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

1. Raziskovalna organizacija (*Research organisation)*:

Univerza v Ljubljani, Biotehniška fakulteta

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

Polona Zalar; polona.zalar@bf.uni-lj.si

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

1.05 Biokemija in molekularna biologija

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

slo: Mikrobna razgradnja izbranih plastičnih polimerov

Plastika je eden od najbolj perečih okoljskih problemov današnjega časa. Okoljsko sporno ni samo njeno kopičenje v naravi, ampak tudi celoten postopek njene proizvodnje. Proizvedemo jo s polimerizacijo različnih monomerov v dolge verige ponavljajočih se molekul, med katerimi so najpogostejše: polietilen (PE), polipropilen (PP), polivinilklorid (PVC), polietilen tereftalat (PET), poliuretan (PU), polistiren (PS) in poliamid (PA) [1], [2]. Ocenjena proizvodnja plastičnih polimerov se približuje okrog 400 milijonom ton na leto [1], v naslednjih 20 letih naj bi se podvojila in do leta 2050 skoraj početverila [3].
Raziskave na področju mikroorganizmov, ki razgrajujejo plastiko, že potekajo, vendar so še v začetnih fazah [4]. Kot kandidatni organizmi za razgradnjo so bile v povezavi z okoljskimi dejavniki identificirane tako bakterije kot tudi glive. Plastika na račun delovanja abiotskih in biotskih faktorjev preide skozi tri različne faze: biodeterioracijo, depolimerizacijo, ter potencialno asimilacijo in mineralizacijo [5]. Ker je različnih polimerov plastike veliko, se bomo v predlagani raziskavi osredotočili le na izbrane, povezane bodisi z barvami ali plastičnimi utrjevalci v restavratorstvu-konservatorstvu (akrilne emulzije in disperzije, poliestrske in alkidne smole). Eden od izbranih polimerov bo poliuretan (PU), polimer organskih enot, povezanih s karbamatom, ki se kot dodatek barvam uporablja še v penah, izolacijskih materialih in tekstilnih premazih. Doslej so poročali o biorazgradnji PU na osnovi estrov, ni pa še znanih encimov, ki bi delovali na PU na osnovi etrskih vezi [1]. Med bakterijami so najboljše razgrajevalce našli v rodu *Pseudomonas, P. putida* naj bi razgrajeval PU z relativno visoko hitrostjo [6]. Drugi potencialni razgrajevalci pa so še: *Comamonas acidovorans, Bacillus subtilis* in *Alicycliphilus* sp. [1]. Dokazali so tudi delovanje encimov kutinaz, ki sicer delujejo tudi na polietilen tereftalat (PET - uporaba v plastenkah, folijah, vlakna v tekstilni industriji) [7]. Poliuretan naj bi razgrajevale tudi številne glive: *Pestalotiopsis microspora* (z metalohidrolazo), *Fusarium solani, Candida ethanolica, C. rugosa* (z lipazo), več vrst kompleksa *Cladosporium cladosporioides, Aspergillus fumigatus,* in *A. flavus* (domnevno z esterazami), *Penicillium chrysogenum, P. griseofulvum, Xepiculopsis graminea, Leptosphaeria sp., Agaricus bisporus,* in *Marasmius oreades* [1], [8], [9].
Delo mladega raziskovalca bo zajemalo naslednje tehnike in korake:

- izolacija gliv in bakterij iz okolij bogatih s cikličnimi ogljikovodiki (sladkovodna onesnažena okolja, plastični predmeti kulturne dediščine, akrilni polimeri, poliuretan) kot potencialnen možen vir ustreznih encimov za razgradnjo plastike
- identifikacija izoliranih mikroorganizmov z molekularnimi pristopi
- izvedba presevnih testov za razgradnjo izbranih plastičnih polimerov (poliuretan) na izolatih, postavitev testov za razgradnjo akrilnih polimerov uporabljenih v restavratorstvu – konservatorstvu;
- presejanje dodatnih gliv potencialnih razgrajevalk plastike iz izbora sevov v Ex zbirki
- izbor za razgradnjo PU, akrila najobetavnejših sevov gliv/bakterij (predvidoma do 4)
- *de novo* sekvenciranje genomov izbranih sevov (s platformo BGI-Seq 500)
- sekvenciranje transkriptomov vseh izbranih sevov (v triplikatih) po rasti na izbranem plastičnem polimeru v primerjavi z rastjo na gojišču brez polimera; za identifikacijo kandidatnih genov, ki sodelujejo pri plastični razgradnji, bomo uporabili kombinacijo genomskih in transkriptomskih podatkov
- izražanje potencialno uporabnih encimov v heterolognem sistemu

Pogoji za izbor: Zaključen magistrski študij Molekulske in funkcionalne biologije ali Mikrobiologije, ali Biotehnologije, ali Biokemije. Zaželjeno je dobro znanje angleškega jezika, samoiniciativnost, komunikativnost, izkušnje z laboratorijskim delom, predznanje na področju bioinformatike.

