Experiment Details

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| Department Name | Biotechnology |
| Class | S.Y.BTech |
| Semester | 3rd |
| Subject Name | Microbial Technology |
| Experiment No. | 01 |
| Experiment Name | Monochrome staning |

Version History

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| Sr. No. | Version Number | Created By | Approved By | Date |
| 1 |  | Rameshwari Arun Metil | Mrs. Medha Petkar | 12/10/2020 |
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AIM:

To stain bacteria by simple (monochrome) staining method

THEORY:

Bacteria are microscopic organisms that cannot be seen with unaided eye. They can be seen even in unstained preparations such as a wet mount or hanging drop preparation but the morphology is not clear. Bacteria are colorless and when suspended in saline they don’t offer any contrast. Besides, bacterial motility makes it difficult to observe the morphology clearly. Hence, bacteria have to be stained to observe them. The dyes often used are toxic chemicals that kill the bacteria. Simple staining is also called as monochrome staining or positive staining.Examples of simple stain are Methylene blue, Safranin, Malachite green, acid fuchsine and Crystal violet etc. In simple staining procedure cell are uniformly stained.

**MECHANISM –** On cell surface ‘-’ charge is present. When we apply basic stain having ‘+’ charge on chromophore group(Basic stain) it react with cell surface charge and get deposited on it and cell get stained

CHEMICALS:

Loeffer’s Methylene blue stain - Methylene blue chloride powder – 0.3 g , 95% Ethanol - 30 ml, 0.1% KOH – 100 ml

PRE TEST:

1. What is stain

2. What is need of staining bacteria

3. What is suspension

4. What is saline

5.Why bacteria are suspended in saline

PROCEDURE:

1. Take a clean grease free glass slide and a circle is marked on one side of the slide using a wax pencil or a glass marker pen (It is labeled with the name of the organism if more samples are there).
2. A small portion of culture suspension is spread evenly within the circle to produce a smear.
3. Once a uniform smear is prepared, it has to be air dried.
4. The smear must be held above the Bunsen flame at comfortable height to fix it (Smears can be fixed physically or chemically).
5. After heat fixation the slide is placed on the staining rack and flooded with a particular stain and this stain is allowed to react for three minutes.
6. Further the slide is washed under running water gently.
7. The slide is air dried and observed under oil immersion lens.
8. Note dowm the sodium thiosulphate added which gives the DO concentration.
9. Take down the DO level of a given sample and plot conc. Against time.

**DIAGRAM:**

POST TEST:

1. Why oil is used as mounting media to visualise bacteria?

2. How much magnification of microscope is used to visualize cells

3. Types of stain based on chromophore group

4. Why basic stains are used to stain bacterial cells

5. Two names of acidic stain

6. Two names of basic stain

7. Two names of neutral stain

8. Which oil is preferd as mounting media

REFERENCES:

KIT Biotechnology engineering department S.Y. BTech