LAB WORK 8.

Subject: Staining Microbial Structures: Inclusions.
Session Purpose: Methods for detection and visualization of intracellular polymers stored.
Objectives:
1.To determine volutin in the cells of *Saccharomyces*.
2.To determine the granules of polyglucose in the cells of Bacteria and Yeast.
3.Detection offat inclusionsin*Bacillus.*
4.Stained fat inclusions.
5. Performexercises 1-4.

Lab Exercise 1.Detection and visualization of Polyphosphate (volutin granules) in *Yeast*.

Methodical instructions: To identify volutin in *yeast* usually used the following method.

Procedure:

1. Fixed smear stained with methylene blue (Loeffler's methylene blue staining) for 3 min.
2. The dye is poured, the drug is washed with water and without drying, applied to smear a small drop of 1% solution of sulfuric acid.
3. A smear covered with a coverslip.

Volutinappears the form of drops of *blue-purple* color on the *little-blue* background of the cytoplasm.

Lab Exercise 2.Detection and visualization of Polyphosphate (volutin granules) in *Bacteria.*

Methodical instructions: Volutin detected by the method of coloring Omelyansky. Coloring is based on the metachromatic granules of low solubility in acid solutions.

Procedure:

1. To skim the slide is prepared thin smear of bacteria, it is dried in air and fixed over the burner flame.
2. On the fixed smear Ziehl's solution is poured and stained the cells for 0.5 min without heating.
3. The dye is poured, the drug was washed with water and additionally stained with methylene blue (1:40) for 20-30 sec.
4. The drug is again washed with water and dried.

When properly stained grains volutin are *red* and clearly visible agains the background of *blue* cytoplasm.

Lab Exercise 3.Granules of polyglucose.(Glycogen, starch, granulosis).

Methodical instructions: Glycogen inclusions in cells of the well to investigate in *Saccharomyces cerevisiae* and *Bacillus mycoides* one-two-day age. To detect an object in granulosis cane use enrichment culture of *Clostridium.* These substances are detected microchemical processing cells Lugol's iodine solution.

3 a. Glycogen, starch (in *Saccharomycescerevisiae*).

Procedure:

1. A drop of cell suspension test organisms on a slide add a drop of Lugol's solution.
2. The drug is covered with a coverslip.

Granules of *starch* substances stained *blue*, and the pellets *glycogen* - a *russet.*

3 b. Granulosis (in *Clostridium butyricum*, *Cl. butylicum, Cl. Pasteurianum*).

Procedure:

1. Apply a drop of microbial culture on a glass slide.
2. Add a drop of enrichment cultures Lugol'ssolution .
3. Covered with a coverslip, on which is placed a drop of immersion oil.

In places the cells, which contain *granulosis*, there is a *blue* color.

Lab Exercise 4.Stained fat inclusions.

Procedure:

1. Apply a drop of microbial suspension on a slide.
2. Add a drop of solution of Sudan III.

Sudan III was dissolved in fat inclusions of the bacterial cell, turning them into an *orange-red*, the cytoplasm of the cell remains colorless.

Equipment:

- Microscope
- Slide
- Several cover glasses
- Dropper bottle of water
- Disinfectant tray
- Culture of Yeats and Bacteria in slant tubes
- Inoculation loop
- Burnerflame
- Immersion oil
- Staining material:

- Loeffler's methylene blue
- Ziehl's solution
- Lugol's solution
- Sudan III.