

**UČNI NAČRT PREDMETA / COURSE SYLLABUS**

**Predmet:** Mikrobiološke metode v agroživilstvu  
**Course title:** Methods in microbiology in agro-food industry

Študijski program in stopnja Study programme and level	Študijska smer Study field	Letnik Academic year	Semester Semester
Interdisciplinarni doktorski študijski program BIOZNANOSTI 3. stopnja	Agroživilska mikrobiologija	1,2	1,2,3,4
Interdisciplinary Doctoral Study Programme in BIOSCIENCES 3rd cycle	Agrifood microbiology	1,2	1,2,3,4

**Vrsta predmeta / Course type**

individualno raziskovalni predmet /  
individual research course

**Univerzitetna koda predmeta / University course code:**

Predavanja Lectures	Seminar Seminar	Vaje Tutorial	Klinične vaje work	Druge oblike študija	Samost. delo Individ. work	ECTS
/	/	10	/	15	100	5

**Nosilec predmeta / Lecturer:** Nosilec: doc. dr. Martina Turk

**Jeziki / Languages:**

**Predavanja / Lectures:**  
**Vaje / Tutorial:** slovenski / angleški  
Slovene / English

**Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti:** **Prerequisites:**

Splošni pogoji za vpis na doktorski študij.

General requirements for the enrolment in PhD program.

**Vsebina:**

**Content (Syllabus outline):**

Agroživilska mikrobiologija je veja aplikativne mikrobiologije, sestavljena iz (a) agrikulturne mikrobiologije, ki proučuje pretežno rastlinske in živalske bolezni, mikroorganizme, povezane z rastlinami in vpletenost mikroorganizmov v razgradnjo organskega materiala s kroženjem hranil, ter (b) živalske mikrobiologije, ki se ukvarja s konserviranjem hrane, uporabo mikroorganizmov za povečanje kvalitete hrane, njen poudarek pa je na ocenjevanju varnosti hrane. Obe področji agroživilske mikrobiologije sta tesno povezani z okoljsko mikrobiologijo.

Pri predmetu Mikrobiološke metode v agroživilstvu bo poudarek na agroživilski mikrobiologiji. Študentke in študenti se bodo seznanili s tehnikami, ki se uporabljajo za preučevanje morfologije, fiziologije, biokemije, genetike in molekularne biologije mikroorganizmov, tako na ravni posamezne celice, čistih kultur kot tudi mikrobnih združb. V okviru predmeta bodo sodelujoči laboratoriji predstavili izbor metod, ki jih uporabljajo pri svojih raziskavah. Delo bo eksperimentalno in bo potekalo v izbranih laboratorijih.

Študentke in študenti se bodo spoznali z:

- osamitvijo mikroorganizmov in njihovo kvantifikacijo v kompleksnih vzorcih, kot so hrana in tal,
- določanjem mikrobiološke kvalitete (neoporečnosti) in sledljivosti hrane,
- taksonomsko karakterizacijo mikroorganizmov z uporabo (a) klasičnih metod (temelječih na biokemijskih, fizioloških in morfoloških lastnostih) in (b) metod molekularne biologije (polimorfizem konformacije enojne verige (SSCP), polimorfizem dolžin pomnoženih fragmentov (AFLP), poliakrilamidna gelska elektroforeza v temperaturnem gradientu (TGGE), polimorfizem dolžin restriksijskih fragmentov (RFLP), elektroforeza v pulzirajočem polju (PFGE), tipiziranje na osnovi multilokusnih sekvenc (MLST)...); študentke in študenti se bodo tudi naučili, kako izvesti filogenetske analize na teh podatkih,
- določitvijo odpornosti mikroorganizmov na fizikalne in kemijske dejavnike okolja,
- določevanjem mikrobne aktivnosti čistih kultur in mikrobnih združb v tleh, aktivnem blatu in drugih habitatih z merjenjem emisij CO<sub>2</sub>, metana, N<sub>2</sub>O; merjenjem aktivnosti nitrifikacije in denitrifikacije; določevanjem aktivnosti pomembnih encimov, kot so na primer fenol oksidaze, celulaze, hemicelulaze in hitinaze,

