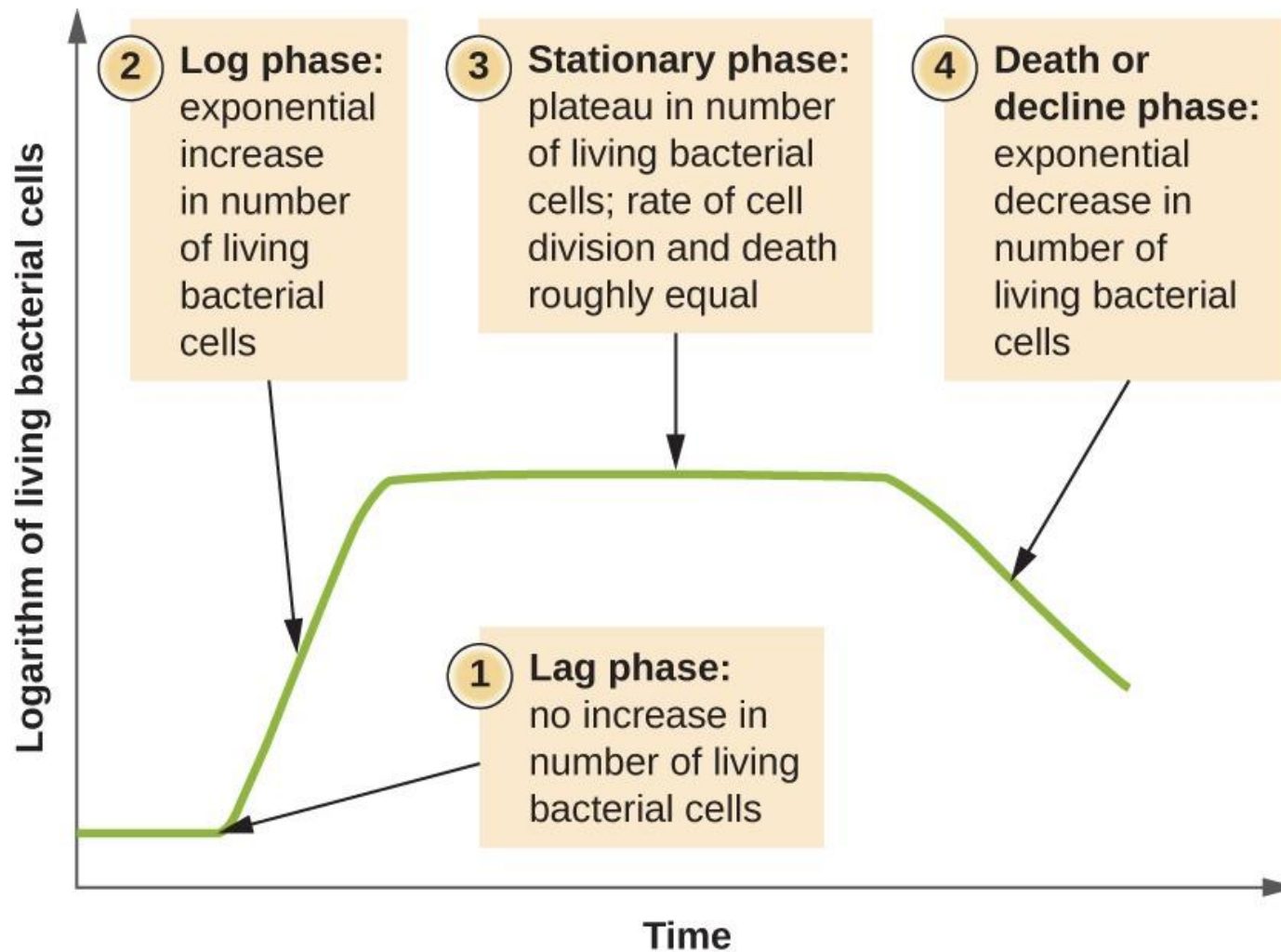
- In stationary phase total number of valuable organism remain constant
- There is a balance between cell division and cell death
- In bacteria stationary phase is achieved at a population level of around 10^9 cells per ml
- Microbial population enters into the stationary phase due to
 - Depletion of essential nutrients
 - Limited availability of oxygen for aerobic organism
 - Accumulation of toxic waste products
- Spore formulation occur in this phase



