

# *The UK-Ukraine University Twinning Grant*

## **Susceptibility of *Escherichia coli* isolates to a new class of anti-bacterial agent**



INSTITUTE FOR  
INFECTION &  
IMMUNITY

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**Roman Kutsyk**

# The UK-Ukraine University Twinning Initiative



A £5 million investment, by Research England is led by Cormack Consultancy Group and UUKi, designed to support Ukrainian universities and researches.

It is intended to help UK and Ukrainian universities share resources and assistance to benefit Ukrainian institutions, staff and students.

**Name of lead HEI (UK): St. George's, University of London**

**Name of lead applicant: Prof. Julian Ma**

**E-mail address of lead applicant: [jma@sgul.ac.uk](mailto:jma@sgul.ac.uk)**

**Role/job title: Hotung Professor of Molecular Immunology / Director of the Research Institute for Infection and Immunity**



**Name of lead HEI (Ukraine): Ivano-Frankivsk National Medical University**

**Name of lead applicant: Prof. Roman Kutsyk / E-mail address of lead applicant:**

**[rkutsyk@ifnmu.edu.ua](mailto:rkutsyk@ifnmu.edu.ua)**

**Role/job title: Head of the Department of Microbiology, Virology and Immunology**

**Lead UK - Ukraine twin details**



## Deliverables

- A characterized panel of 30-100 clinical *E. coli* isolates from Ukraine, including antibiotic sensitivities to 6 front-line antibiotics.
- Sensitivity and resistance profiling of the *E. coli* panel to three recombinant colicins.
- Evaluation of colicin combinations to cover resistant strains.
- Determination of any correlation between antibiotic resistance and colicin resistance.
- Genomic analysis of antibiotic and colicin resistant isolates to elucidate linked mechanisms of AMR.



## Susceptibility of *Escherichia coli* isolates to a new class of anti-bacterial agent

- ▶ This research addresses the discovery and development of new anti-microbial compounds.
- ▶ Bacteriocins are well known anti-bacterial proteins, but high yield expression has only recently been achieved using plant-based biomanufacturing. The potential for economical production at commercial scale, now allows further investigation of bacteriocins as adjuncts or alternatives to conventional antibiotics.
- ▶ The **objective** of this research is to collect a panel of *Escherichia coli* clinical isolates and to test **susceptibility** to three recombinant **colicins** individually or in combinations. We will determine whether antibiotic and colicin resistance might be linked and provide data to support further clinical development of plant-derived colicins.

# Nomad Bioscience GmbH as an Investment Case

Nomad is a privately held German company with ownership controlled by the original founders; no investors at present  
Between 2008 (founding) and 2022, Nomad has spent EUR 36.7 million for its operations; expects to spend EUR 2.5 - 3.0 million more by Q3 2023 (expected investor entry)

Nomad financed its operations through contract research (over EUR20 million), use of part of its proceeds from the sale of its subsidiary Icon Genetics to Denka (EUR 85 million), licensing and grants

Apart from conducting contract research, mostly for Bayer and

Monsanto, Nomad was developing several projects:

- Bacteriocins as antibacterial biologics for medicine
- Antiviral lectins as preventative therapeutics
- Sweet and taste modifying proteins for food market (now at Nambawan)
- Antibacterial proteins for food safety and veterinary (now at Nambawan)

