

BACTERIAL IDENTIFICATION

Simple Staining

Aim

To compare the morphological shapes and arrangements of bacterial cells

Principle

In simple staining, bacterial smear is stained with a single reagent, which produces a distinctive contrast between the organism and its background. A simple stain that stains the bacteria is the direct stain. The purpose of simple staining technique is to determine cell shape, size arrangement of bacterial cells. Simple staining is performed by using basic stains which have different exposure time (Crystal Violet 20-60 s, Carbol fuschin 15-30 s and Methylene blue 1-2 minutes).

Procedure

- Clean glass slide was taken and was washed and dried.
- Bacterial smears were prepared from the bacterial cultures.
- The slide was kept on the staining tray and 5 drops of stain was added for a designed period.
- The extra stain was poured off and the smear was washed gently under slow running tap water.
- The slide was then blot dried using blotting paper.
- The slide was then examined under 10X, 45X and oil immersions objects respectively.

Observation

On the basis of microscopic observation, bacteria appeared blue, violet and red respectively depending on the stain taken.

Differential staining

Differential staining requires the use of at least 3 chemical reagents that are applied sequentially to a heat fixed smear. Its function is to impart its colour to all cells. In order to establish a colour contrast, the second reagent used is the decolorizing agent. Based on the chemical composition of cellular components the decolorizing agent may or may not remove the primary stain from the entire cell or from any cell structure. The final reagent is the counter stain. Following discoloration, if the primary stain is not washed out, the counter stain cannot be absorbed and neither the cell nor its components will retain the colour of the primary stain. If the primary stain is removed, the decolorized cellular components will accept and assume the contrasting colour of the counter stain. In this way, cell type or their structure can be distinguished from each other. On the basis of the stain that is retained the most important differential stain used in bacteriology is the Gram stain.

Gram Staining

Aim

To differentiate two principal groups of bacteria

Principle

The Gram stain, a differential stain was developed by Hans Christian Gram, a Danish physician, in 1884. Gram staining classifies bacteria into 2 major groups, Gram positive and Gram negative bacteria. The Gram stain reaction is based on the difference in the chemical and physical composition of bacterial cell wall. Gram positive cells have a thick peptidoglycan layer, whereas peptidoglycan layer in Gram negative cells is much thinner and surrounded by outer lipid containing layer.

In Gram negative, the higher amount of lipid in the formation of large pores thus facilitating the leakage of crystal violet-iodine complex and resulting in the decolonization of the bacterium which later takes this complex counter stain. In contrast, the Gram positive cell wall are thick and composed mainly of proteins and cross linked mucopeptide, when treated with alcohol it causes dehydration and closure of the cell wall pores thereby not allowing the loss of complex and cell retains primary stain. The bacteria which retain the primary stain appear dark blue or violet and not decolorized when stained with Gram's method are called Gram positive, where as those that lose the crystal violet used counter stain, saffranin appear red are called as Gram negative.

The Gram stain uses different reagents in the order, crystal violet, iodine solution, alcohol and saffranin.

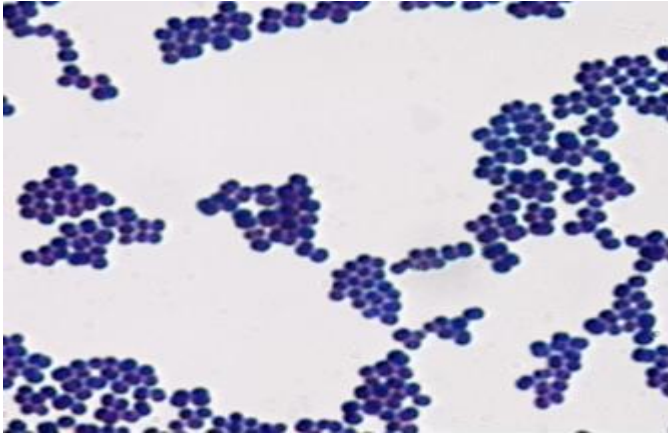
Procedure

- Thin smear was prepared of the given bacterial species on a clean glass slide.
- Let the smear dry.
- Heat fixed smear.
- Hold the smear using the slide rack.
- Covered each smear with crystal violet for 1 minute.
- Washed each slide with distilled water for few seconds using wash bottles.
- Covered each smear with Gram's iodine solution for 1 minute.
- Gently washed with distilled water.
- Decolorized with 95%.
- Washed the slide with distilled water and drained.
- Counter stain was applied saffranin for 30 seconds.
- Washed with distilled water and blot dried with absorbent paper.
- The stained slides were air dried and observed under the microscope.

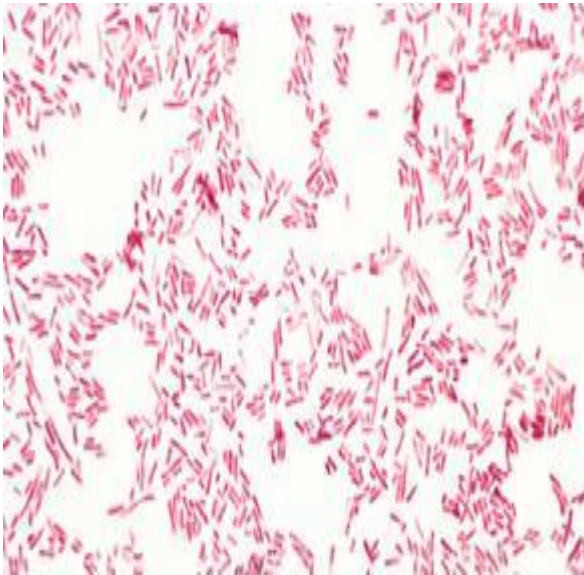
Observation

- Examined the slides microscopically using oil immersion objective.
- Identified the Gram reaction of the given cultures and classified it and described the morphology and arrangement of cells.

Those bacteria that appear blue are referred to as Gram positive and these appearing pink are described as Gram negative.



Gram Positive



Gram Negative

Fig. 11. Gram Staining