University of Zagreb Faculty of Agriculture
Svetošimunska cesta 25, 10000 Zagreb
Phone: +385 (0)1 2393 777
E-mail: dekanat@agr.hr
Web: www.agr.unizg.hr

Methods in soil molecular microbiology (146070)

Course coordinator

Prof. Mirna Mrkonjić Fuka, PhD

Course description

Soil can be considered as a reservoir for biodiversity. The number of microorganisms can reach more than one billion of microbial cells per g soil but most of the microorganisms still remains unknown since only 10 % of species living in soil have been isolated and characterized. Methods based on total DNA/RNA soil extraction and polymerase chain reaction (PCR) provide detection technologies with high levels of specificity and sensitivity regardless of physical and chemical characteristics of the soils. They have revolutionized our understanding of the composition, phylogeny, physiology, and function of microbial communities in soil. Application of molecular methods in soil microbiology provides an understanding of influence of soil management on microbial genetic resources such providing valuable information for a sustainable use of land for agriculture. In the frame of this course students will be introduced to PCR technology and application of PCR based methods in soil microbiology and will be introduced to the importance of polyphasic approach in microbial ecology that includes application of different fingerprinting methods (e.g. denaturing gradient gel electrophoresis (DGGE), terminal restriction length polymorphysms (T-RFLP), arbitrarly primed PCR (AP-PCR), clone libraries, sequencing) as well as methods used for quantification of total or specific functional microbial groups (e.g. Real-Time PCR, MPN-PCR).

ECTS: **3.00**

E-learning: **L1**

Teaching hours: 30
Lectures: 22
Laboratory exercises: 8
Seminar: 0

Lecturer

- Prof. Mirna Mrkonjić Fuka, PhD
- Prof. Michael Schloter, PhD

Associate teacher for exercises

- Asst. Prof. Irina Tanuwidjaja, PhD

Grading

Sufficient (2): 60-70 %
Good (3): 71-80 %
Very good (4): 81-90 %
Excellent (5): 91-100 %

Type of course

- Graduate studies / Environment, agriculture and resource management (Elective course, 4 semester, 2 year)

University of Zagreb Faculty of Agriculture
Svetošimunska cesta 25, 10000 Zagreb
Phone: +385 (0)1 2393 777
E-mail: dekanat@agr.hr
Web: www.agr.unizg.hr

General competencies

This course introduces students to basic molecular- microbiological methods in studying microbial diversity in soil, food and water. Students will gain necessary theoretical and practical knowledge that could be applied for microbial ecology studies.

Types of instruction

- Lectures
  Classical lectures will be supplemented to particular study problems that should be solved in small student's groups. Independent student's work and argumentation will be especially encouraged.
- Laboratory practice/exercises
  Laboratory work will be based on PCR methods to quantify bacteria in soil and to identify bacteria from soil or sediments. The practical work will be organized in small students' groups.

Learning outcomes

Learning outcome	Evaluation methods
To apply molecular methods in soil microbiology	Oral and written exam
To explain application of biomarkers in soil microbiology	Oral and written exam
To explain advantages and biases of polymerase chain reaction (PCR) technology	Oral and written exam
To characterize and detect soil microbial populations by PCR	Oral and written exam
To apply procedure and methods for DNA/RNA extraction from bacterial cultures, soil and sediment	Oral and written exam
To assess soil microbial indicators by PCR methods	Oral and written exam
To integrate different knowledge in the area of soil ecology	Oral and written exam

Working methods

Teachers' obligations

Participation in all the forms of teaching as lectures and consultation, provide teaching materials and assure active communication with the student throughout the semester

Students' obligations

Regular attendance and active participation in lectures and exercises

Methods of grading

University of Zagreb Faculty of Agriculture
Svetošimunska cesta 25, 10000 Zagreb
Phone: +385 (0)1 2393 777
E-mail: dekanat@agr.hr
Web: www.agr.unizg.hr

Evaluation elements	Maximum points or Share in evaluation	Grade rating scale	Grade	Direct teaching hours	Total number of average student workload	ECTS
Oral exam	50 %	˂ 60 % 60-70 % 71-80 % 81-90 % 91-100 %	Insufficient (1) Sufficient (2) Good (3) Very good (4) Excellent (5)	15	45	1.5
Written exam	50 %	˂ 60 % 60-70 % 71-80 % 81-90 % 91-100 %	Insufficient (1) Sufficient (2) Good (3) Very good (4) Excellent (5)	15	45	1,5
Total	100 %			30	90	3

