The **State Doctoral Examination**

The **State Doctoral Examination (SDE)** checks whether the student´s general theoretical knowledge of microbiology is of a sufficient depth and width regarding the current state of knowledge in this scientific field. The purpose of the examination is also to test the student´s scientific abilities, *i.e.*, to comprehend the essence of any scientific problem and to propose their own creative solutions.

A short (*maximum 10 min*) presentation by the student, which introduces the topic of the respective doctoral project to the the members of the committee is a necessary part of the SDE (*the SDE begins with this presentation*).

The SDE further consists of an oral examination focused on the student´s knowledge on two subjects:

***1) the obligatory subjects:***

* Physiology of Microorganisms
* Genetics of Microorganisms

***2) the elective subject***(*the student selects one option among these stated below, in accordance to his/her doctoral project*):

* Molecular Biology
* Medical Microbiology
* Ecology of Microorganisms

The SDE focuses on three levels of student´s knowledge: 1) good general theoretical background and orientation in the whole field of microbiology; 2) knowledge on various methodical approaches utilized in microbiology, particularly these related specifically to his/her own research (their principles, various advantages and disadvantages); 3) advanced and detailed knowledge on the current state of scientific topics directly related to his/her doctoral project.

**Topics of obligatory and elective subjects:**

**Subject Physiology of microorganisms**

1. Characteristics of bacteria and archaea, historical milestones of bacterial physiology, strategies in physiological research of bacteria, model organism of bacterial physiology - *Escherichia coli*. Laboratory pure cultures and bacterial communities in nature.

2. Bacterial cell structure and function.

Chemical composition and structure of the bacterial cell, DNA, nucleoid and genomes of bacteria. Flagella, pili and fimbriae, capsules, S - layers, outer membrane of gram - negative bacteria, cell wall and periplasmic space, cytoplasmic membrane, cytoplasm and cytoplasmic compartments, storage materials. Bacterial motility.

3. Transport in bacteria.

Bacterial transport, lipids, membrane proteins, ionophores as models, types of transport, diffusion, primary and secondary transport, ABC transporters, phosphotransferase system, iron transport, native conformation of proteins, chaperones and chaperonins, protein transport to CPM, periplasm, outer membranes, protein secretion into the environment and bacterial secretion systems (Type I SS - Type VIII SS).

4. Bacterial nutrition, energy, catabolism and anabolism.

Bacterial nutrition, glycolysis and its alternatives, Krebs cycle, production and transformation of energy in the bacterial cell, aerobic respiration.

5. Biosynthesis and growth

Molecular composition of bacteria, nitrogen and sulphur assimilation, biosynthesis of amino acids, nucleotides, lipids, biosynthesis of cell wall structures, growth of individual cells, cell cycle, growth of bacterial population, continuous cultivation.

6. Metabolic diversity

Anaerobic fermentation, anaerobic respiration, chemolithotrophy, phototrophy.

7. Regulation in bacteria

Signal transduction and two-component systems. Regulation of enzyme synthesis, global regulation, regulation of enzyme activity

8. Microorganisms in the environment - effects of temperature, water activity, oxygen, pH on the growth of microorganisms.

9. Yeast life cycle.

10. Aging, apoptosis and necrosis in yeast.

11. Physiology of fungi and their role in the environment.

**Literature – bacteria and *Archaea***:

Kim B.H., Gadd, G.M.: Prokaryotic Metabolism and Physiology (2019), Cambridge University Press

Michael T. Madigan, Bender K.S., Buckley D.H., Satley W.M., Stahl D.A.: Brock Biology of Microorganisms, 15th Edition (2019), Pearson Education, 16th Edition (2021)

**Literature – yeasts**:

Yeast: Molecular and Cell Biology, 2nd Edition, Horst Feldmann (Editor) 2012, Wiley-Blackwell

**Subject Microbial Genetics**

**Bacteria and Archaea**

1. Basic characteristics of prokaryotic chromosome - differences between archaea and bacteria. Explanation and rules of heredity in haploid microorganisms.

2. Basic genetic terminology and nomenclature. The importance of lateral transmission of genetic information in the evolution of prokaryotes.

