

Kratek opis usposabljanja mladega raziskovalca (*Short description of the Young Researcher's training*)

1. Raziskovalna organizacija (*Research organisation*):

Univerza v Ljubljani, Fakulteta za elektrotehniko
(*University of Ljubljana, Faculty of Electrical Engineering*)

2. Ime, priimek in elektronski naslov mentorja (*Mentor's name, surname and email*):

prof. dr. Tadej Kotnik, tadej.kotnik@fe.uni-lj.si

3. Šifra in naziv raziskovalnega področja (*Research field*):

2.06.07 Biomedicinska tehnika (*Biomedical Engineering*)

4. Kratek opis usposabljanja mladega raziskovalca (*Short description of the Young Researcher's training*):

Navedite tudi morebitne druge zahteve, vezane na usposabljanje mladega raziskovalca (npr. znanje angleškega jezika, izkušnje z laboratorijskim delom, potrebne licence za usposabljanje...).

slo:

Raziskovalna tematika usposabljanja mladega raziskovalca oz. mlade raziskovalke (v nadaljevanju: MR) bo **preučevanje elektropermeabilizacije bakterij za vnos in ekstrakcijo molekul**.

Elektropermeabilizacija (tudi elektroporacija, v nadaljevanju: EP) je že uveljavljena na več področjih medicine, v biotehnologiji pa se uveljavlja šele v zadnjem desetletju – najobetavneje za gensko transformacijo mikroorganizmov, inaktivacijo patogenih organizmov, ekstrakcijo biomolekul in hitro sušenje biomase. Na uporabo EP bakterij za vnos in ekstrakcijo molekul se bo osredotočalo tudi usposabljanje MR, ki ga bodo tvorili trije delovni sklopi:

(1) **Vnos antibiotikov v bakterije**. Odpornost (rezistenca) bakterij proti antibiotikom postaja vse bolj pereč problem tako v humani kot veterinarski medicini, MR pa bo izpostavljal bakterije različnih za človeka nepatogenih sevov različnim antibiotikom in primerjal bakteriostatični oz. bakteriostatični učinek na odporne in neodporne seve z EP in brez nje.

(2) **Ekstrakcija DNA iz bakterij**. Med poglobljenostjo poznavanja vnosa DNA v bakterije in njihove transformacije z reverzibilno EP ter poznavanja iztekanja DNA iz bakterij zaradi ireverzibilne EP je velik razkorak, MR pa bo preučil (i) kako na hitrost izločanja in količino izločene DNA vplivata jakost in trajanje električnega polja, način dovajanja razelektritev (kontakten ali obločen) in razlika med ovojnicama Gram negativnih in pozitivnih bakterij, (ii) kako hitro izločena DNA razpada v medijih s sestavo, podobno naravnim habitatom različnih mikroorganizmov, (iii) ali na hitrost razpadanja vpliva gostota mikroorganizmov, ter (iv) ali na hitrost razpadanja DNA vplivajo te endonukleaze in način dovajanja razelektritev.

(3) **Kombinacija ekstrakcije in vnosa DNA za horizontalni prenos genov (HGT) med bakterijami**. Kombinacija elektroekstrakcije DNA iz enega seva bakterij in vnos te DNA v bližnje druge seve bakterij, ki jo povzroči udar nevihtne strele v naravne habitate, je verodostojen naraven abiotski mehanizem HGT med njimi, HGT pa je eden najpomembnejših vzrokov odpornosti bakterij na antibiotike; MR bo na osnovi spoznanj o na EP temelječem vnosu in iztekanju makromolekul, pridobljenih v sklopih (1) in (2), izvedel serijo poskusov, v katerih bo dva seva bakterij izpostavil emulaciji udara strele in preučeval, ali je prišlo do prenosa genov in njihovega izražanja v bakterijah prejemnicah genov.

Vsak sklop bo od MR zahteval seznanitev s specifičnimi eksperimentalnimi metodami gojenja in inkubacije preučevanih mikroorganizmov, detekcije in kvantifikacije vnosa in ekstrakcije molekul, pri vnosu DNA pa tudi izražanja genov. Raziskovalna skupina (1538-004) in raziskovalni program (P2-0249), katerih član je mentor, imata na voljo ekipo, opremo, potrebna dovoljenja in znanje za vse te eksperimentalne metode. Pričakovana predznanja kandidata so:

- osnovno poznavanje dela s mikrobiološkimi (bakterijskimi) kulturami;
- aktivno znanje angleškega jezika.

eng:

The research work of the Young Researcher (hereinafter: YR) will focus on studying **electro-permeabilization of bacteria for uptake and extraction of molecules**.

While electroporation (also electroporation, hereinafter: EP) is an established method in several areas of medicine, many of its applications in biotechnology are only starting to emerge; among the most promising of these are heritable genetic modification of microorganisms (electrotransformation), inactivation of pathogens, extraction of biomolecules and fast drying of biomass. The research of the YR will focus on the use of EP of bacteria for uptake and extraction of molecules, consisting of three work packages:

(1) **Uptake of antibiotics into bacteria.** The increasing resistance of bacteria to antibiotics is rapidly becoming a serious concern both in human and veterinary medicine, and the YR will be exposing bacteria non-pathogenic to humans to various antibiotics and compare their bactericidal or bacteriostatic effect on antibiotic-resistant and antibiotic-susceptible strains, with EP and without it.

(2) **Extraction of DNA from bacteria.** There is a substantial gap between the considerable breadth and depth of studies that have been dedicated to DNA uptake into bacteria and their consequent transformation by means of reversible EP, and the scarce studies focusing on release of DNA from bacteria. The YR will investigate (i) how the rate and amount of DNA release are affected by the strength and duration of the electric field, the exposure mode (via electrodes in contact with the sample, or via a gap discharge), and the difference between the envelopes of Gram-negative and -positive bacteria, (ii) how rapidly the released DNA degrades in various media emulating natural habitats of different microorganisms, (iii) whether DNA degradation rate is affected by the density of microorganisms, and (iv) whether this rate is affected by those endonucleases and/or the exposure mode.

(3) **Combination of DNA extraction and uptake for horizontal gene transfer (HGT) among bacteria.** A combination of DNA electroextraction from one bacterial strain and uptake of thus released DNA into nearby strains, triggered by lightning strokes into natural habitats, is a plausible natural abiotic HGT mechanism, while HGT is now recognized as a key path of the spread of antibiotic resistance. The YR will, using the knowledge on EP-based uptake and release of large molecules acquired in work packages (1) and (2), perform a series of experiments in which two bacterial strains will be exposed to a lightning stroke emulation, and study the extent of DNA transfer, and possibly expression, in the recipient bacteria.

Each work package will require of the YR to master specific experimental techniques of culturing and incubation of the studied microorganisms, detection and quantification of uptake and release of molecules, as well as of gene expression. The research group (1538-004) and its research programme (P2-0249) of the mentor possess the team, the equipment, the required permissions and knowledge for all these experimental methods. The candidates are expected to:

- have a basic knowledge of work with microbiological (bacterial) cultures;
- active knowledge of English language.