

Molecular methods in microbial agroecology (152098)

Nositelj predmeta

[prof. dr. sc. Mirna Mrkonjić Fuka](#)

Opis predmeta

Microorganisms are an integral part of different environments such as soil, food and water. Nowadays, it is impossible to investigate the microbial diversity of any ecosystem without the knowledge and application of molecular microbiological methods. The aim of this course is to familiarize students with the basic molecular microbiological methods in the analysis of soil, water and food, and to enable them to apply and integrate the acquired knowledge in research in agroecology. Lectures will focus on understanding the basic molecular - microbiological methods and their application in complex analysis in agroecology and will be an introduction to the experimental work in laboratory. In the frame of this course students will get the practical knowledge into DNA isolation followed by PCR technology and application of PCR based methods in agroecology. They will be introduced to the importance of polyphasic approach in microbial agroecology that includes application of different fingerprinting methods (e.g. denaturing gradient gel electrophoresis (DGGE), terminal restriction length polymorphisms (T-RFLP), arbitrarily 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). Identification of species and strains of microorganisms relevant to agriculture will be determined by a variety of molecular techniques (PCR - RFLP , RAPD , rep - PCR). The theoretical principles of DNA sequencing and the application of “deep sequencing” (NGS sequencing) in microbial agroecology and the role metagenomics and metagenetics will be explained.

ECTS: **6.00**

E-učenje: **R1**

Sati nastave: 60

Predavanja: 30

Vježbe u praktikumu: 28

Seminar: 2

Izvođač predavanja

- [prof. dr. sc. Mirna Mrkonjić Fuka](#)

Izvođač vježbi

- Irina Tanuwidjaja, mag. ing. agr.

Ocjenjivanje

Dovoljan (2): 60-70 %

Dobar (3): 71-80 %

Vrlo dobar (4): 81-90 %

Izvrstan (5): 91-100 %

Vrsta predmeta

- Graduate studies / [MS Courses taught in English](#) (Izborni predmet, 2. semestar, 1. godina)

Opće kompetencije

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.

Oblici nastave

- 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.

Polaganje ispita

Elementi praćenja	Maksimalno bodova ili udio u ocjeni	Bodovna skala ocjena	Ocjena	Broj sati izravne nastave	Ukupni broj sati rada prosječnog studenta	ECTS bodovi
Written exam	50 %	< 60 % 60-70 % 71-80 % 81-90 % 91-100 %	Nedovoljan (1) Dovoljan (2) Dobar (3) Vrlo dobar (4) Izvrstan (5)	30	90	3
Oral exam	50 %	< 60 % 60-70 % 71-80 % 81-90 % 91-100 %	Nedovoljan (1) Dovoljan (2) Dobar (3) Vrlo dobar (4) Izvrstan (5)	30	90	3

Elementi praćenja	Opis	Rok	Nadoknada
Written exam		Exam period	
Oral exam		Exam period	

Tjedni plan nastave

1. The role of cell in microbial ecology- L- The role of cell in microbial ecology studies. Biases connected to culture depending methods (isolation and culturing)
2. DNA isolation. L- Basic principles of DNA isolation from soil, food and water. Lab- DNA isolation from soil and poor cultures
3. Polymerase chain reaction (PCR). L- Polymerase chain reaction- basic principles, variation of basic PCR. Lab- PCR application.
4. Identification of indigenous strains of bacteria and yeasts L- The significance of studies on indigenous strains from soil and traditional food. Application of indigenous strains in biotechnology. Lab- PCR application.
5. Identification of indigenous strains of bacteria and yeasts. L- Identification of bacteria at the species and strain (ecotype) level (16S rRNA genes, functional genes, PCR RAPD). Lab- Results analysis.
6. Identification of indigenous strains of bacteria and yeasts. L- Identification of yeasts at the species and strain (ecotype) level (18S rRNA gen, ITS regija PCR RFLP, RAPD, rep- PCR). Lab- PCR application.
7. Identification of indigenous strains of bacteria and yeasts. Lab- Analysis of fingerprints and cluster analysis.
8. Diversity analysis and quantification of microbial populations in different agroecosystems. L- Fingerprinting (DGGE, SSCP, T-RFLP) and quantification (Real-Time PCR i MPN PCR) of microbial communities in agroecology. Lab- The total bacterial number estimation by MPN PCR.
9. Diversity analysis and quantification of microbial populations in different agroecosystems. L- Metagenomics and metagenetics. Analysis of MPN PCR results and comparison to Real-Time PCR. Lab- AP-PCR.
10. Cloning. L- The basic principle of cloning and importance for agrobiodiversity. Lab- PCR products preparation for ligation into vector.
11. Cloning. Lab- ligation into vector and transformation.
12. Analysis of clones and inserts. Lab- Plasmid DNA isolation
13. Analysis of clones and inserts. Lab- Endonuclease digestion of plasmids and PCR analysis of inserts.
14. Analysis of clones and inserts. P- Introduction to sequencing. Analysis of sequences by open databases (NCBI i RDP)
15. Seminars. S- Students will get particular problem in microbial agroecology and will suggest and argue different method that can be used to solve it.

Obvezna literatura

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

Preporučena literatura

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 nitrogen cycle, Elsevier, 2007