Reference:

[1] D. Danso, J. Chow, and W. R. Streit, "Plastics: environmental and biotechnological perspectives on microbial degradation," *Appl. Environ. Microbiol.*, vol. 85, no. 19, Jul. 2019, doi: 10.1128/AEM.01095-19.

[2] Science Advice for Policy by European Academies (SAPEA), *A Scientific Perspective on Microplastics in Nature and Society | SAPEA*. Berlin: SAPEA, 2019.

[3] Ellen MacArthur Foundation, "The New Plastics Economy: Rethinking the future of plastics," 2016.

[4] M. Crippa *et al.*, *A circular economy for plastics*. Brussels: European Commission, 2019.

[5] N. Lucas, C. Bienaime, C. Belloy, M. Queneudec, F. Silvestre, and J.-E. Nava-Saucedo, "Polymer biodegradation: Mechanisms and estimation techniques – A review," *Chemosphere*, vol. 73, no. 4, pp. 429–442, Sep. 2008, doi: 10.1016/j.chemosphere.2008.06.064.

[6] Y.-H. Peng, Y. Shih, Y.-C. Lai, Y.-Z. Liu, Y.-T. Liu, and N.-C. Lin, "Degradation of polyurethane by bacterium isolated from soil and assessment of polyurethanolytic activity of a *Pseudomonas putida* strain," *Environ. Sci. Pollut. Res.*, vol. 21, no. 16, pp. 9529–9537, Aug. 2014, doi: 10.1007/s11356-014-2647-8.

[7] J. Schmidt *et al.*, "Degradation of polyester polyurethane by bacterial polyester hydrolases," *Polymers (Basel).*, vol. 9, no. 12, p. 65, Feb. 2017, doi: 10.3390/polym9020065.

[8] I. Brunner, M. Fischer, J. Rüthi, B. Stierli, and B. Frey, "Ability of fungi isolated from plastic debris floating in the shoreline of a lake to degrade plastics," *PLoS One*, vol. 13, no. 8, p. e0202047, Aug. 2018, doi: 10.1371/journal.pone.0202047.

[9] J. R. Russell *et al.*, "Biodegradation of Polyester Polyurethane by Endophytic Fungi," *Appl. Environ. Microbiol.*, vol. 77, no. 17, pp. 6076–6084, Sep. 2011, doi: 10.1128/AEM.00521-11.

eng: Microbial degradation of selected plastic polymers

Plastic is one of the most urgent environmental problems of our time. Not only its accumulation in nature is ecologically controversial, but also the entire process of its production. It is produced by polymerizing various monomers into long chains of repeating molecules, the most common of which are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyurethane (PU), polystyrene (PS) and polyamide (PA) [1], [2]. The estimated production of plastic polymers is approaching 400 million metric tonnes per year [1], which is expected to double in the next 20 years and almost quadruple by 2050 [3].

Research on plastics-degrading micro-organisms is already under way, but is still in its infancy [4]. Both bacteria and fungi have been identified as candidate organisms for plastic degradation in conjunction with environmental factors. Plastics in the environment passes through an order of events, which can be divided into three stages: biodeterioration, depolymerization, and potential assimilation and mineralization [5]. Since there are many different polymers of plastics, the proposed research will focus only on selected ones, which are either related to paints or to plastic consolidants in restoration- conservation (acrylic emulsions and dispersions, polyester and alkyd resins). One of the selected polymers will be polyurethane (PU), a polymer of organic units associated with carbamate, typically used in foams, insulation materials, textile coatings, as well as in paint. Biodegradation has so far been reported for the ester-based PU, but no enzymes acting on ether-based PU are currently known [1]. Among bacteria, *Pseudomonas* spp. are the best known degraders, starting with two lipases from *Pseudomonas chlororaphis*. *Pseudomonas putida* was reported to degrade PU at relatively high rates [6]. *Comamonas acidovorans*, *Bacillus subtilis* and *Alicycliphilus* sp. are other potential degraders [1]. Some cutinases acting on PET have been shown to be sufficiently promiscuous to also degrade PU [7]. A number of fungi have also been reported to degrade polyurethane: *Pestalotiopsis microspora* (with a metallo-hydrolase), *Fusarium solani*, *Candida ethanolica*, *Candida rugosa* (with a lipase), several species from *Cladosporium cladosporioides* complex, *Aspergillus fumigatus*, and *Aspergillus flavus* (presumably with esterases), *Penicillium chrysogenum, Penicillium griseofulvum, Xepiculopsis graminea*, *Leptosphaeria sp*., *Agaricus bisporus,* and *Marasmius oreades* [1], [8], [9].