3. Regulation of gene expression and its regulation at the level of transcriptional, posttranscriptional, translational and posttranslational. Methods of study.

4. Homologous and site-specific recombination.

5. Mobile genetic elements: Structure, function and types of mobile elements of bacteria and archaea. Models of transposition mechanisms and its regulation. Influence of mobile elements on prokaryotic diversity.

6. Plasmids: Genetic determinants of natural plasmids of bacteria and archaea, their properties, classification. Mechanisms of plasmid replication regulation. Plasmids as a tool of genetic manipulation.

7. Bacterial conjugation: Mechanism of conjugative transfer and genetic determinants needed for conjugative transfer. Chromosome mobilization. Interspecies transmission of genetic information.

8. Transformation: Natural competence, (G + and G- bacteria). Integration of donor genetic material, discrimination of heterologous DNA.

9. Bacteriophages: Genome structure and genetics of basic bacteriophages (Lambda, P1, T4, T7, ssDNA bacteriophages, bacteriophage Mu). Bacteriophage bacterial cell infection: lytic and lysogenic response, bacteriophage replicative strategies, useful derivatives used in genetics. Archaea viruses (STIV).

10. Transduction: Nonspecific transduction: basic mechanisms. Specific transduction: basic characteristics, use in bacterial genetics.

11. Mutations and mutagenesis: specific, non-specific mutagenesis, mutation rate, types of mutations, allelic exchange, methods of selection and isolation of mutants, culture media. Mutants used in bacterial genetics (auxotrophy, suppressors, conditional mutations).

12. Principles of genetic analysis. Methods of genetic analysis, dominance and recessivity of alleles, partial diploidity, complementary genetic analysis. Genetic and physical mapping. Genetic analysis in representative archaea (*Sulfolobus spp*.).

13. Genetic approaches in model microorganisms: problems in genetic manipulation, restriction modification systems.

14. Methods of global analysis: transcriptome, proteome, analysis of regulatory proteins.

15. Analysis of microbial populations: Metagenomic approaches, cultivation-free approaches.

**Yeasts**:

16. Plasmids, transposons and prions in yeasts.

**Literature – bacteria and *Archaea***:

Molecular Genetics of Bacteria, Snyder, L. and Champness, W. 4th Ed, (ASM Press 2013), 5th Ed, (ASM Press 2020)

**Literature –yeasts:**

**Yeast: Molecular and Cell Biology,** 2nd Edition, Horst Feldmann (Editor) 2012, Wiley-Blackwell

**Subject Molecular biology**

Molecular biology and genetics and -omics - subject of study and methodology.

Molecular evolution in the sense of "RNA world", "RNA and protein world", and "DNA world"

History of modern molecular biology.

Structure, topology and function of DNA.

RNA structure, conformation and function.

Complementarity of bases, WC and non-WC pairing

Central dogma of molecular biology and function of information biopolymers.

Structure, conformation and functional classification of proteins.

Gene, genome and chromosome in bacterial, archaeal and eukaryotic cells.

Nucleosome and chromatin; chromatin remodelling.

Cell cycle, chromosome duplication and segregation.

DNA replication, replication fork enzymes and replication accuracy.

DNA damage and repair (reparative) DNA synthesis.

Homologous and site-specific recombination.

Mobile elements (transposons) and transpositions.

V (D) J recombination.

Archival and bacterial transcription and RNA polymerases.

Eukaryotic RNA polymerases and transcription factors.

Posttranscriptional modification and modification of RNA.

Primary transcript splicing mechanisms; alternative splicing.

Pre-tRNA splicing; translational splicing.

RNA editing; reading frame shift; missense, nonsense and frameshift mutations.

Structure of mRNA and translation in archaea, bacteria and eukaryotes.

Regulation of transcription in archaea and bacteria.

Regulation of transcription in a eukaryotic cell.

tRNA as a model of RNA structure and its function in translation.

Small RNAs and their functions in posttranscriptional modifications.

Aptamer properties of RNA and riboswitches.

RNA interference and the role of siRNA and miRNA in gene silencing.

Structure and function of ribosomes.

Initiation, elongation and termination of translation.

Genetic code.