SENESCENCE AND DEATH

- Decline in viable count following stationary phase
- Nutrient depletion and accumulation of toxic waste is responsible for death phase



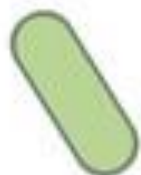


GENERATION TIME/ DOUBLING TIME

- The time required to double the number of microorganism in a population
- Because the population is doubling in every generation
- The increase in population always 2^n where n is the number of generations
- So the population increase is exponential or Logarithmic
- Let N_0 is the initial population number
- N_t is the population at time t
- $N_t = N_0 \times 2^n$



N_0



N_1



N_2



N_t



t_0



t_1



t_2



t_n



CONTINUOUS CULTURE SYSTEM

- A microbial population can be maintained in the exponential growth phase and at constant biomass concentration for long periods in continuous culture system
- Two major types of continuous culture system commonly used
 - 1) Chemostat
 - 2) Turbidostat



CHEMOSTSAT

- Sterile medium is fed into the culture vessel at the same time media containing microorganism is removed
- One essential nutrient is limiting in chemostat
- Dilution rate is constant
- Chemostat is stable and effective at lower dilutions

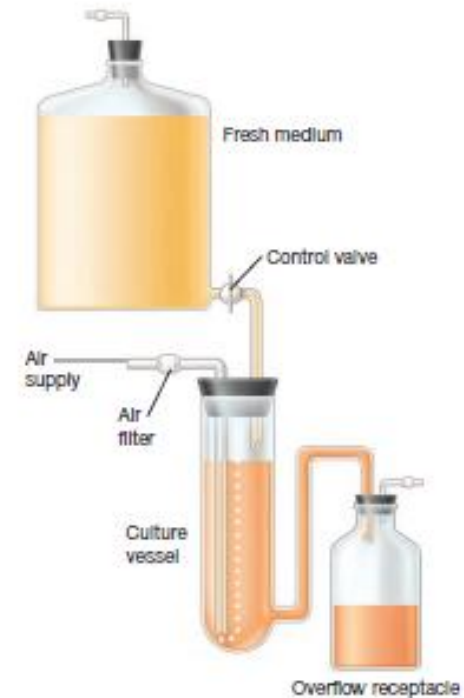


Figure 6.16 A Continuous Culture System: The Chemostat. Schematic diagram of the system. The fresh medium contains a limiting amount of an essential nutrient. Growth rate is determined by the rate of flow of medium through the culture vessel.



TURBIDOSTAT

- It has a photocell that measures the absorbance or turbidity of the culture in the growth medium
- The flow rate of media through the vessel is automatically regulated to maintain predetermined turbidity or cell density
- In turbidostat all the nutrients will be in excess
- Turbidostat operates best at high dilution rates



CLASSIFICATION OF BACTERIA BASED ON ENVIRONMENTAL FACTOR REQUIREMENT

Based on solute and water activity

- **Osmotolerant** : able to grow over a wide range of water activity or osmotic concentration

Eg: *Staphylococcus aureus*

- **Halophile**: requires high level of Sodium chloride usually above 0.2 M to grow

- Eg, *Staphylococcus aureus*

Halobacterium



PH

- **Acidophile**: Growth optimum between pH 0 to 5.5
Eg: Sulfolobus
- **Neutrophile**: Growth optimum between pH 5.5 and 8.0
Eg : *Escherichia coli*
- **Alkalophile**: Growth optimum between pH 8.0- and 11.5



