Prokaryotes and Protozoa

Aim:

1. **Prokaryotes**
   Bacterial smear, Gram staining
2. **Yeasts**
   Saccharomyces
   Saccharomyces smear, staining of the smear
3. ***Protozoa***
   Hay infusion – native preparation
   Flagella, ciliated and amoeboid movement
4. **Staining and morphology of lactic acid bacteria of the *Streptococcus*, *Lactobacillus*, *Bifidobacterium* genera. Preparation of the fixed slide.**

You know that cell organisms separate into two groups: ***Prokaryotes*** and ***Eukaryotes***

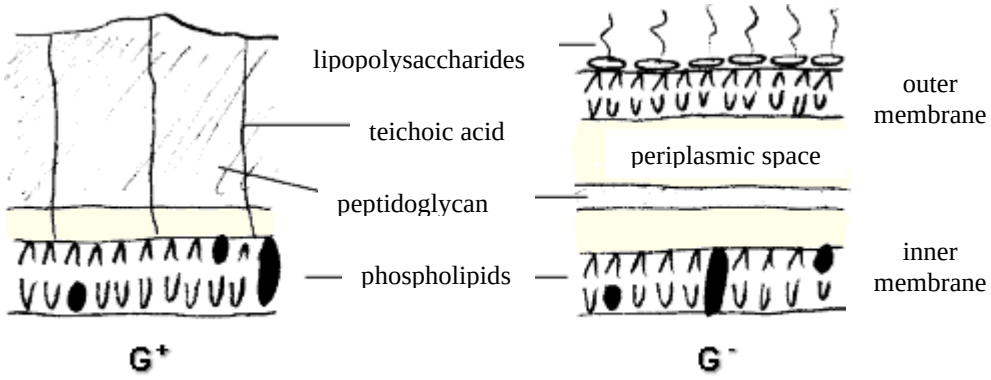
Prokaryotes have nucleus consisting of only one chromosome in the form of circular molecular of DNA without membrane, we describe it as a nucleoid. They don't have mitochondria. Their reproduction is asexual by cell division.
This group consists of: bacteria, cyanobacteria and archea.

Eukaryotes have nucleus consisting of more chromosomes divided by membrane. There are mitochondria, organelles and cytoskeleton in cytoplasm. Their reproduction is asexual (mitosis) or sexual.
When prokaryotes got the ability of phagocytosis it was turning point when prokaryotes developed into eukaryotes.

Prokaryotes – bacterial cell
Bacterial cell consists of structures, which we can separate into two groups:
- In common structures: nucleoid, cytoplasm, ribosome, cytoplasmic membrane and cell-wall.
- Other structures: cot, flagella, cilia, endospore, inclusion.

Every bacterium has cell-wall, which is very strong and it saves bacteria from effects of outer environment.
Cell-wall consists of peptidoglycan and teichoic acid.
Gram-positive bacteria have thicker cell-wall (about 15-20 nm) and they don't have lipids.

Cell-wall of gram negative bacteria is thinner (about 10 nm), but more complex, because there is outer membrane over peptidoglycan.



The cell wall of gram-positive and gram-negative bacterial cells

Task: Preparation of bacterial smear and it's dying according to Mr. Gram

Materials: bacterial culture, slide, bacteriological loop, microscope, Pasteur pipette

Chemicals: Gram I, Gram II (Lugol solution), safranin or fuchsin, acetone, alcohol, immersion oil

Method:

1. Put a drop of distilled water on the slide.
2. Take a little quantity of bacteria from bacterial culture and spread these bacteria in water.
3. Preparative must get dry.
4. Set the preparative in acetone for 10 minutes.
5. Put Gram I on dry preparative and leave it for 4 minutes.
6. Pour the dye into the dish.
7. Put Lugol solution (Gram II) on preparative for 4 minutes. Until the preparative gets black colour.
8. Wash down preparative carefully with alcohol and distilled water.
9. Put the fuchsine on preparative for 2 minutes.
10. Wash down the preparative with water
11. Dry the preparative.
12. Watch the preparative under the microscope.

Gram's dying

Gram's dying is important for separation of gram-positive and gram-negative bacteria. These are two main groups of bacteria.
Mr. H. C. J. Gram developed that method in 1884 and we use it so far.

Gram-positive bacteria are dyed with triphenylmethane dye (Gram I) and they stained in iodine solution (Gram II), there rise complex of dye and iodine solution. This complex is washed by organic solvent from cell-wall of Gram-negative bacteria together with lipids. Gram-negative bacteria are dyed with lighter dye (fuchsine or safranin).
G^+ are blue.
G^- are red.