Agro-food microbiology is a branch of applicative microbiology composed of (i) agricultural microbiology, studying mainly plant and animal diseases, plant-associated microorganisms and involvement of microbes in the degradation of organic matter and nutrient cycling, and (ii) food microbiology, which deals with food preservation, the use of microbes to increase food quality and evaluation of food safety. Both fields of agro-food microbiology are tightly connected with environmental microbiology.

Methods in microbiology in agro-food industry will be presented with the emphasis on agro-food microbiology. Students will familiarise themselves with the techniques used for the study of morphology, physiology, biochemistry, genetics, and molecular biology of microorganisms, from the single cell, pure cultures to the microbial communities. Within the framework of the course, the participating microbiology laboratories will introduce to students a selection of methods, which are used in their research. Experimental work will be carried out in the selected labs.

Students will learn:

- to isolate microorganisms and to quantify them in complex samples, such as food or soil,
- to assess microbiological quality and the traceability of food products,
- to taxonomically characterize microorganisms applying (i) classical (based on biochemical, physiological, and morphological traits) and (ii) molecular biology methods (single strand conformation polymorphism analysis (SSCP), amplified fragment length polymorphism (AFLP), temporal temperature and denaturing gradient electrophoresis (TTGE, DGGE), restriction fragment length polymorphism (RFLP), pulsed field gel electrophoresis (PFGE), multi locus sequence typing (MLST), etc.); students will also learn how to perform phylogenetic analyses on those data,
- to characterize the resistance of microorganisms to physical and chemical factors of the environment,
- to determine the microbial activity of pure cultures and in microbial communities soil, active sludge and other habitats by measuring the emissions of CO<sub>2</sub>, methane, N<sub>2</sub>O; measuring the nitrification and denitrification activities; determination of the activities of important enzymes like phenol oxidase, cellulase, hemicellulase, and chitinase,

- uporabo metod molekularne biologije, kot je verižna reakcija s polimerazo (PCR in RT-PCR) pri ugotavljanju avtentičnosti živil (mleko, meso),
- uporabo podatkov s področij genomike, transkriptomike in proteomike za reševanje težav, povezanih s proizvodnjo hrane,
- ugotavljanjem občutljivosti mikroorganizmov za protimikrobna sredstva in odkrivanjem mehanizmov odpornosti za antibiotike in druge protimikrobne snovi pri ocenjevanju potencialne nevarnosti mikroorganizmov iz hrane/krme ter okolja za zdravje ljudi/živali/rastlin,
- določevanjem toksičnosti in genotoksičnosti snovi z uporabo mikroorganizmov (kometni test),
- uporabo različnih pristopov pri proučevanju nastanka mikrobnih biofilmov, sposobnosti pritrditve na površine in odpornosti na parametre okolja v povezavi s proizvodno-prehransko verigo.

- to use molecular biology methods, such as polymerase chain reactions (classical and real-time PCR) in food authenticity studies (milk, meat),
- to use data from genomics, transcriptomics, and proteomics in solving problems connected to food production,
- to test for antimicrobial susceptibility and discovering mechanisms of resistance to antibiotics and other antimicrobial substances in the evaluation of potential hazard of microorganisms from food/feed and environment for the human/animal/plant health,
- to determine the toxicity and genotoxicity of substances with the use of microorganisms (comet assay),
- to use different approaches for the investigation of microbial biofilm formation, their adhesive abilities and resistance in relation to surface characteristics and other environmental parameters, related to food production chain.