TEMPERATURE

- **Psychrophile**: Grows well at 0°C and has an optimum growth temperature of 15°C or lower
Eg: *Bacillus psycrophilus*
- **Psychrotroph**: can grow at 0-7°C has an optimum growth temperature between 20-30°C and maximum around 35°C.
Eg: *Listeria monocytogenes*
Pseudomonas flourescens
- **Mesophile** : Has optimum growth around 20-45°C
Eg: *Escherichia coli*
- **Thermophile**: Can grow at 55°C or higher. Optimum temperature of growth often between 55-65°C
- **Hyper thermophile**: has an optimum temperature between 80-113°C



PRESSURE

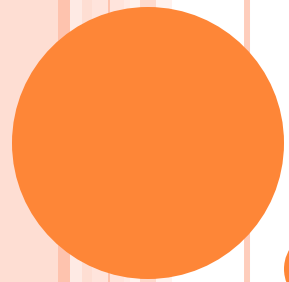
- **Barophilic:** Grow more rapid at high hydrostatic pressures



OXYGEN REQUIREMENT

- **Obligate aerobe**: Completely dependent on atmospheric O₂ for growth
Eg : micrococcus
Pseudomonas
Mycobacterium
- **Facultative anaerobe**: Does not require oxygen for growth, but grows better in its presence
Eg. *E. Coli*
- **Aerotolerant anaerobe**: grows equally well in presence or absence of O₂
Eg. *Streptococcus pyogenes*
- **Obligate anaerobe**: Do not tolerate O₂ and dies in its presence
Eg. Clostridium, Bacteriodes
- **Microaerophile**: Require O₂ levels below 2-10% for growth and is damaged by atmospheric level O₂
Eg. Campylobacter
Treponema pallidum





PATHOGENICITY AND VIRULENCE

PATHOGENICITY AND VIRULENCE

- Any organism that produce disease is called **pathogen**
- The ability to cause disease is called **pathogenicity**



- **Primary pathogen**: Any organism that cause disease in healthy host by direct interaction
- **Opportunistic pathogen**: Refers to an organism that is part of host's normal microbiota, but is able to cause disease when the host is immunocompromised or when it has gained access to other tissue sites
- **Latent stage**: there is no shedding of organism and no symptoms present in the host
- **Virulence** refers to the degree or intensity of pathogenicity



Pathogenicity vs Virulence

- Pathogenicity is the quality or state of being pathogenic, the potential ability to produce disease
- Pathogenicity is a qualitative term, an "all-or-none" concept
- Virulence is the disease producing power of an organism the degree of pathogenicity within a group or species
- Virulence is a term that quantifies pathogenicity

In two minutes



VIRULENCE FACTORS

- The individual characteristic that contributes to virulence are called virulence factors