Saccharomyces

Saccharomyces are heterotrophic eukaryotic organisms. We classify them into mushrooms. They have ability to ferment monosaccharide and produce alcohol and CO_2.

They have a very thick cell-wall (about 25 nm). It's complicated heterogeneous polymer, which consists of glucan, mannan, proteins, lipids, chitin and phosphate.

Typical signs of cell-wall saccharomyces are scars. Scar is produced during reproduction after separation of daughter cell from mother cell.

Their reproduction can by asexual or sexual (meiosis). These two phases go on in the cycle.

Task: Preparation of saccharomyces smear and dying

Materials: bacterial culture, slide, bacteriological loop, microscope, Pasteur pipette

Chemicals: Gram I, Gram II (Lugol solution), safranin or fuchsin, acetone, alcohol, immersion oil

Method:

1. Put a drop of distilled water on the slide.
2. Take a little quantity of bacteria from bacterial culture and spread these bacteria in water.
3. Preparative must get dry.
4. Set the preparative in acetone for 10 minutes.
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11. Dry the preparative.
12. Watch the preparative under the microscope.

Protozoa

To this group of microorganisms belong such the organisms, which have body consisting from only one cell, which is vital. Body of protozoa is consists of the same essential materials like body of metazoan. The main component is water (up to 90% of weight). Protozoa have very various anatomies, but all of them have cytoplasm and nucleus. The cytoplasmic membrane consists of proteins and lipids. This membrane directs penetrating of different compounds, ions, and foods into the cell.

Nuclei can have various forms (ball shaped, egg shaped, horse-shoe shaped). They reproduce by mitosis.

Apart from essential cell structures protozoa have other specific structures.

For example:

1. Skeleton (plate, pellicle)
2. protective organelles

3. organelles of movement
4. organelles of digestion
5. organelles of osmoregulation
6. organelles of senses

Protozoa have asexual or sexual reproduction. The life cycle of protozoa consists of turning phases of asexual or sexual reproduction followed by sporogonia. Important biological specialty of many protozoa is ability of encystations. It helps them survive bad condition.

Task: Preparation of native preparative from hay infusion

Materials: Slide, cover glass, Pasteur pipette, injection, microscope

Chemicals: NaCl solution (8 %)

Method:
1. Put a drop of hay infusion on slide and cover it by cover glass.
2. Watch protozoa under the microscope.

- Try to identify protozoa, which you found.
- Watch their movement.
- Get air under the cover glass by the help of injection and watch oxygenotaxis.
- Put salt solution near the cover glass. You can see movement of protozoa so-called chemotaxis (positive or negative).
- Describe and comment your results.

Staining and morphology of lactic acid bacteria of the *Streptococcus, Lactobacillus, Bifidobacterium* genera. Preparation of the fixed slide.

We host a wide range of bacteria in our intestine, and there is increasing scientific evidence supporting the view that maintaining a healthy gut microflora may provide protection against digestive and intestinal problems, including gastrointestinal infections and inflammatory bowel disease. (See Mattilda - Sandholm and Saarela (2000).

Modern lifestyle is getting rougher and more stressful, which can interfere with the function of bacteria in the gastrointestinal tract (GI tract). It is estimated that on average one in five regularly suffer from some form of gastrointestinal related problems. These problems may also contribute to a diet rich in processed refined foods, and on the contrary it was shown that probiotics these problems and helps to prevent them in all age groups of the population (Alm et al. (1993), Black et al., (1989 & 1995) Langhendries et al. (1995), Saavedra et al. (1994 & 1998).

Lactic acid bacteria

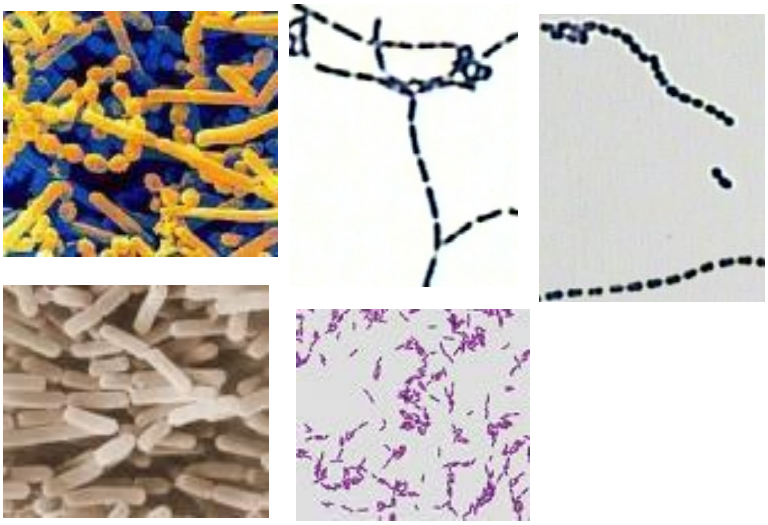
They are used to produce fermented dairy products