Posttranslational protein modifications.

Basics of genetic engineering. Cloning of molecules, cells and organisms.

DNA sequencing and genomic analysis.

Restriction-modification systems in bacteria.

Plasmids, viruses and molecular vectors.

Modification of information biopolymers and gene silencing.

Model organisms in molecular biology.

Main methods of contemporary molecular biology.

Transgenic organisms.

Literature – molecular biology:

T.D. Pollard a kol. Cell Biology, 3rd Edition, Elsevier Health Sciences, 2016

B. Alberts a kol., Molecular biology of the cell, 6th Edition, Garland Publishing, Inc., 2014

H. Lodish a kol., Molecular cell biology, 8th Edition, W.H. Freeman and company, 2016

H. Lodish a kol., Molecular cell biology, 9th Edition, W.H. Freeman and company, 2021

B. Lewin, Genes XII, 12th Rdition, Jones and Bartlett Publishers, 2017

T. A. Brown, Gene Cloning and DNA Analysis: An Introduction; Blackwell Publishing Incorporated, 7th Edition, 2016

J.D. Watson a kol., Recombinant DNA 3rd. Edition, CSHL Press, 2007

**Subject Medical Microbiology**

**General**:

Natural antibacterial immunity

Acquired (adaptive) antibacterial immunity

Passive immunization and non-specific immune support

Types of vaccines, vaccination

Methods of sterilization and disinfection

Mechanisms of action of antimicrobials

Antimicrobial susceptibility testing

Development of bacterial resistance to antimicrobials

Mechanisms of bacterial resistance to antimicrobials

Physiological bacterial flora of the human body

Biofilms and regulation of bacterial flora

Pathogenicity and virulence of bacteria

Bacterial virulence factors

Bacterial exotoxins

Bacterial enterotoxins

Bacterial superantigens

Principles of collection of clinical materials

Possibilities of detection and identification of bacteria

Cultivation examination of clinical materials

Properties and cultivation of anaerobic bacteria

Serological examinations, skin tests

**Special part - bacteria** :

Neurotoxic clostridia

Histotoxic clostridia

Non-sporulating anaerobic bacteria

Conditionally-pathogenic enterobacteria

Yersinia pestis and plague

Salmonella, Shigella

Vibrio cholerae and cholera

Haemophilus, Pasteurella

Pseudomonas aeruginosa

Bordetella

Brucella, Bartonella

Francisella tularensis

Campylobacter

Helicobacter

Legionella

Neisseria gonorrhoeae

Neisseria meningitidis

Treponema pallidum

Leptospira

Borrelia

Staphylococcus aureus

Coagulase-negative staphylococci

Streptococcus pyogenes

Streptococcus agalactiae and viridizing streptococci

Streptococcus pneumoniae

Enterococcus

Bacillus anthracis and Bacillus cereus

Corynebacterium diphtheriae

Listeria monocytogenes

Mycobacterium tuberculosis

Atypical mycobacteria

Actinomycetes and actinomycosis

Rickettsia, Chlamydia, Mycoplasma

Bacterial nosocomial infections

**Special part – viruses, parasites, fungi:**

Poxviruses

Varicella virus and herpes zoster

Herpes simplex virus

Cytomegalovirus

Papillomaviruses

Adenoviruses

Influenza viruses

Poliomyelitis virus

Rhinoviruses

Causative agents of diarrheal viral diseases

Viruses transmitted by arthropods

Tick-borne encephalitis viruses

Rabies virus

Flaviviruses and arenaviruses

Coronaviruses and SARS

Filovir

Agents of viral hepatitis

Epstein and Barr virus

Human immunological deficiency viruses

Infectious agents without nucleic acids - prions

Trypanosomes, sleeping sickness

Leishmania, leishmaniasis

Trichomonas vaginalis

Entamoeba histolytica

Amoebas, Naegleria fowleri

Toxoplasma gondii

Plasmodium, malaria

Tapeworm

Enterobius vermicularis, Ascaris lumbricoides

Filaria

Trichinella spiralis, Dracunculus medinensis

Transmission of infectious diseases by arthropods

Mycosis agents, Candida albicans

**Literature – Medical Microbiology:**

Julák J.: Úvod do lékařské bakteriologie.

