

## Opis delovnega mesta mladega raziskovalca/ke (*Description of the Young Researcher's position*)

1. Članica UL (*UL member*):

Biotehniška fakulteta/Biotechnical Faculty

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

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3. Raziskovalno področje (*Research field*):

1.05 Biokemija in molekularna biologija

4. Opis delovnega mesta mladega raziskovalca/ke (*Description of the Young Researcher's position*):

Vključuje morebitne dodatne pogoje, ki jih mora izpolnjevati kandidat/ka za mladega raziskovalca/ko, ki niso navedeni v razpisu za mlade raziskovalce.

*sl:* Vpliv nadzora gostitelja med lizogenijo na horizontalni prenos fagov

Določeni lizogeni fagi se integrirajo v funkcionalne genske lokuse, jih inaktivirajo in posledično spremenijo fenotip bakterij. Primer takšnih genov so geni, ki vplivajo na tvorbo biofilma pri bakterijah rodu *Bacillus* sp.. Biofilmi so večcelične skupine bakterij, obdanih z eksopolimeri, za katere je znano, da so lahko tudi ovira za fage. Zato ima lahko integracija fagnega DNK v gene, povezane s tvorbo biofilma, zelo zanimive posledice za prenos faga in za interakcije med fagom in gostiteljem – te trenutno ostajajo neznane.

Program usposabljanja mladega raziskovalca bo vseboval naslednje raziskave:

- 1) vpliv gena za integracijo faga na lastnosti biofilma gostitelja,
- 2) učinek komponent biofilmskega matriksa na razpršitev in stabilnost fagnih delcev
- 3) učinek komponent biofilmskega matriksa na prenos fagov med gostitelji

*Bacillus subtilis* je modelni organizem za proučevanje različnih oblik biofilmov, na molekularni in ekološki ravni ga širše proučujemo na Katedri za mikrobnno ekologijo in fiziologijo Biotehniške fakultete Univerze v Ljubljani.

V zbirki imamo lizogene seve *B. subtilis*, kjer je profag integriran v genetski lokus *spvM*, povezan s tvorbo biofilma. Imamo tudi naravne izolate, ki niso lizogeni in kjer je ta lokus neprekinjen. Nazadnje hranimo v zbirki tudi bakterije z okvarjenim *spvM* genom. V okviru projekta bo kandidat/kandidatka preučeval/preučevala vlogo produkta gena *spvM* na sestavo in biofizikalne lastnosti biofilma, in bo ob tem pridobil/pridobila izkušnje s: HPLC-SEC, reologijo, meritvami površinske energije, mikroskopijo ali z optično pinceto.

Vpliv gena *spvM* na bakterijski fitness bomo preučili v ekološko pomembnih pogojih. Ocenili bomo vlogo različnih komponent biofilma (vključno s produktom gena *spvM*) pri zajemanju fagnih delcev z uporabo klasičnih testov fagne aktivnosti, fluorescenčne mikroskopije s fluorescenčno označenimi fagi ter mikrofluidike. Raziskovalec bo izdelal tudi nove bakterijske

seve z umetno spremenjenim vezavnim mestom faga in z označeno fagno DNK, da bi preizkusil vpliv *spvM* na prenosa faga. Profag, ki nas zanima, se izreže iz bakterijsk DNK v matični celici spor, kar omogoča, da se *spvM* sestavi in lahko izraža pred sporulacijo. Zato bo v okviru projekta preizkušena tudi vloga produkta *spvM* na spore glede na a) biofizikalne lastnosti spor, z uporabo metod, kot so meritve površinske energije, odpornost na peroksid, sposobnost spor za interakcijo z EPS in b) mešanje spor z uporabo fluorescenčne mikroskopije za sledenje v času; c) prenos fagov preko spor z uporabo fluorescenčne mikroskopije za slednje v času, FACS in qPCR.

Kandidat/kandidatka bo razširil znanje na področju biologije bakterij in fagov, kvantitativnih metod za preučevanje sestave biofilmov, molekularne mikrobiologije vključno z fagnim inženiringom, izolacije DNK in kvantitativne analize, mikroskopije ter sortiranja celic. Program od kandidata zahteva osnovno znanje iz področja mikrobiologije, molekularne biologije in biokemije. Rezultati raziskovalnega programa bodo lahko odgovorili na temeljna vprašanja o razmerju med fagi in gostitelji. Testirali bodo hipotezo o manipulaciji gostitelja preko integracij v funkcionalni genski lokus.

