STAINING OF BACTERIA

Gram Staining technique :

1. First of all **H.C. Gram** differentiated bacteria on the basos of staining.

2. In the first step of this method bacteria are stained with **Crystal Violet** and then **KI solution.**
3. After staining, bacteria are washed with Acetone or Ethyl alcohol. After washing some bacteria retain the stain and some bacteria are decolourised.
4. Bacteria which retain stain (violet or purple) are called Gram(+) and bacteria which decolourise are known as Gram(-). Gram (-) bacteria are counter stained by **saffranine.**

Ziehl Neilson staining tech.

1. In this method first of all bacteria are stained with the phenolic solution of **basic fuschin** (carbol fuchsin) and then washed with alcoholic solution of HCI or H_2SO_4.
2. If bacteria retain the stain after washing, the they are called "Acid fast" while the bacteria in which stain is lost are known as "Non acid fast". Non acid fast bacteria are counter stained by **methylene blue.**
3. Quantity of wax is more is acid fast and less in non acid fast

STAINING OF BACTERIA

Most of the bacteria are heterotrophic but some are autotrophic. On the basis of nutrition bacteria are classified into following three categories.

Autotrophs

These bacteria use light or chemical energy for their own food synthesis.

On the basis of **source of energy** autotrophs are of following two types

(i) Photosynthetic autotrophs -

- These bacteria use light energy for food synthesis.
- In these bacteria photosynthesis is non oxygenic.
- Photolysis of water does not take place here, so hydrogen is received from other sources like inorganic sulphur compound (H_2S, Thiosulphate) or organic compound (Amino acids, Isopropyl alcohol Fatty acid).

  On the basis of **hydrogen donor,** photoautotrophs are further classified into following two categories.

(a) Photo-lithotrophs - Hydrogen donor is inorganic sulphur containing compounds viz. H_2S, Thiosulphate. This category includes only two types of bacteria

-Purple sulphur bacteria - e.g. *Chromatium*
- Green sulphur bacteria - e.g. *Chlorobium, Thiothrix*

(b) Photo - organotrophs - Hydrogen donor is organic compounds viz. Isopropyl alcohol, amino acids, fatty acids.

This category involves only one type bacteria.
- Purple non sulphur bacteria - e.g. *Rhodospirillu, Rhodopseudomonas*

(ii) Chemosynthetic autotrophs -

- These are nonphotosynthetic autotrophs i.e., photosynthetic pigments are absent.
- They use chemical energy instead of light energy for food synthesis.
- Chemical energy is obtained from oxidation of inorganic or organic compounds.
  On the basis of **oxidised compound** chemoautorophs are further classified into following two Categories

(a) Chemo-lithotrophs - These bacteria oxidise the inorganic compounds and release energy which is used for food synthesis.

This category includes following types of bacteria.

- Iron bacteria - These bacteria convert Ferrous compounds into Ferric compounds
  $Fe^{+2} \longrightarrow$ + energy
  e.g. ***Ferrobacillu, Leptothrix***

- Sulphur bacteria - convert the H_2S into Sulphur and water.
  $2H_2S + O_2 \longrightarrow 2S + 2H_2O$ + energy
  e.g., ***Thiobacillus, Beggiatoa***

- Carbon bacteria - Convert CO into CO_2
  $2CO + O_2 \longrightarrow 2CO_2$ + energy
  e.g. Bacillus oligocrbophylus

- Nitryfying bacteria - They oxides nitrogenous compounds and obtain energy.
  ● Nitrite bacteria - Converts ammonia into Nitrite
  e.g. Nitrosomonas

  ● Nitrate bacteria - Convert nitrite into nitrates.
  e.g. Nitrobacter

- Hydrogen bacteria - Convert the hydrogen into water
  $4H32 + CO_2 \longrightarrow CH_4 + 2H_2O$ + energy
  e.g. Bacillus pentotrophus, Hydrogenomonas

(b) Chemo - organotrophs - These bacteria oxidise the organic compounds and released energy is used for food synthesis.

This group includes only one type of bacteria

- Methano bacteria - These are found in the marshy places. They convert CH_4 into CO_2.
  e.g. methanomonas

Heterotrophs

- Most of the bacteria are heterotrophic i.e., they can not manufacture their own food.

- They receive their own food from **dead organic matter** or **living organism.**

The are following types

(i) Saprotrophic bacteria - These bacteria obtain food from dead and decarying organic matter.

These are of two types.

(a) Obligate saprotrophic - These bacteria obtain food only from dead organic matter. These are completely saprophytic.
e.g. Bacillus vulgaris, Clostridium botulinum

(b) Facultative parasite - These are normally saprophytic in nature, but in the absence of dead organic matter they can become parasite.
e.g. Pseudomonas, Staphylococcus

(ii) Parasitic bacteria - They obtain their food from living organism

These are of two types

(a) Obligate parasite : They always remain parasitic.
e.g. Mycobacterium leprae

(b) Facultative Saprotrophic - They are normally parasitic in nature but in the absence of living host, they may become saprophytic.
e.g. Mycobacterium tuberculosis

Symbiotic bacteria

These bacteria convert atmospheric nitrogen into nitrogenous compounds like Amino acid NO_3 or Salts of ammonia
e.g. Rhzobium

RESPIRATION

On the basis of respiration bacteria are of two types

Aerobic bacteria

These are of two types

(A) Obligate aerobic - These are completely aerobic and die in the absence of O_2
eg. Azotabacter

(B) Facultative anaerobic - These are normally aerobic bacteria but can survive in the absence of O_2
eg. Acetobacter aceti
Clostridium tetani

Anaerobic bacteria

These are of two types.

(A) Obligate annerobic - These are completely anaerobic bacteria and do not have capacity of aerobic respiration.
eg. Clostridium botulinum

(B) Facultative aerobic - These are normally anaerobic but also have capacity of aerobic respiration.
eg. Fermentation bacteria accept ***Acetobacter aceti***