Karolinum, Praha, 2006. ISBN 80-246-1270-4 (only bacteriology, detailed).

Julák J., Pavlík E.: Lékařská mikrobiologie pro zubní lékařství.

Karolinum, Praha, 2010. ISBN 978-80-246-1141-9 (sufficient for non-medics, includes the essentials of virology, parasitology, mycology, prions)

Hurych J., Štícha R. et al.: Lékařská mikrobiologie – repetitorium. 2. vydání,

Triton, Praha 2021, ISBN: 987-80-7553-900-7 (excellent textbook, written by medics for medics). <https://www.tridistri.cz/lekarskamikrobiologie-repetitorium-2.vydani?ItemIdx=1>

Bednář M., Fraňková V., Schindler J., Souček A., Vávra J.: Lékařská mikrobiologie.

Marvil, Praha, 1996 (older but useful for the overview of pathogens).

Beneš J. a kol.: Infekční lékařství.

Galén, Praha, 2009. ISBN 978-80-7262-644-1 (clinical aspects of infectious diseases).

Carey R.B., Schuster M.G., McGowan K.L.: Lékařská mikrobiologie v klinických případech.

Triton, Praha 2011. ISBN: 978-80-7387-480-3 (model clinical cases of infectious diseases)

**Subject Ecology of Microorganisms**

**General topics:** Ecology of micro- and macro-organisms

Niche and habitat of microorganisms, biogeography of microorganisms, cultivable and uncultivable microorganisms

Concept of species in microorganisms

Taxonomic and functional diversity

Biofilms and microbial mats

Relationship of microorganisms to environmental conditions

**Metabolism of microorganisms**: Redox transformation of organic substances in oxic and anoxic environments. Phototrophy and chemolithotrophy

**Carbon cycle:** decomposition of organic matter, utilization and production of CO2 and methane

**The nitrogen cycle:** Nitrogen sources in the environment, nitrogen fixation, ammonification, nitrification and denitrification

**Functions of microorganisms** **and methods** of their study: Isolation and quantification of microorganisms, biomarkers, quantification of microbial processes.

**Genes** used for taxonomic analysis of the microbial community, sequence database

Molecular methods for qualitative and quantitative analysis of genetic diversity

Analysis of microbiome composition. Sequencing methods, metagenomics and metatranscriptomics.

**Interaction** of microorganisms: Spread of microorganisms. Predation, competition, mutualism.

**Fungi** in the ecosystem: morphology and genetics, spread of fungi.

Specific features of the physiology and ecology of fungi, reactions to external conditions.

The importance of fungi in the ecosystem: decomposition and symbiosis with plants, interaction with other organisms.

**Microorganisms in soil**: soil as an ecosystem, living and non-living soil components.

Trophic relationships in the soil, flow of energy and carbon in the soil community. Relationships between above- and below-soil surface parts of the ecosystem. Microbiology of deep subsurface environments.

**Microorganisms in fresh waters, seas and oceans**.

Standing water: characteristics of the ecosystem, vertical stratification, light, temperature, nutrients. Organisms and functional groups, food relationships. Energy and carbon flow, microbial loop. Migration. Competition.

Flowing water: Ecosystem characteristics, horizontal stratification. The carbon cycle. Organisms and functional groups. Energy and carbon flow. The microbial loop.

Seas and oceans: characteristics of the environment, eutrophication and OMZ, characteristic microorganisms, piezophiles, environment of hydrothermal springs.

**Genetic equipment** of microorganisms: Sequencing of bacterial genomes, genomic islands. Horizontal gene transfer

Production of biologically active substances.

**Literature - Ecology of Microorganisms:**

Madigan M. et al.: Brock Biology of microorganisms, Pearson (15 edition, 2017) ISBN-10 ‏ : ‎ 9780134261928, Pearson (16. edition, 2021) ISBN-10 ‏ : ‎ 1292404795

Madsen E.L.: Environmental Microbiology\_ From Genomes to Biogeochemistry. Wiley-Blackwell (2nd ed., 2015). ISBN-10 ‏ : ‎ 1118439635