#### ŽELENI KANDIDAT/KANDIDATKA:

- ima opravljen univerzitetni študij druge stopnje oz. kot zahteva nacionalna zakonodaja, na področju mikrobiologije, biologije, biokemije, biotehnologije ali drugih naravoslovnih ved.
- ima izkušnje z gojenjem bakterij in fagov.
- ima visoko motivacijo za dodatno izobraževanje v Sloveniji in v tujini, predstavitev raziskav na domačih in mednarodnih konferencah.
- ima odlično znanje angleškega jezika (pisno in pogovorno).
- ima sposobnost za samostojno delo in učenje, a hkrati za delo v večji skupini.

#### DODATNE PREDNOSTI:

- Izkušnje z genskim inženiringom bakterij in fagov, izolacijo in kvantificiranjem biopolimerov.
- Izkušnje z modelnim organizmom *Bacillus subtilis*.
- Izkušnje pri pisanju izvirnih znanstvenih člankov v angleščini.

#### *Eng: Impact of host control during lysogeny on phage horizontal transmission*

Certain group of temperate phages integrate into functional loci, inactivating them and transiently altering bacterial phenotypes. An example of such loci can be biofilm-associated genes in *Bacillus*. Biofilms are multicellular assemblies of bacteria embedded in exopolymers, which are known to serve as barriers against phages. Therefore, phage integration into biofilm-associated genes can have very interesting implications for phage transmission and for phage-host interactions – currently these remain largely unknown.

The training program for the young researcher will be related to study of:

- 1) impact of phage attachment gene on biofilm properties,
- 2) effect of biofilm matrix components on dispersal and stability of phage particles
- 3) effect of biofilm matrix components on phage transmission between hosts

*Bacillus subtilis* is a model organism to study various forms of biofilms, it is broadly studied at the molecular and ecological level at the Chair of Microbial Ecology and Physiology, Biotechnical Faculty, University of Ljubljana.

We hold lysogenic *B. subtilis* strains, where the prophage of interest is integrated into biofilm-associated genetic locus *spsM*. We also carry natural isolates that are non-lysogenic, and where this biofilm-associated locus is intact. Finally, we carry the locus mutant ( $\Delta$ *spsM*). During the project, role of *spsM* gene product on biofilm composition and biophysical properties of biofilm will be tested, allowing the young researcher to master methods such as: HPLC-SEC, rheology, surface energy measurements, microscopy imaging or optical tweezers.

Effect of *spsM* gene on bacterial fitness will be examined under series of ecologically relevant conditions. Role of different biofilm components (incl. *spsM* product) on capturing of phage particles will be assessed, using classical phage activity assays, fluorescence microscopy with fluorescently labelled phage particles, as well as microfluidics, applying different flow rates. The researcher will also construct engineered bacterial strains, with artificially altered phage attachment gene, and trackable phage DNA in order to test effect of *spsM* disruption of phage transmission. The prophage of interest undergoes excision in the spore mother cell, allowing the *spsM* to reintegrate and be expressed prior to sporulation. Therefore, in final part of the project, role of *spsM* product on spores will be tested considering a) spore biophysical properties, using methods such as surface energy measurements, resistance to peroxide, the spores ability to interact with EPS and b) spore mixing using time-lapse fluorescence microscopy; c) phage transmission via spores, using time-lapse fluorescence microscopy, FACS and qPCR.

The candidate will expand knowledge in bacteria and phage biology, quantitative methods in biofilm components research, molecular microbiology incl. phage engineering, DNA isolation and quantitative analysis, bacterial populations imaging, cell sorting. The program requires the young researcher to have basic knowledge in microbiology, molecular biology and biochemistry. The results of the research program will provide answers to fundamental questions of phage-host relationship, testing the parasite manipulation hypothesis via integration into functional host genes.

#### A DESIRED CANDIDATE HAS:

- completed second level university course or. as required by national legislation, in the field of microbiology, biology, biochemistry, biotechnology or other natural sciences.
- experience with bacteria and phage cultivation.
- high motivation for additional training in Slovenia and abroad, presentation of research at domestic and international conferences.
- excellent knowledge of English language (written and conversational).
- ability to work and learn independently, but at the same time to work in a larger group.

#### ADDITIONAL ASSETS:

- Experience with bacteria and phage genetic engineering, isolation and quantification of biopolymers.
- Experience with *Bacillus subtilis* model organism.
- Experience in writing original scientific papers in English.

