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MiCoBion

Microbial Communities in Biomedical and Environmental Areas, and Systems Biology

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Organized summer school at BIOCEV

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PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
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CO	Confidential, only for members of the consortium (including the Commission Services)	

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Executive Summary

This document provides a summary of a completed task within Work Package WP1 *Training*, namely: T1.6 *Summer school* led by the Charles University (CUNI). It contains description of the content of the organized school, presents details about the lecturers and participants, and provides detail about the event outcomes and benefits brought to CUNI.

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1. Introduction

Deliverable D1.6 Organized s*ummer school at BIOCEV* is associated with task T1.6. The objective of this task was to provide training in modern approaches to investigate biology of parasitic protists (bioinformatics, reverse genetics, proteomics, biochemistry, and advanced bioimaging). The task consisted of a two weeks-long event organized in a format of morning lectures and previous day result discussions, and afternoon experimental exercises. In the event, lectures and specialists from Catholic University of Leuven (KUL), University of Paris (UP), the European Molecular Biology Laboratory (EMBL), and Charles University (CUNI) (2 invited experts, 2 CUNI experts) and 12 young scientists from CUNI were included. However, due to acute COVID-19 infection, an invited lecturer from UP, was replaced by an expert from CUNI, and invited specialist from EMBL by expert from University in Dundee, who is a member of the MiCoBion External Advisory Board (EAB).

2. Summer School Participants

The attendees of the course were young scientists and students of CUNI. Twelve participants were accepted for the course, unfortunately one of them was COVID-19 infected shortly before the course and was unable to attend to summer school. The team of lecturers from CUNI included senior researchers Jan Tachezy, and Pavel Doležal and young scientists Zdeněk Verner, Petr Rada, Luboš Voleman, and Ravi Ravi Kumar Narayanasamy.

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3. Summer School Content

Lectures:

Monday July 11th

Welcome participants

Jan Tachezy (CUNI): Introduction *Trichomonas vaginalis* projects

Pavel Dolezal (CUNI): Introduction *Giardia intestinalis* projects

Tuesday July 12th

Aleš Benda (CUNI): Light microscopy, fluorescence microscopy, confocal microscopy, superresolution.

Wednesday July 13th

David Liebl (CUNI): Electron microscopy

Thursday July 14th

Philippe Van den Steen, Katholic University of Leuven, Belgium: Malaria immunology (on line)

Friday July 15th:

Mark Field, University of Dundee, UK: How trypanosomes express genes to succeed.

Monday July 18th

Jean-Michel Camadro, UP - JMI, Paris, France (replaced by Karel Harant, CUNI)**:** Mass spectrometry, principles and applications

Experimental training was performed in a format of two weeks projects. The participants were divided into couples to combine more and less experienced participants, and each couple was given a specific question/task in frame of more general objectives to be solved within two weeks. Two human parasites were selected as model organisms: *Trichomonas vaginalis* and *Giardia intestinalis.* Every morning, the progress in each project was discussed and following experimental steps were suggested. All members of the CUNI team assisted to participants in experimental work during the day. The last day all participants presented achieved results for general discussion.

Objective 1: Multiphasic approach to investigate biology of *Trichomonas vaginalis*

Specific objective (SO)1. Cell localization and function of small TIM proteins in *Trichomonas vaginalis* hydrogenosomes

Task 1: Immunofluorescence microscopy of trichomonads expressing TvTimA, TvTimC and TvTimAC.

Task 2: Detection of small Tims in *T. vaginalis* subcellular fractions using mass spectrometry

SO2. Elucidation of hydrogenosomal membrane potential

Task 1: Investigation of membrane potential using flow cytometry

Task 2. Investigation of membrane potential in living cells using epifluorescent microscope Nikon Spinning Disc.

SO3. The gene knockout using CRISPR-Cas9

Task 1-4: Four different genes required for the protein import machinery in the hydrogenosomal TIM complex.