#### **Temeljni literatura in viri / Readings:**

Revijalni in eksperimentalni članki s področja, tekoča periodika, druga učna gradiva, ki jih bo priskrbel izbrani raziskovalec/izvajalec.  
Current scientific periodicals and literature from the field and text books, provided by the selected researcher.

#### **Cilji in kompetence:**

- seznaniti študentko ali študenta z naborom metod, ki se uporabljajo v agroživilski mikrobiologiji in ji/mu omogočiti izvedbo izbrane metode v laboratoriju. Izbrana metoda bo omogočila študentu rešitev specifičnega eksperimentalnega problema, ki se bo pojavil v okviru njenega/njegovega raziskovalnega dela,
- posredovanje ključne znanstvene literature iz področja izbrane znanstvene metode, vključno s posredovanjem lastnega raziskovalčevega znanja,
- pomoč pri analizi podatkov pridobljenih z izbrano metodo,
- rešitev ali izboljšava rešitve specifičnega problema s pridobljenimi rezultati.

#### **Objectives and competences:**

- to provide the student with the range of methods used in agro-food microbiology and enable her/him to perform a selected method in a chosen laboratory. The selected method will enable the student to solve a specific experimental problem that will appear in the context of her/his research work,
- to mediate key scientific literature from the field of the chosen method, including mediation of the lecturer's own experience,
- to supply help with the analysis of data obtained with the chosen method,
- to enable or improve the solution of a specific problem with the obtained results.

**Predvideni študijski rezultati:**

Znanje in razumevanje:  
Poznavanje izbrane mikrobiološke metode, sposobnost razlage principov na katerih izbrana metoda temelji in interpretacija pridobljenih rezultatov.

**Intended learning outcomes:**

Knowledge and understanding:  
Familiarity with the chosen microbiological method, capability to explain the principles or laws on which the method is based and interpretation of the obtained results.

**Metode poučevanja in učenja:**

V okviru eksperimentalnega laboratorijskega dela bodo udeleženi raziskovalci pomagali pri izvedbi izbrane metode kot tudi pri vpogledu v področje lastnih raziskav, pri katerih si pomagajo z izbrano metodo.

**Learning and teaching methods:**

Within the framework of experimental lab work the participating researchers will provide help in realization of the chosen method as well as providing specific insight into their field of research in which they use the this method.

<b>Načini ocenjevanja:</b>	Delež (v %) / Weight (in %)	<b>Assessment:</b>
- ocena kompetenc pri eksperimentalnem delu v laboratoriju	60%	- evaluation of the competences at the experimental work in the lab
- ustna predstavitev projekta po zaključenem eksperimentalnem delu	40%	- oral presentation of the project after the completion of the experimental work

**Reference nosilca / Lecturer's references:****Martina Turk**

- GOSTINČAR, Cene, GUNDE-CIMERMAN, Nina, TURK, Martina. Genetic resources of extremotolerant fungi : a method for identification of genes conferring stress tolerance. *Bioresource technology*, ISSN 0960-8524. [Print ed.], 2012, vol. 111, str. 360-367.
- MICHÁN, Carmen, MARTÍNEZ, Jose L., ALVAREZ, María C., TURK, Martina, SYCHROVA, Hana, RAMOS, Jose. Salt and oxidative stress tolerance in *Debaryomyces hansenii* and *Debaryomyces fabryi*. *FEMS yeast research*, ISSN 1567-1356. [Print ed.], 2013, vol. 13, issue 2, str. 180-188.
- TURK, Martina, PLEMENITAŠ, Ana, GUNDE-CIMERMAN, Nina. Extremophilic yeasts : plasma-membrane fluidity as determinant of stress tolerance. *Fungal biology*, ISSN 1878-6146, 2011, vol. 115, no. 10, str. 950-958.
- GOSTINČAR, Cene, TURK, Martina, GUNDE-CIMERMAN, Nina. The evolution of fatty acid desaturases and cytochrome b5 in eukaryotes. *The journal of membrane biology*, ISSN 0022-2631, 2010, iss. 1-3, vol. 233, str. 63-72.
- GOSTINČAR, Cene, TURK, Martina, PLEMENITAŠ, Ana, GUNDE-CIMERMAN, Nina. The expressions of  $\Delta^9$ - $\Delta^{12}$ -desaturases and an elongase by the extremely halotolerant *Hortaea werneckii* are salt dependent. *FEMS yeast research*, ISSN 1567-1356. [Print ed.], 2009, vol. 9, issue 2, str. 247-2566.