Evaluation elements	Description	Deadline	Recoupment
Oral exam		Exam period	

University of Zagreb Faculty of Agriculture
Svetošimunska cesta 25, 10000 Zagreb
Phone: +385 (0)1 2393 777
E-mail: dekanat@agr.hr
Web: www.agr.unizg.hr

Weekly class schedule

1. Introduction to methods in microbiology L - Culture dependent methods. Culture independent methods. Biases and errors of culture dependent methods in soil microbiology.
2. Organization of microbial cells L - Organization of microbial cells. Differences in cell wall structure. Genome organization of prokaryotic and eukaryotic cells. Molecular biomarkers.
3. Preparation of genomic DNA from microbial cultures L - Isolation of microorganisms. Microbial growth. Methodology of DNA isolation from bacterial cultures. Optimized procedures for DNA extraction (Gram positive, gram negative bacteria, plasmid and chromosomal DNA)
4. PCR technology and application feasibility L - Principles of PCR technology. Advantages and biases of PCR methods. Constructions of primers for specific microbial groups. Specificity of PCR technology. PCR contaminants.
5. Direct extraction of DNA/RNA from soil and sediment L - Soil sampling and general soil characteristics. Principles of DNA and RNA extraction from soil and sediments. Optimized procedures for simultaneously recover of DNA and RNA.
6. Specific DNA sequences for detection of microorganisms in soil L - Functional groups (functional genes) and phylogenetic markers (16S rRNA, 18S rRNA, ITS). Tracing of respective microbial groups in time and space.
7. Characterisation of the diversity of soil microbial communities: fingerprinting methods L - Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. Diversity pattern of soil microbial communities by terminal restriction fragment length polymorphisms (T-RFLP). Application of DGGE and T-RFLP in soil functional microbiology.
8. Cloning and modification of PCR products from soil L - Assessment of microbial diversity by clone library construction. Rarefaction analysis. Shot gun cloning and metagenomic.
9. DNA sequencing in bacterial systematics L - Nucleic acid sequencing methods. Sanger' s method. Next generation sequencing. Application in soil ecology.
10. Quantification of microbial populations by PCR methods Pe-L - Principles of Real-Time PCR assay. Application of Real-Time PCR in microbial ecology. Abundance of genes involved in transformation of nutrients in soil.
11. Polyphasic approach in soil microbiology: dynamic of microbial communities in time and space Pe-L - Appplication of molecular methods and PCR technology: microbial functions, functioning and potentialities in soils. Microbial respond to soil management practice
12. Extraction of total DNA from soil Lab - Extraction of total DNA from different soil types
13. Characterization of the diversity of soil microbial communities by AP-PCR Lab - AP- PCR od soil samples. Cluster analysis of obtained fingerprints
14. Quantification of total microbial communities in soil by Real-Time PCR Lab - Enumeration of total number of bacteria in soil by Real-Time PCR
15. Most probable number PCR (MPN-PCR) to quantify total microbial communities in soil Lab - Estimation of total number of microorganisms in soil by MPN-PCR

Obligatory literature

1. de Brujin F.; Molecular microbial Ecology, volume I and II, Wiley Blackwell, 2011
2. van Elsas, Trevors J.T., Wellington E.M.H.: Modern soil microbiology, Marcerl Dekker, Inc.,1997
3. Kowalchuk G., de Bruijn F., Head I., Akkermans A., van Elsas J. D.: Molecular Microbial Ecology Mannual, Kluwer Academic Publisher, 2004.

University of Zagreb Faculty of Agriculture
Svetošimunska cesta 25, 10000 Zagreb
Phone: +385 (0)1 2393 777
E-mail: dekanat@agr.hr
Web: www.agr.unizg.hr

Recommended literature

1. Gerhardt Ph., Murray R.G.E., Wood W., Krieg N.: Methods for General and Molecular Bacteriology. American Society for Microbiology. 1994.
2. Bothe H., Ferguson S., J., Newton W.E.: Biology of nitorgen cycle, Elsevier, 2007

Similar course at related universities

- Molecular biological methods in food analysis, BOKU
- Molecular Environmental Soil Science, College of agriculture and life sciences, NC State University