Eg: Capsule, Pili, and toxins

- Virulence is characterised by the characteristic of pathogen

a) Invasiveness

b) Infectivity

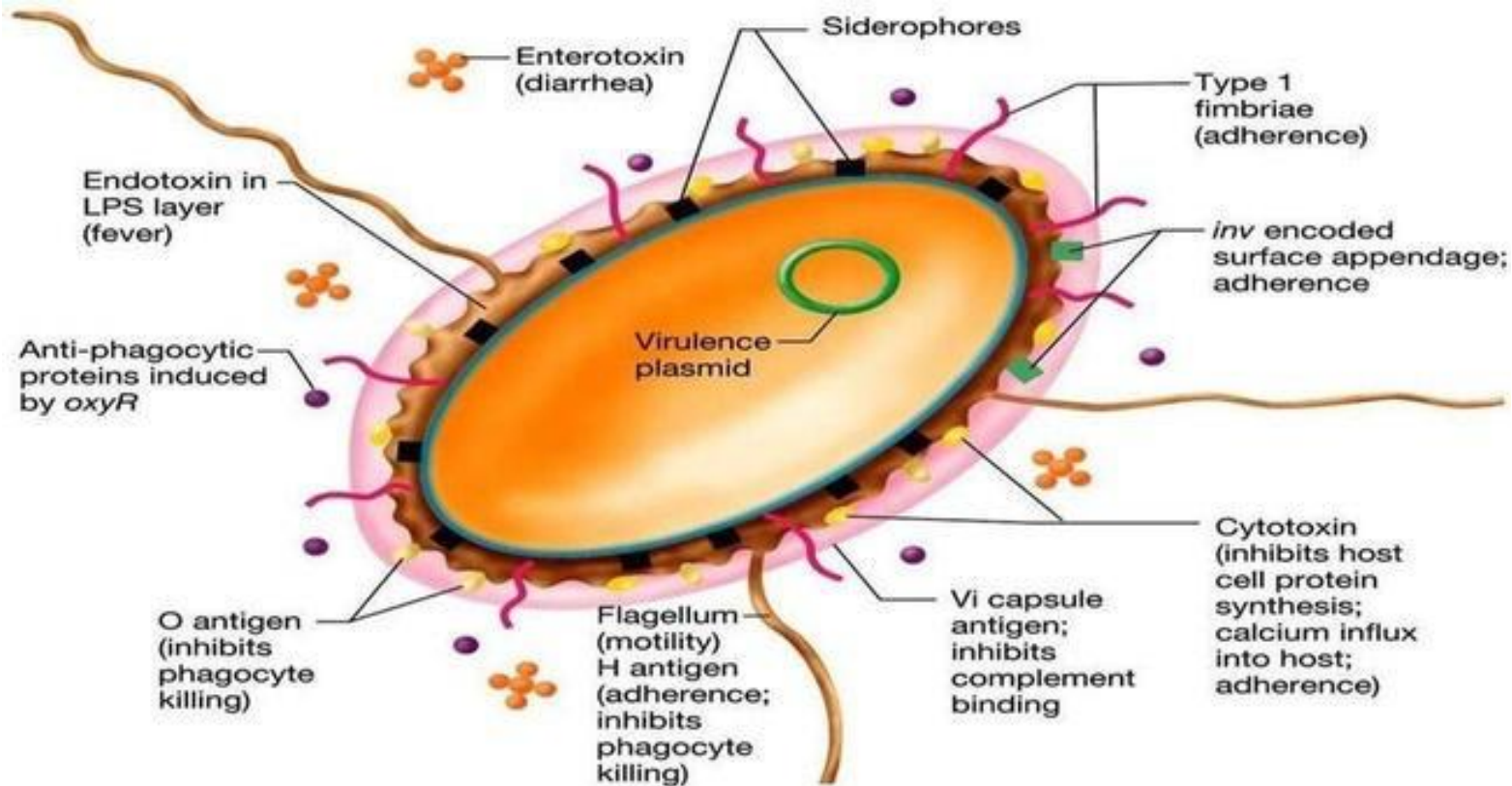
c) Pathogenic potential



- **Invasiveness** : is the ability of organism to spread to adjacent /other tissues
- **Infectivity** is the ability of organism to establish a focal point of infection
- **Pathogenic potential**: refers to the degree that the pathogen causes damage
- **Toxigenicity**: is the pathogens ability to produce toxins, chemical substance that damage the host and produce disease



VIRULENCE FACTORS OF BACTERIA



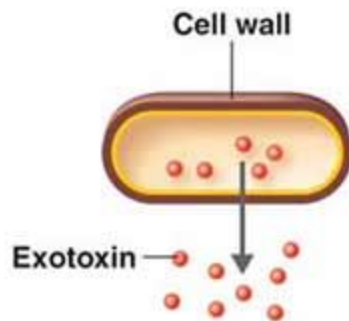
PATHOGENICITY ISLANDS

- The genes that encode major virulence factors in many bacteria are found in large segments of DNA called pathogenicity islands which carry genes responsible for virulence
Eg: Yersinia, Salmonella
- **Intoxications**: Are disease that result from specific toxin produce by bacteria
- **Toxaemia** is a condition caused by toxins that have entered into the blood of host
- Toxins produced by bacteria is classified into two groups: **exotoxins and endotoxins**