6. GOSTINČAR, Cene, OHM, Robin, KOGEJ, Tina, SONJAK, Silva, TURK, Martina, ZAJC, Janja, ZALAR, Polona, GRUBE, Martin, SUN, Hui, HAN, James, SHARMA, Aditi, CHINIQUY, Jennifer, NGAN, Chew Yee, LIPZEN, Anna, BARRY, Kerrie, GRIGORIEV, Igor, GUNDE-CIMERMAN, Nina. Genome sequencing of four *Aureobasidium pullulans* varieties : biotechnological potential, stress tolerance, and description of new species. *BMC genomics*, ISSN 1471-2164, 2014, vol. 15, str. 1-28.

**UČNI NAČRT PREDMETA / COURSE SYLLABUS**

**Predmet:** Sociomikrobiologija  
**Course title:** Sociomicrobiology

Študijski program in stopnja Study programme and level	Študijska smer Study field	Letnik Academic year	Semester Semester
Interdisciplinarni doktorski študijski program BIOZNANOSTI 3. stopnja	<b>Agroživilska mikrobiologija</b>	1,2	1,2,3,4
Interdisciplinary Doctoral Study Programme in BIOSCIENCES 3rd cycle	<b>Agrifood microbiology</b>	1,2	1,2,3,4

**Vrsta predmeta / Course type**

individualno raziskovalni predmet /  
individual research course

**Univerzitetna koda predmeta / University course code:**

Predavanja Lectures	Seminar Seminar	Vaje Tutorial	Klinične vaje work	Druge oblike študija	Samost. delo Individ. work	ECTS
/	20	20	/	10	200	10

**Nosilec predmeta / Lecturer:**

Nosilec: prof. dr. Ines Mandić Mulec

**Jeziki / Languages:**

**Predavanja / Lectures:** slovenski / angleški  
Slovene / English  
**Vaje / Tutorial:** slovenski / angleški  
Slovene / English

**Pogoji za vključitev v delo oz. za opravljanje študijskih obveznosti:**

Splošni pogoji za vpis na doktorski študij

**Prerequisites:**

Enrolment to the PhD program.

**Vsebina:**

**Content (Syllabus outline):**

Sociomikrobiologija je novo hitro se razvijajoče področje mikrobiologije, ki preučuje različne skupinske odzive mikroorganizmov.

Glavni vsebinski sklopi:

- Kooperacija mikroorganizmov
- Sorodstvena in skupinska selekcija
- Sorodstveno prepoznavanje/diskriminacija
- Goljufi in mehanizmi, ki stabilizirajo kooperacijo
- Seks pri bakterijah in adaptacije
- Agresija in antibiotiki kot signali
- Socialne interakcije v strukturiranih agroživilskih okoljih
- Ekologija socialnih interakcij v agroživilskih okoljih
- Biokontrola: mikrob in rastlina

Sociomicrobiology is a new and rapidly developing field of microbiology which addresses various aspects of microbial group behaviour.

Content:

- Cooperativity of microorganisms
- Kin and group selection
- Kin recognition/discrimination
- Cheating and mechanisms that stabilize cooperation
- Spite and antibiotics as signals
- Bacterial sex and adaptation
- Social interactions in structured agro-food environments
- Ecology of social interactions in agro-food environments
- Biocontrol: plant / microb

#### **Temeljni literatura in viri / Readings:**

Predmet je zasnovan na novejših revijalnih (Nature Review Microbiology, Current Biology, Trend in Microbiology, ISME journal) in tekočih eksperimentalnih člankih s področja.

