

**Cvičení 1**  
**Detekce bakterií**  
**Počítání bakterií**  
**Preparáty**  
**LUCIA G**  
**Makrofoto**

## **1) Makrofoto kolonií**

### **2) Preparáty**

– zviditelnění buňky, pomoc při identifikaci – morfologie buňky a typ buněčné stěny (Gram, acidorezistence, Giemsa..), sporulace...

- a) Mikroskopie v jasném poli (barvené preparáty – nejčastěji Gramovo a negativní barvení)
- b) Morfologie buňky a shluků, porovnávání velikosti buněk (prokaryota X eukaryota; bacily mezi sebou)

Použité bakteriální kmeny – od každého jedna miska:

- I) V rámci jednoho rodu,
  - a) G+ tyčky různé tvary, bacily 48h kultivace se spory:

*Bacillus thuringiensis* CCM 19 (G+ tyčky)

*Bacillus sphaericus* CCM 1615 (G+ tyčky)

*Bacillus mycoides* CCM 145 (G+ tyčky)

*Bacillus cereus* CCM 2010 (G+ tyčky)

*Bacillus megaterium* CCM 2007 (G+ tyčky)

*Bacillus subtilis* CCM 2216 (G+ tyčky)

*Lactobacillus curvatus* CCM 7558T – zakřivené

*Lactobacillus rhamnosus* CCM 1825T – typické tyčky

*Lactobacillus casei* subsp. *casei* CCM 7088T - zakřivené

*Lactobacillus brevis* CCM 3805T – krátké tyčky

- b) pokud bacilární – hledáme spory, fázový kontrast

### II) větvení buněk

*Lactobacillus delbrueckii* subsp. *bulgaricus* CCM 7190T – pro tento pokus se bude kultivovat ve vysokém sloupci M6 media – lze pozorovat větvení

*Streptomyces griseus* ssp. *griseus* CCM 2386 – vlákna, G+

*Nocardia carnea* CCM 2756 – krátká rozpadavá vlákna

### III) Archaea:

*Haloarcula hispanica* CCM 3601T

*Haloflex mediterranei* CCM 3361T

IV) cyklus: tyčka - kok

*Arthrobacter crystallopoietes* CCM 2386 (G+ tyčky a koky) – z kultivace 12h, 19h a 24h

V) eukaryota

*Saccharomyces cerevisiae* – kvasinka, eukaryotní buňka, pro fázový kontrast

VI) vliv media na morfologii buňky, zároveň nácvik barvení z tekutého media – pozor na oplach ze sklíčka!!

*Streptococcus mutans* CCM 7409T z krevního agaru a z tekutého media  
nácvik barvení 1 rodu (jednou tyčky, jednou koky)

VII) G- tyčky

*Serratia marcescens* CCM 303(G- tyčka)

*Escherichia coli* CCM 3954

VIII) Gramem nebarvitelné a palisády – smíšený preparát s *Micrococcus luteus*

*Mycobacterium phlei* CCM 5639

*Corynebacterium glutamicum* CCM 2428

IX) G+ koky nebo krátké tyčky

*Azotobacter vinelandii* CCM 289 (slizovité kolonie, pouzdra)

*Leuconostoc mesenteroides* CCM 1803 (slizovité kolonie, pouzdra)

*Sporosarcina ureae* CCM 860 – G+ tetrády

*Micrococcus luteus* CCM 169 - G+ koky

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Barvení:

Spirochetes can be stained with a variety of silver stains such as the Warthin-Starry, Dieterle and Steiner stains. Finally, due to the large number of gastrointestinal biopsies in routine practice, a large number of stains are available for visualization of the Gramnegative bacillus, *Helicobacter pylori*. These include Giemsa, Alcian yellow - toluidine blue, Diff-Quik, Genta, and Sayeed stains. A large number of laboratories prefer immunohistochemistry for identification of *Helicobacter pylori*. The Giemsa stain highlights several protozoa such as toxoplasma, leishmania, plasmodium, trichomonas, cryptosporidia and giardia. Ameba can be highlighted by the PAS stain due to their large glycogen content. Histochemical stains for fungi are discussed separately in this publication.

Modifications: The Brown-Hopps and Brown-Brenn stains are modifications of the Gram stain and are used for demonstration of gram negative bacteria and rickettsia.

