LAB WORK 11.

Subject: Morphology of eukaryotic.

Session Purpose: To familiarize with the basic characteristics of microscopic fungi, yeasts to study the morphology of fungi and yeasts, to determine the genus of fungi.

Objectives:

1. In vivo preparations view of culture of fungi *Mucor, Penicillium*and*Aspergillus*. Sketch the mycelium and spore bodies.
2. Determine the generic identity of unknown fungi, using the provided key.
3. View the colonies of fungi grown on solid nutrient medium in Petri dishes at low magnification microscope.
4. Prepare stained with methylene blue preparation "crushed drop" the yeast *Saccharomyces* genus and *Rhodotorula*, determine the shape of cells, to identify budding, living and dead cells. Sketch the yeast cells.
5. Performexercises 1, 2.

Lab Exercise 1.The study of the morphology of Mold.

Methodical instructions: Colonial morphology, the structure of the mycelium and sporophores location can be studied by looking at the colonies of fungi grown on solid nutrient medium in Petri dishes at low magnification microscope.

Morphological structure of hyphomycetes studied in squashed droplet, which is separated by dissecting needles small portion of mycelium from fruit-bearing hyphae and putting it in a drop of water, straighten, cover with a coverslip and mikroskopiruyut.

Procedure:

1. First, browsing the drug 8x lens, but after the discovery of fruiting hyphae establish a 40x lens. Morphology blastomycetes studied in smears under immersion lens.
2. Fixing and painting of the aerial mycelium of fungi.
3. On the aerial mycelium of the fungus that developed on solid nutrient medium in a petri dish, put 2 drops of 96^{o} alcohol, then immediately 2 drops of concentrated acetic acid.
4. Stained with alcohol-water solution of gentian violet in 1:40 2-3 minutes.
5. Then poured into the dye, washed with water and covered with colored mycelium coverslip area, consider it under low magnification microscope.

To familiarize yourself with the form of spores and mycelia of fungi make the drug "mark" (see above). This drug is also useful for studying
natural arrangement of cells in colonies of microorganisms.Genus of fungus determined by a key Nikitinsky- Aleeva, the results described in the form:

Genus	Type ofmycelium	Fertilehyphae	Type ofspores	The location ofspores	Picture

The key to the genera of fungi belonging:

1. Fungi reproduce sporangiosporami inside the sporangia - 2.
Fungi reproduce conidia formed on the outside of the special conidiophores, sometimes directly on the mycelium - 5.
2. Sporangiophores bearing sporangia, usually simple, rarely simple branching. Sporangia are all the same - 3. Sporangiophores branching. Sporangia of two kinds: large - on the main axis, and small (sporangioli) - on the side branches - 4.
3. Sporangiophores single, simple, sometimes branched. Sporangia small or large, is always homogeneous, colorless or colored. Spores roundish or elliptical, smooth, colorless or

greyish - *Mucor*.Sporangiophores are shrubs growing on stolons from one center, with large black heads. Spores, rounded ovate, wrinkled - *Rhizopus.*

4. Bush branches are whorled on the main axis Sporangiophores in one or more tiers. Designated branches nerazduty.Spores cylindrical, and ellipsoidal, colorless - *Thamnidium*.

Bush branches diverging from the blistering on the main axis Sporangiophores. The branches of the second order is also moving away from swollen areas. Sporangioli sit on small swellings of the terminal branches. Spores ellipsoidal or spherical, colorless - *Chaetostilum*.

5. Conidia are formed on conidiophores much different from the ordinary vegetative hyphae - 6.

Conidiophores and differ little from ordinary hyphae, or none at all, and conidia are formed directly on the mycelium - 14.

6. Conidiophores branched profusely in various ways - 7.

Conidiophores not branched or sometimes branched, but only slightly. Branching is simple, forked or kustoobraznoe - 9.

7. Branching tree - 8. Branching racemose or repeatedly forked, conidia are in chains, smooth, colorless or colored, round - *Penicillium*.

8. Conidiophores treelike branching, large. The branches are arranged randomly, the conidia at the ends of the branches of shrubs developed, colorless, ovoid, smooth - *Botrytis*.