#### **Cilji in kompetence:**

Študent se skozi problemsko zasnovan seminar in eksperimentalno delo seznanja s koncepti in naborom metod, ki se uporabljajo v sociomikrobiologiji in so predstavljene v okviru vsebine predmeta. Omogoči se mu izvedba in nudi pomoč pri reševanju eksperimentalne problematike vezane na področje sociomikrobiologije in prirejene individualnim potrebam študenta ter so lahko del doktorata vpisanega študenta.

#### **Objectives and competences:**

Student learns through seminar work fundamental methods and concepts (described above) which are used in sociomicrobiology. Student is also provided with an opportunity to work in the lecturer's laboratory and is supervised in solving the experimental problems in sociomicrobiology. These could also be part of student's thesis project.

#### **Predvideni študijski rezultati:**

Študent spozna in razume osnovne koncepte in teorijo v sociomikrobiologiji in uporabo teh konceptov v medicini, biotehnologiji in ekologiji. Študent se nauči zasnovati in izvesti eksperiment s področja sociomikrobiologije. Študent ima možnost izvajati del eksperimentov vezanih na doktorsko tezo v laboratoriju nosilke predmeta (v dogovoru z mentorjem). Pri tem lahko uporabi razpoložljivo opremo in modelne sisteme, ki jih rutinsko uporabljamo v laboratoriju.

#### **Intended learning outcomes:**

Student gains an insight and understands the basic concepts in sociomicrobiology and application of this knowledge in medicine, biotechnology and ecology. Student learns how to design and execute an experiment in the field of sociomicrobiology. Student can perform part of the thesis project in the lecturer's laboratory ( in

- Zasnova eksperimenta v sociomikrobiologiji
- Modelni mikrobn sistemi
- Priprava rekombinantnih sevov
- Fluorescenčne mikroskopije
- Fluorometrija
- Reometrija
- Metagenomski pristopi

agreement with the student's thesis supervisor). He may use model systems and laboratory equipment that are routinely applied in the laboratory.

- Design of an experiment in sociomicrobiology
- Modelni mikrobn sistemi
- Preparation of recombinant strains
- Fluorescent microscopy
- Fluorometry
- Rheometry
- Metagenomic approaches

#### Metode poučevanja in učenja:

Konzultacije, pomoč pri zasnovi in izvedbi eksperimentalnega projekta. V okviru predmeta je možna uporaba raziskovalnih orodij, ki smo jih razvili v laboratoriju za proučevanje sociomikrobiologije, za potrebe raziskovalnega dela doktorskega študenta iz področja sociomikrobiologije.

#### Learning and teaching methods:

Consultations and experimental project in the laboratory of the lecturer. Student may use research tools developed for the study of sociomicrobiology in our laboratories for his/her PhD project related to sociomicrobiology or subjects related under the content of the course.

#### Načini ocenjevanja:

Delež (v %)  
/  
Weight (in %)

#### Assessment:

Seminar- pisni Eksperimentalni del in pisno poročilo	20 % 80 %	Seminar Eksperimentalni projekt in the written report)
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#### Reference nosilca / Lecturer's references:

##### Prof. dr. Ines Mandić Mulec

1. OSLIZLO, Anna, ŠTEFANIČ, Polonca, DOGŠA, Iztok, **MANDIĆ-MULEC, Ines**. Private link between signal and response in *Bacillus subtilis* quorum sensing. Proceedings of the National Academy of Sciences of the United States of America, ISSN 0027-8424, 2014, vol. 111, no. 4, str. 1586-1591, ilustr., doi: 10.1073/pnas.1316283111. [COBISS.SI-ID 4358008].