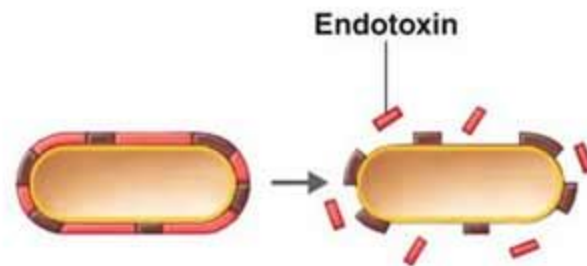


EXOTOXIN AND ENDOTOXIN

Differences Between Exotoxins and Endotoxins



(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.



(b) Endotoxins are the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.



EXOTOXINS

- They are soluble heat labile proteins that are released into the surroundings as the bacterial pathogen grows
- In general exotoxins are produced by Gram positive bacteria, although some Gram negative bacteria also produce exotoxins
- Exotoxins are usually encoded by plasmids and prophages
- Exotoxins are among the most lethal substances known: Botulinum toxin
- They are heat labile and can be inactivated by at 60-80°C



- Exotoxins are highly immunogenic and can stimulate production of neutralising antibodies called antitoxins
- The toxin protein can also be inactivated by formaldehyde, iodine and other chemicals to form immunogenic toxoids

Tetanus toxoid

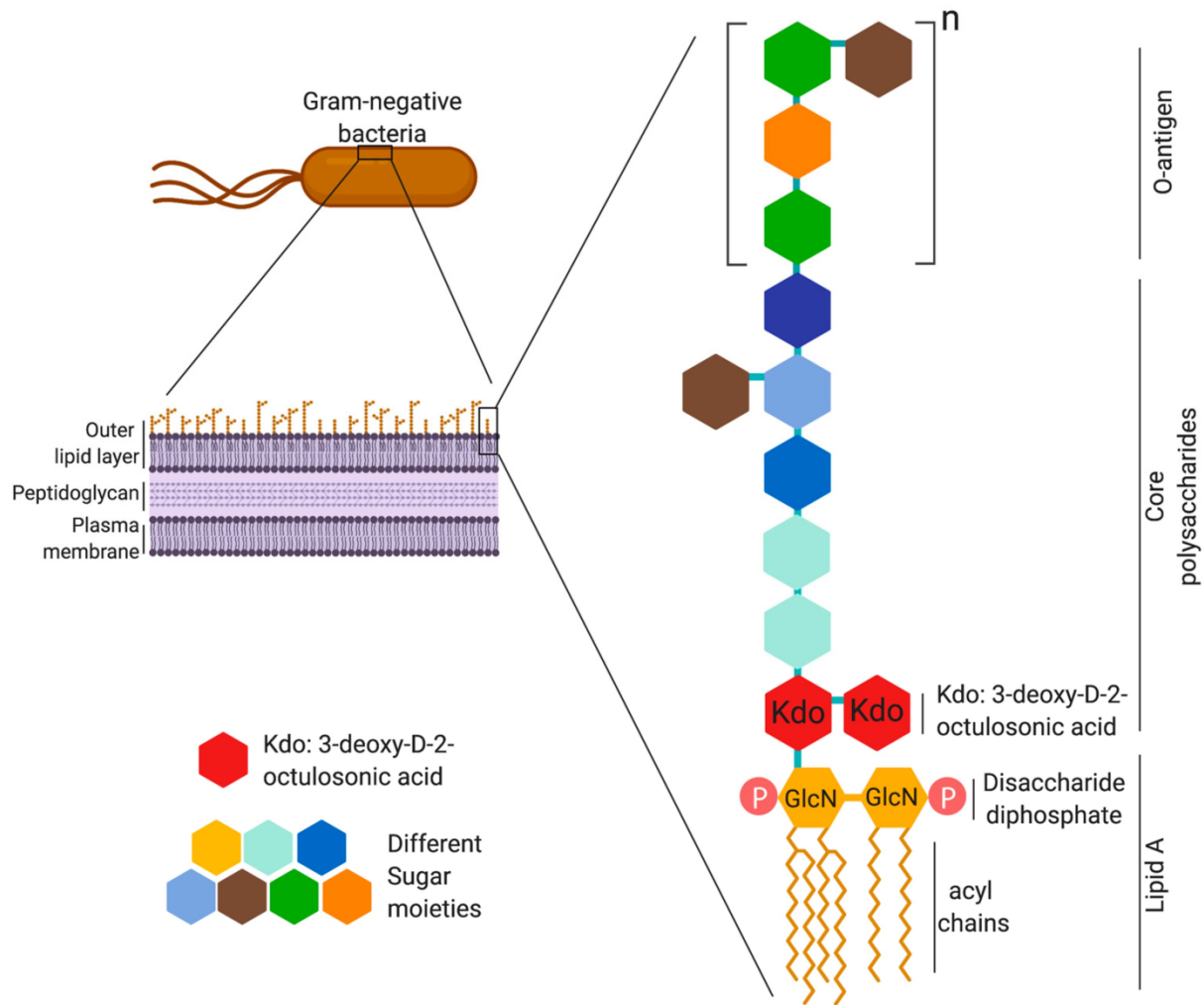
- Anthrax toxin
- Tetanus toxin
- Botulinum toxin
- Cholera toxin