Conidiophores treelike branched. The branches are whorled. Conidia formed singly or in batches, elongated-ovate or elliptical, colorless or slightly colored - *Verticillium*.

9. Conidiophores unbranched, long, with large pear-shaped clumps of conidia at the end. Conidia two-celled, hyaline or pinkish.Colonies of the fungus a yellowish-pink - *Trichotecium*.Conidiophores and conidia of some form - 10.

10. Conidiophores branched. Branching rarely simple forked - 11. Simple (just as an exception branching) are at the end of conidiophores clavate or vesicular swelling and sometimes swelling is absent - 12.

11. Conidia large, irregularly shaped, dotted with warts, arranged in chains, painted in brown. Colonies at first and then the mucous fluffy, with a strong odor of hydrogen arsenic - *Acaulium*.

Conidia hyaline, long, serpoobraznye, multicellular (with transverse septa), sometimes there are a chain of one another or appear directly on the mycelium. Colonies of the fungus are colored in pink (especially the underside) - *Fusarium.*

12. The ends of the conidiophores clavate or swollen and saccately range covered by sterigmata bearing conidial chains - 13.

Extension on the end is often missing, sterigmata are only at the top of konidkonostsev but do not grow on the sides. Conidia small rounded, smooth, hyaline, sterigmata are in chains - *Cytromyces*.

13. Sterigmata covering club-shaped swellings, simple, unbranched, bear conidial chains. Conidia round, smooth or prickly, stained or discolored - *Aspergillus.*

Sterigmata branching form to saccately inflated conidiophore two tiers. The top is a chain of conidia. Conidia smooth, rounded, painted - *Sterigmatostis*.

14. Conidia are formed directly on the mycelium - 16.

Conidia are formed on conidiophores, slightly different from the ordinary vegetative hyphae - 15.

15. Conidiophores are visible only when cultured in the (hanging drop). Conidia easily fall apart - 17.

Place of formation of conidia are visible in ordinary microscopic preparations - 18.

16.Odnokletochnye conidia hyaline, veretenovkdnye or round-oblong. Conidia slimy, black - *Dematium.*

Conidia unicellular, ovoid, drozhzheobraznye. Young colonies are similar to yeast, then become shaggy - *Monilia*.

17. Conidia are obtained simply by dividing the mycelium and is easy to fall off, colorless, rectangular, sometimes united in short chains - *Oidium.*

Conidiophores long, multicellular. Conidia irregular(Long, rounded or limonoobraznye), painted in a light olive-green color - *Cladosporium.*

18. On the slow-growing colonies no real conidiophores. Small, shiny, yellow-brown conidia formed very long chains at the ends of ordinary hyphae - *Catenularia.*

Large multicellular, round-pear-shaped or acuminate-oblong conidia are formed singly or in short chains on short lateral branches of vegetative hyphae, conidiophores play a role - *Alternaria.*

Lab Exercise 2.Morphology of yeast.

Methodical instructions: The shape of vegetative cells of yeast varied: round, oval, ovoid, cylindrical, lemon, etc. Many yeast reproduce by budding, in which the cell surface formed small bump - kidney, which was gradually increased to the size of the mother cell. At the same time or successively formed buds on different sides of the mother cell is plural or multilateral budding.

Procedure:

1. Prepare the yeast preparations "crushed drop".

2.Stained with methylene blue. When the microscope with 40x objective to determine the shape of yeast cells, the presence of the living and the dead, and the number of budding cells. Stained yeast cells are dead.

3.The results recorded in Table.

Table - The morphology of yeast cells

Theshapeofcells	The number ofbudding cells,%	The number ofdead cells,%

Equipment:

- Microscope
- Slide
- Several cover glasses
- Dropper bottle of water
- Disinfectant tray
- Inoculation loop
- Burnerflame
- Culture of Yeast in slant tubes
- Petri dishes with cultures of Mold
- Gloves
- Filter paper
- Staining material:

- Gentian violet 1:40
- Methylene blue