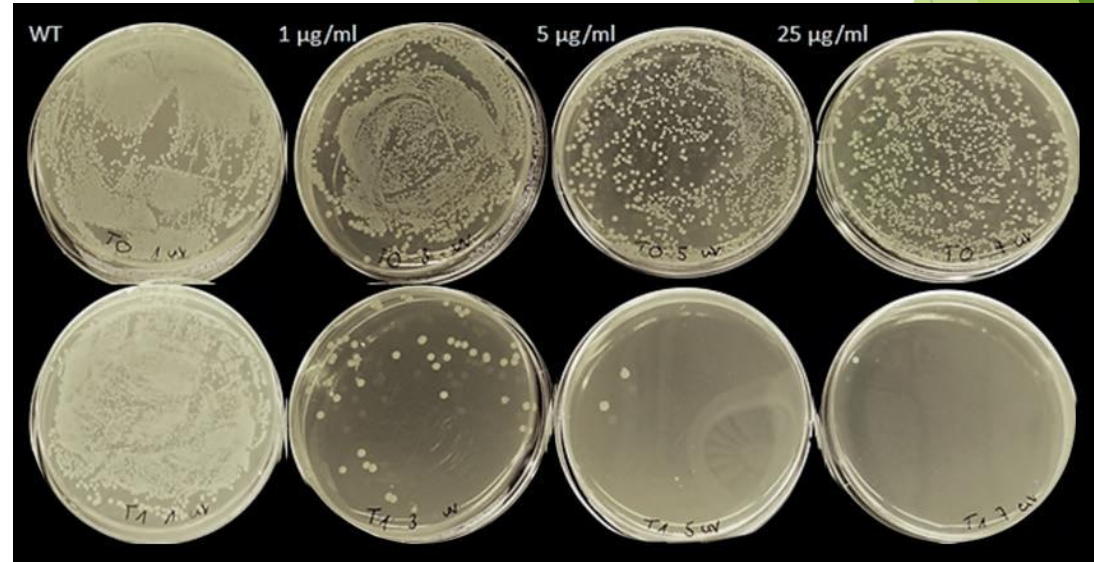
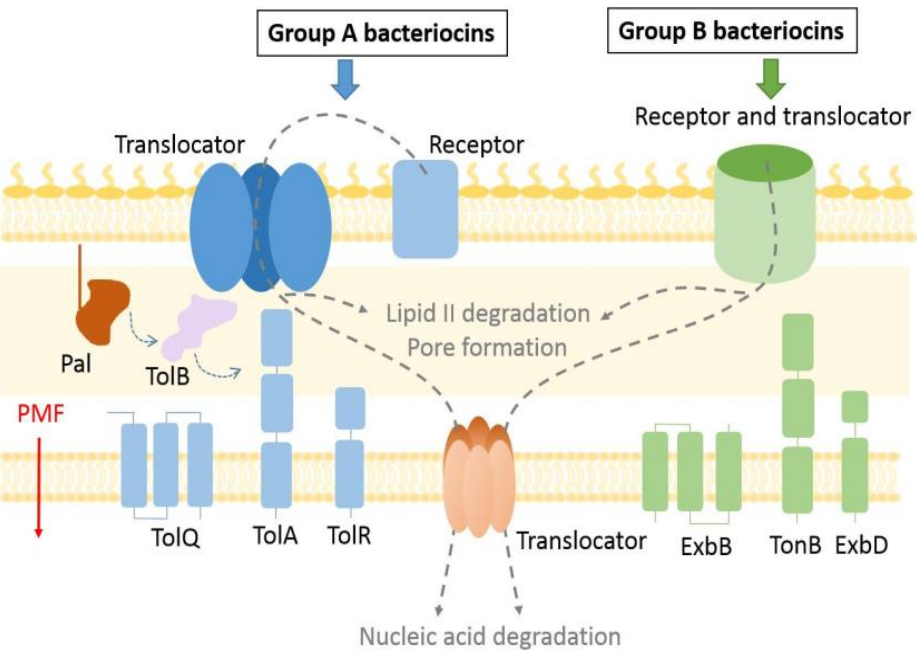
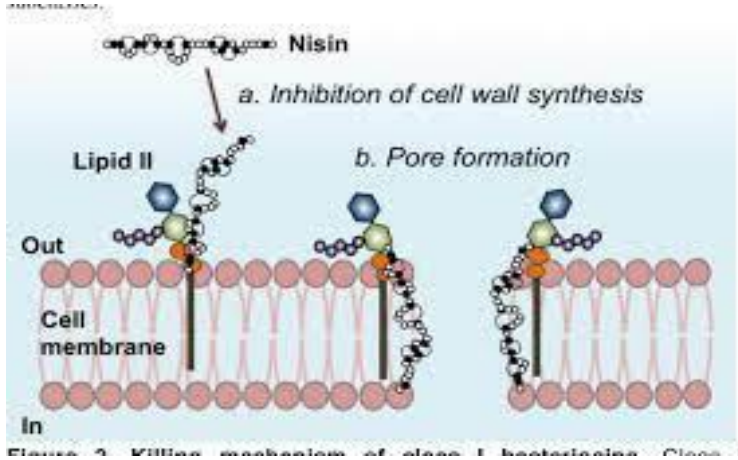
Nomad's proposed investment case is around Bacteriocins as medicine biologics; other projects have already been spun off (now with Nambawan); whereas Antiviral lectins program will be licensed out by Q3 2023 (prior to investors stepping in)