ENDO TOXIN

- Endo toxin is the lipopolysacharide of Gram negative bacteria. So it is produced only by Gram negative bacteria
- It is bound to the bacterium and released only when the microorganism dies
- The toxic component of lipopolysacharide is Lipid A





GENERAL CHARACTERISTIC OF BACTERIAL ENDOTOXIN

- Heat stable
- Toxic (nanogram amounts)
- They are weakly immunogenic
- Generally similar despite the source
- Capable of producing general systemic effects like fever, shock, blood coagulation , weakness, diarrhoea, inflammation, intestinal haemorrhage, fibrinolysis
- The test used for detection of endotoxin is Limulus amoebocyte lysate test (LAL test)



DIFFERENTIATION BETWEEN ENDOTOXIN AND EXOTOXIN

| Characteristic | Exotoxins | endotoxins |
|----------------------|--|---|
| Chemical composition | protein | Lipopolysacharide complex on outer membrane Lipid A portion is toxic |
| Disease examples | Botulism Diphtheria Tetanus | Gram negative infection |
| Effect on host | Highly variable between different toxins | Similar for all endotoxins |
| Fever | Usually do not produce fever | Produce fever by induction of IL-1 and TNF |
| Genetics | Frequently carried by extra chromosomal genes such as plasmids | Sythesiszed directly from chromosomal genes |

Heat stability

**More heat sensitive
and inactivated at
60-80°C**

Heat stable at 250°C

CONTD..

Immune response

Anti toxins provide
host immunity,
Highly antigenic

Weakly immunogenic
and immunogenicity
associated with
polysaccharide

Location

Usually excreted
outside the living cell

Part of outer
membrane of Gram
negative bacteria

Production

Produced by both Gram
positive and Gram
negative bacteria

Found only on Gram
negative bacteria and
released on bacterial
death

Toxicity

Highly toxic and fatal
in nanogram quantities

Less potent and less
specific than exotoxin.
It causes septic shock

Toxoid production

Converted to antigenic
non toxic toxoids are
used for immunization

Toxoids can not be
made



THANK YOU