Giemsa Stain

The Giemsa is used to stain a variety of microorganisms including bacteria and several protozoans. Like the Gram stain, the Giemsa stain allows identification of the morphological characteristics of bacteria. The Giemsa stain is also useful to visualize *H. pylori*. The Giemsa also stains atypical bacteria like rickettsia and chlamydiae which do not have the peptidoglycan walls. The Giemsa stain is used to visualize several protozoans such as toxoplasma, leishmania, plasmodium, trichomonas, cryptosporidia and giardia. Principles: The Giemsa stain belongs to the class of polychromatic stains which consist of a mixture of dyes of different hues which provide subtle differences in staining. When methylene blue is prepared at an alkaline pH, it spontaneously forms other dyes, the major components being azure A and B. Although the polychromatic stain was first used by Romanowsky to stain malarial parasites, the property of polychromasia is most useful in staining blood smears and bone marrow specimens to differentiate between the various hemopoietic elements. Nowadays, the Giemsa stain is made up of weighted amounts of the azures to maintain consistency of staining which cannot be attained if methylene blue

is allowed to "mature" naturally. Modifications: The Diff-Quick and Wright's stains are modifications of the Giemsa stain.

#### Silver Stains (Warthin Starry Stain, Dieterle, Steiner Stains)

Utility of the Stains: Silver stains are very sensitive for the staining of bacteria and therefore most useful for bacteria which do not stain or stain weakly with the Grams and Giemsa stains. Although they can be used to stain almost any bacteria, they are tricky to perform and are therefore reserved for visualizing spirochetes, legionella, bartonella and H. pylori.

Principles of Staining: Spirochetes and other bacteria can bind silver ions from solution but cannot reduce the bound silver. The slide is first incubated in a silver nitrate solution for half an hour and then "developed" with hydroquinone which reduces the bound silver to a visible metallic form. The bacteria stain dark-brown to black while the background is yellow

#### Auramine O- Rhodamine B Stain

The auramine O-rhodamine B stain is highly specific and sensitive for mycobacteria. It also stains dead and dying bacteria not stained by the acid-fast stains. The mycobacteria take up the dye and show a reddishyellow fluorescence when examined under a fluorescence microscope.

(krystalová viole[ a jod reagují uvnitř buňky, čímž vzniká sloučenina obsahující velké molekuly, které u grampozitivních bakterií neprojdou zpečetěnou membránou a nejsou rozpustné v alkoholu) a rozdílné vlastnosti protoplazmy (vnější vrstva u grampozitivních bakterií je grampozitivní a obsahuje magnesium ribonukleát, u gramnegativních bakterií tato vnější vrstva a magnesium ribonukleát chybí). Bylo zjištěno, že GRAMOVA reakce souvisí s vlastnostmi ribonukleových kyselin (ponořením buněk do cholátu sodného je GRAMOVA reakce anulována a opětovně obnovena působením magnesium ribonukleátu). Ribonukleáza specificky odstraní grampozitivní charakter buňky. Nemění souvisí GRAMOVA reakce s lipidy, mastnými kyselinami, bílkovinami, polysacharidy a fosforečnanovými estery. GRAMOVA reakci může též ovlivnit působení oxidací činidel, žlučových solí, antibiotik, UV záření, lysozymu či mechanického poškození.

### **3) Detekce bakterií – metody molbiol, biochem, imunochem**

#### **Immunodetection of inactivated Francisella tularensis bacteria by using a quartz crystal microbalance with dissipation monitoring.**

Francisella tularensis are very small, gram-negative bacteria which are capable of infecting a number of mammals. As a highly pathogenic species, it is a potential bioterrorism agent. In this work we demonstrate a fast immunological detection system for whole F. tularensis bacteria. The technique is based on a quartz crystal microbalance with dissipation monitoring (QCMD), which uses sensor chips modified by a specific antibody. This antibody is useful as a capture molecule to capture the lipopolysaccharide structure on the surface of the bacterial cell wall. The QCMD technique is combined with a microfluidic system and allows the label-free online detection of the binding of whole bacteria to the sensor surface in a wide dynamic concentration range. A detection limit of about  $4 \times 10^3$  colony-forming units per milliliter can be obtained. Furthermore, a rather short analysis time and a clear discrimination against other bacteria can be achieved. Additionally, we demonstrate two possibilities for specific and significant signal enhancement by using antibody-functionalized gold nanoparticles or an enzymatic precipitation reaction. These additional steps can be seen as further proof of the specificity and validity.

### **4) Počítání buněk bakterií**

– preparát, komůrky

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Všímáme si: tvar buněk, vyklenutí (způsobeno sporami?? vyklenují centrálně či terminálně?)  
poměru šířky a délky buněk

Co ovlivňuje vzhled buněk v preparátu: stáří kultury?? živná půda?? zvětšení??