Part 1: Gene target selection

Part 2: Designing guide RNA

Part 3: Genscript-gRNA Order and cloning

Part 4: Homology Directed Repair (HDR) cassette

Part 5: Nucleofection

Part 6: PCR Confirmation

Part 7: Single cell cloning

Part 8: KO Clone PCR and preservation

Objective 2: Multiphasic approach to investigate biology of *Giardia intestinalis*

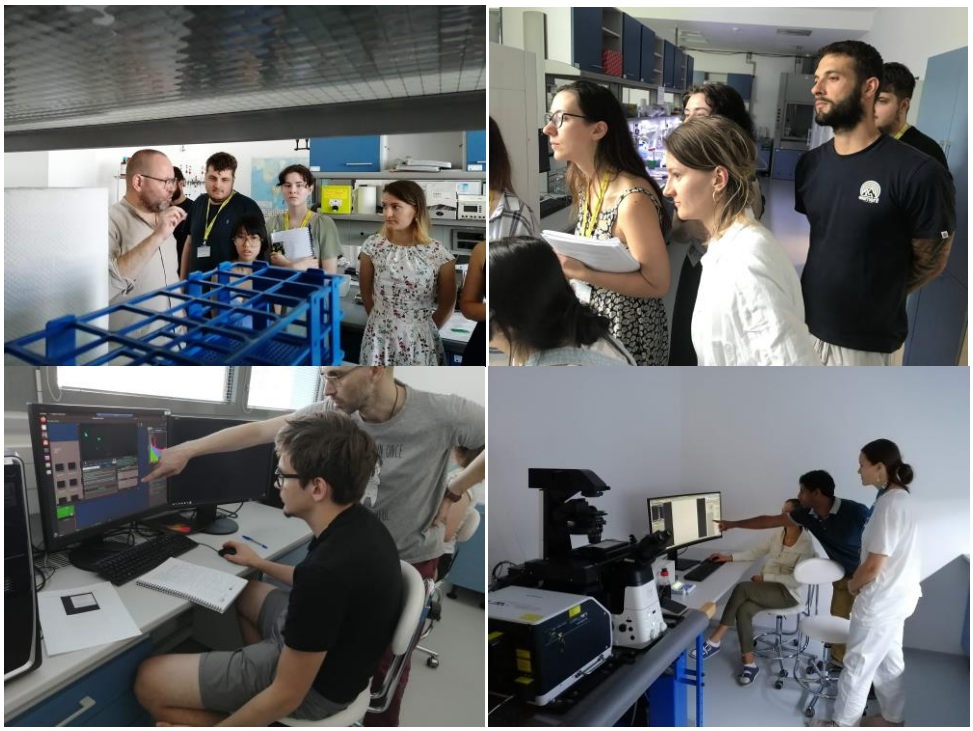
SO 1. Giardia metabolism: Pyruvate:ferredoxin oxidoreductase (PFOR) – key metabolic enzyme

SO 2. Preparations of CRISPR-Cas9 based knockout for TrxR.

SO3. Testing the interaction of recombinant TrxR and Grx5

SO4. CRISPR/Cas9-mediated FISH in *Giardia*?

SO5. Auxin inducible degradation (AID) in *G. intestinalis.*



4. Summer School Outcomes

Professional Experience: The participants gained theoretical knowledge and practical experience in modern approaches for the investigations of parasitic protists. Particularly, they learned preparation of samples for proteomic analysis using mass spectrometry and row data evaluation, functional genomics using CRISPR-Cas9 based reverse genetics, and advanced bioimaging methods including software for data processing.

5. Conclusions

T1.6 *Summer school* was successfully completed.

6. Degree of Progress

The deliverable was 100% fulfilled concerning content of the course and number of participating experts: 9 experts from CUNI, 1 expert from KUL. Experts from EMBL and UP were due to COVID-19 infections replaced by specialists from CUNI and EAB (University of Dundee).

7. Dissemination Level

The Deliverable 1.6 *Organized summer school at BIOCEV* document is a public deliverable.

See also:

https://www.biocev.eu/en/about/projects/micobion.2/summer-school-biology-of-parasitic-protists-practical-course.334