Yoghurt culture: It is used to produce yogurt, yogurt drinks

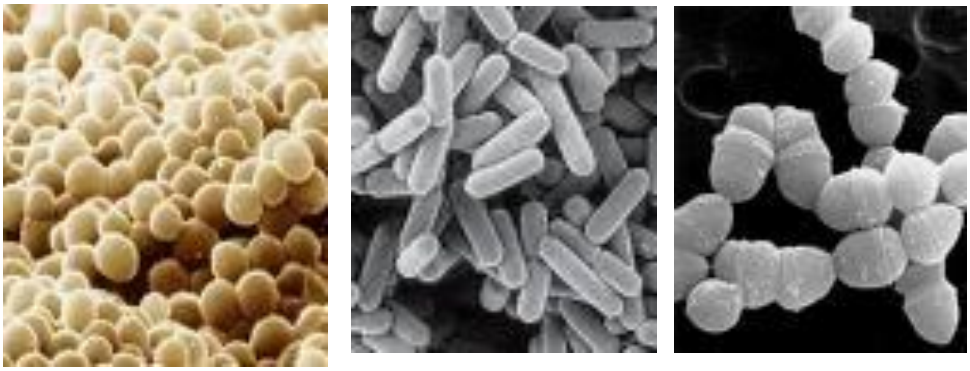
Lactobacillus delbrueckii* subsp. Bulgaricus*, Streptococcus thermophilus

Acidophilous:

It is used to produce acidified milk ***Lactobacillus acidophilus***



Kefir:
It is used to produce kefir milk: *Lactobacillus* sp., *Lactococcus* sp., *Streptococcus* sp., *Kluyveromyces* sp., *Candida* sp.

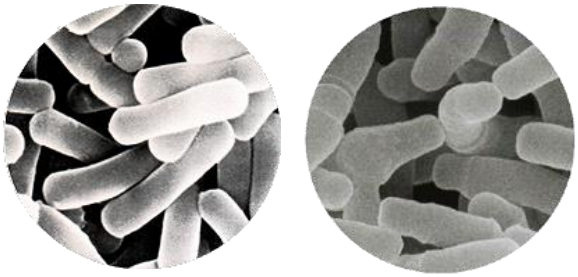


Lactic acid bacteria
Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus salivarius, Lactobacillus plantarum, Lactobacillus delbrueckii subsp. *bulgaricus, Lactococcus lactis, Enterococcus faecium, Streptococcus thermophilus, Pediococcus pentosaceus*

Bifidobacteria
Bifidobacterium animalis subsp. lactis, Bifidobacterium longum subsp. longum, *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum subsp. infantis, Bifidobacterium pseudolongum, Bifidobacterium thermophilum*

Other bacteria
Escherichia coli, Bacillus sp., *Clostridium butyricum*

Microfungi
Saccharomyces sp., *Aspergillus oryzae, Candida pintolopesii*



Criteria for probiotics
required:

- must be the same animal as a consumer
- viable
- capable of withstanding harsh TT
- able to colonize the digestive tract of the consumer

What probiotics should not

- be resistant to antibiotics
- produce toxic substances
- be pathogenic (capable of cau**sing disease)**

Task: To prepare fixed slide of lactic acid bacteria
Utilities: bacterial culture (milk and yogurt), a slide, bacteriological loop, microscope, Pasteur pipette.
Chemicals: Gram I, Lugol's solution, Safranine, acetone, alcohol, immersion oil
Procedure:
For microscopic observation of the morphology of streptococci, lactobacilli and bifidobacteria in yogurt or milk to use an optical microscope. To observe bacteria using immersion objective and a total magnification 1000x/1500x. On the surface degreased slide transfer to drops of water or saline examinee yoghurt sample using a platinum loop and spread evenly in a thin layer. The resultant coating carefully to dry the flame burner and finish 2-3 times through the flame. Thermally fixed slide subsequently to stain by Gram.