2. ŠTEFANIČ, Polonca, DECOROSI, Francesca, VITI, Carlo, PETITO, Janine, COHAN, Frederick, **MANDIĆ-MULEC, Ines**. The quorum sensing diversity within and between ecotypes of *Bacillus subtilis*. Environmental microbiology, ISSN 1462-2912. [Print ed.], 2012, vol. 14, no. 6, str. 1378-1389, doi: 10.1111/j.1462-2920.2012.02717.X. [COBISS.SI-ID 4044408].



3. ŠTEFANIČ, Polonca, **MANDIĆ-MULEC, Ines**. Social interactions and distribution of *Bacillus subtilis* phenotypes at microscale. *Journal of bacteriology*, ISSN 0021-9193, 2009, no. 6, vol. 191, str. 1756-1764, doi: 10.1128/JB.01290-08. [COBISS.SI-ID 3565432].
4. DOGŠA, Iztok, OSIZLO, Anna, ŠTEFANIČ, Polonca, **MANDIĆ-MULEC, Ines**. Social interactions and biofilm formation in *Bacillus subtilis*. *Food technology and biotechnology*, ISSN 1330-9862, 2014, vol. 52, no. 2, str. 149-157. [COBISS.SI-ID 4381816].
5. DURRETT, Russell, MIRAS, Mathieu, MIROUZE, Nicolas, NARECHANIA, Apurva, **MANDIĆ-MULEC, Ines**, DUBNAU, David A. Genome sequence of the *Bacillus subtilis* biofilm-forming transformable strain PS216. *Genome announcements*, ISSN 2169-8287, 2013, vol. 1, no. 3, str. 1-2, e00288-13.
6. LEVIČNIK HOEFFERLE, Špela, NICOL, Graeme, AUSEC, Luka, **MANDIĆ-MULEC, Ines**, PROSSER, James Ivor. Stimulation of thaumarchaeal ammonia oxidation by ammonia derived from organic nitrogen but not added inorganic nitrogen. *FEMS microbiology, ecology*, 2012, vol. 80, issue 1, str. 114-123.
7. **MANDIĆ-MULEC, Ines**, GORENC, Katja, GAMS PETRIŠIČ, Marinka, FAGANELI, Jadran, OGRINC, Nives. Methanogenesis pathways in a stratified eutrophic alpine lake (Lake Bled, Slovenia). *Limnology and oceanography*, 2012, vol. 57, no. 3, str. 868-880. 9. KRAIGHER, Barbara, **MANDIĆ-MULEC, Ines**.
8. AUSEC, Luka, ELSAS, Jan D., **MANDIĆ-MULEC, Ines**. Two- and three-domain bacterial laccase-like genes are present in drained peat soils. *Soil biology & biochemistry*, 2011, vol. 43, issue 5, str. 975-983.
9. **MANDIĆ-MULEC, Ines**, PROSSER, James Ivor. Diversity of endospore - forming bacteria in soil : characterization and driving mechanisms. V: LOGAN, Niall A. (ur.), DE VOS, Paul (ur.). *Endospore : forming soil bacteria*, (Soil biology, ISSN 1613-3382, 27). 1st ed. Berlin; Heidelberg: Springer, 2011, str. 31-59. [COBISS.SI-ID 3897720].
10. JACQUIOD, Samuel, DEMANECHÉ, Sandrine, AUSEC, Luka, XU, Zhuofei, DELMONT, Tom O., DUNON, Vincent, CAGNON, Christine, **MANDIĆ-MULEC, Ines**, VOGEL, Timothy M., SIMONET, Pascal, FRANQUEVILLE, Laure. Characterization of new bacterial catabolic genes and mobile genetic elements by high throughput genetic screening of a soil metagenomic library. *Journal of biotechnology*, ISSN 0168-1656. [Print ed.], 2014, str. [1-12, v tisku], doi: 10.1016/j.jbiotec.2014.03.036. [COBISS.SI-ID 4379512].