Lab class no. 2 – Evaluation of efficacy of disinfectants, evaluation of colonies, Gram staining, isolation inoculation

(in pairs)

Aims:

- *Evaluation of efficacy of disinfectants.*
- *Evaluation of colonies*
- *Gram staining*
- *Microscopy – permanent preparations*

Material

Plastic bacteriological loop

Set of solutions for Gram staining

Glass slides

Ethanol burner

Microscope

Prepared dish Nutrient broth agar and Malt agar for isolation inoculation

Work procedure

1. **Evaluation of efficacy of disinfectants and evaluation of colonies of microorganisms *M. luteus*, *E. coli* and *S. cerevisiae*:**

- count the colonies in the respective quadrants and calculate % of efficacy of all disinfectants for all three microorganisms - put the results of % of efficacy to graphs into your protocols
- further evaluate colonies of all three microorganisms with respect to these parameters:
  a. **size** - colonies are dotted (if smaller than 1 mm) or their size is expressed as diameter in mm
  b. **shape** - round, irregular shape, …
  c. **edges** - straight, wavy, protuberant, …
  d. **profile** - flat, slightly bulging, convex, raised edges, …
  e. **surface** – shiny (S-phase), smooth, opaque, rough (R-phase), wrinkled, …
  f. **transparency** - transparent, translucent, opaque

g. **color** - colorless, white/greyish, production of pigments (*M. luteus* – yellow, *M. roseus, Serratia rubidea* – rose) or change in color of indicator present in medium (lactose fermenting and non-lactose fermenting enterobacterias on selective diagnostic media with lactose)

h. **change in surroundings** - change in color (production of pigments soluble in water, change in media pH – change in color of indicator), on blood agar we can see hemolysis of erythrocytes (partial hemolysis - α, full - β, double - α', greening - viridation)

2. Gram staining of *M. luteus*, *E. coli* and *S. cerevisiae*:

- Transfer small amount of chosen colony with bacteriological loop to a drop of physiological solution on glass slide and let dry
- this preparation then fix using heat - three times pass the glass slide through a flame; reversed side facing the flame!! (mild denaturation and killing of bacteria)
- now add to the preparation few drops of crystal violet for 20 seconds
- without discarding, add few drops of Lugol solution
- discard both solution and add enough Lugol solution and let it act for 20 - 30 seconds
- using a pipet add acetone-ethanol mixture to glass slide and let it flow over the preparation, until it becomes discolored (mixture flowing from preparation is also discolored)
- preparation then wash with distilled water
- then add few drops of safranine solution and let it dye the preparation for 30 - 60 seconds
- finally, wash the preparation and let dry; microscope in oil immersion (objective 100×).

3. Microscopy – permanent preparations

Protocol no. 2 will contain:

- evaluation of colonies of all three microorganisms as described in point no. 1
- procedure of Gram staining, principle, drawing or picture of your own results

- drawing of photo of permanent preparations - describe: cocci, bacilli, G+, G-, aerobic, anaerobic, normal microbiota or not, where we can find them (skin, GIT, …), disease which they can cause