*Biologics instead of small molecules*  
*Truly novel modes of action*  
*Control of Gram-negative pathogens*  
*Highly active against multidrug- and pandrug-resistant pathogens*  
*Precision antibacterials*  
*High efficacy in validated animal models*  
*Effective control of bloodstream infections*  
*Strong, eventually dominant IP position*  
*First-In-Class therapeutics*  
*First-In-Humans clinical trials*  
*Clear path to a major value inflection point*



# Bacteriocins

- ▶ Precision antibacterial biologics invented by nature
- ▶ Evolved naturally by bacteria to fight same or similar bacterial species
- ▶ Highly potent *in vitro* and *in vivo*, rapid acting, multiple modes of action, huge natural diversity, easy to engineer, easy to produce and purify
- ▶ Due to novel modes of action, excellent control of multi-/pan-drug resistant Gram-negative bacteria
- ▶ Destroy only the pathogenic species while sparing gut microbiome



# Collection and identification of *E. coli* clinical strains

## MacConkey agar

a selective and differential culture medium for Gram-negative and enteric bacteria differentiate them based on lactose fermentation. Lactose fermenters turn red or pink on MacConkey agar, and nonfermenters do not change color. The media inhibits growth of Gram-positive organisms with crystal violet and bile salts, allowing for the selection and isolation of gram-negative bacteria. The media detects lactose fermentation by enteric bacteria with the pH indicator neutral red.

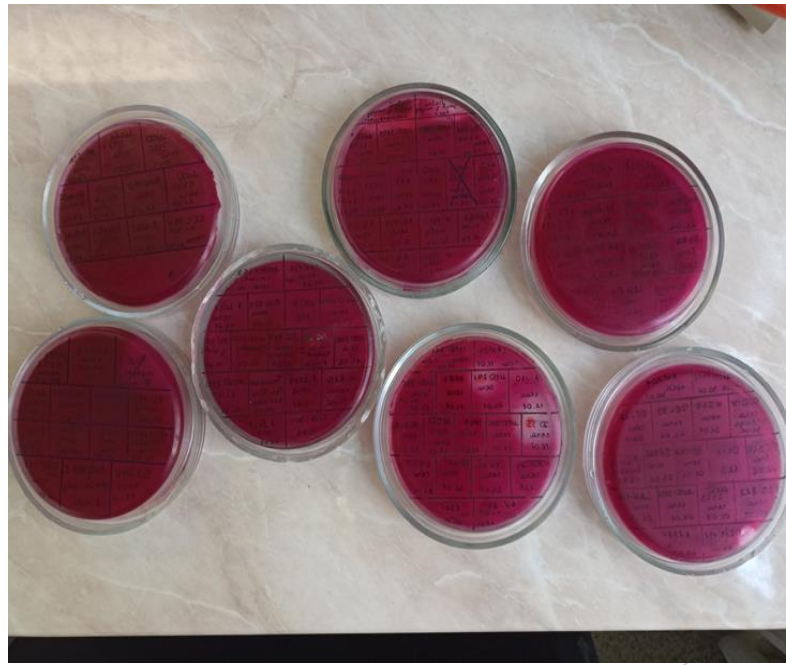


## UriSelect medium

Isolation and identification of both Gram-positive and Gram-negative pathogens in a single plate Chromogenic mix allowing the detection of specific enzymes activities