The work of the young researcher comprises the following steps and includes the following techniques:

- isolation of fungi and bacteria from environments rich in cyclic hydrocarbons (polluted freshwater environments, plastic cultural heritage objects, acrylic polymers, PU) as a potential source of suitable enzymes for the decomposition of plastics;
- identification of isolated microorganisms by molecular approaches;
- implementation of screening tests for the decomposition of selected plastic polymers (PU) on isolates, possible development of tests for the decomposition of acrylic polymers used in restoration - conservation;
- screening of additional fungi (potential plastic decomposers) from the selection of strains in the Culture collection of extremophilic microorganisms Ex;
- selection of the most promising fungal/bacterial strains (for decomposition of PU, acrylic emulsions, polyester and alkyd resins of strains (estimated up to 4);
- de novo sequencing of genomes of selected strains (using the BGI-Seq 500 platform);
- sequencing of transcriptomes of selected strains and comparison between cultures grown on the selected plastic polymer and cultures grown on glucose; a combination of genomic and transcriptomic data is used to identify candidate genes involved in plastic degradation;
- expression of potentially useful enzymes in a heterologous system.

Conditions for young researcher selection: Completed master's degree in Molecular and Functional Biology, or Microbiology, or Biotechnology, or Biochemistry. Good English language skills, initiative, communication skills, experience with laboratory work, knowledge in the field of bioinformatics are desirable.

[1] D. Danso, J. Chow, and W. R. Streit, "Plastics: environmental and biotechnological perspectives on microbial degradation," *Appl. Environ. Microbiol.*, vol. 85, no. 19, Jul. 2019, doi: 10.1128/AEM.01095-19.

[2] Science Advice for Policy by European Academies (SAPEA), *A Scientific Perspective on Microplastics in Nature and Society | SAPEA*. Berlin: SAPEA, 2019.

[3] Ellen MacArthur Foundation, "The New Plastics Economy: Rethinking the future of plastics," 2016.

[4] M. Crippa *et al.*, *A circular economy for plastics*. Brussels: European Commission, 2019.

[5] N. Lucas, C. Bienaime, C. Belloy, M. Queneudec, F. Silvestre, and J.-E. Nava-Saucedo, "Polymer biodegradation: Mechanisms and estimation techniques – A review," *Chemosphere*, vol. 73, no. 4, pp. 429–442, Sep. 2008, doi: 10.1016/j.chemosphere.2008.06.064.

[6] Y.-H. Peng, Y. Shih, Y.-C. Lai, Y.-Z. Liu, Y.-T. Liu, and N.-C. Lin, "Degradation of polyurethane by bacterium isolated from soil and assessment of polyurethanolytic activity of a *Pseudomonas putida* strain," *Environ. Sci. Pollut. Res.*, vol. 21, no. 16, pp. 9529–9537, Aug. 2014, doi: 10.1007/s11356-014-2647-8.

[7] J. Schmidt *et al.*, "Degradation of polyester polyurethane by bacterial polyester hydrolases," *Polymers (Basel).*, vol. 9, no. 12, p. 65, Feb. 2017, doi: 10.3390/polym9020065.

[8] I. Brunner, M. Fischer, J. Rüthi, B. Stierli, and B. Frey, "Ability of fungi isolated from plastic debris floating in the shoreline of a lake to degrade plastics," *PLoS One*, vol. 13, no. 8, p. e0202047, Aug. 2018, doi: 10.1371/journal.pone.0202047.

[9] J. R. Russell *et al.*, "Biodegradation of Polyester Polyurethane by Endophytic Fungi," *Appl. Environ. Microbiol.*, vol. 77, no. 17, pp. 6076–6084, Sep. 2011, doi: 10.1128/AEM.00521-11.