Direct identification of *E. coli* ( $\beta$ -Galactosidase, Indole), *Enterococcus* spp. and *Proteus mirabilis* via revelation and visualization of enzymatic activity of bacteria

Presumptive identification of KESC, Streptococci and Staphylococci (differentiation of *S. saprophyticus*) Inhibition of *Proteus* spp. swarming with an original formulation



Regional clinical hospital



Regional clinical infectious diseases hospital



Dermatovenereological center



Clinical  
bases for  
*E.coli*  
strains  
collection

Regional perinatal center



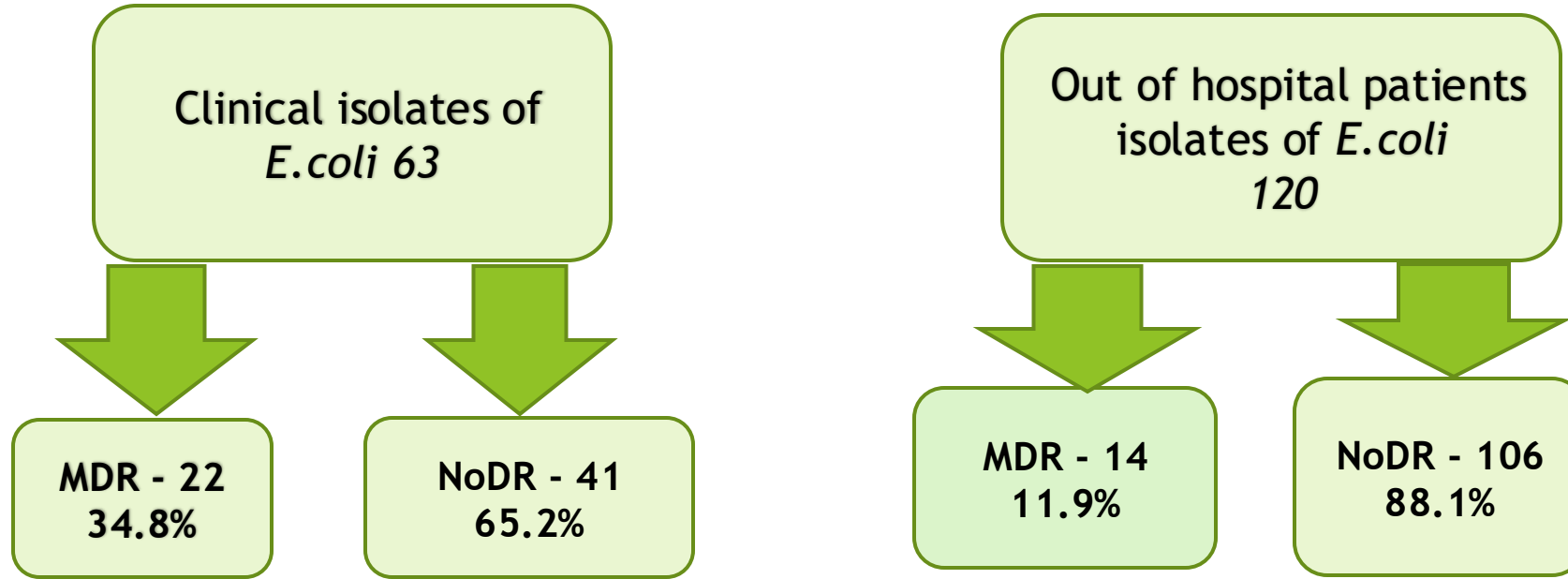
# Institutions for *E.coli* strains identification and determination of sensitivity to bacteriocins

Microbiology, Virology  
and Immunology  
department

25 Hetman Mazepa  
Street



# Isolated *E.coli* strains = 183



## Source of isolated strains:

- ▶ Pus: 13 ( 5.9%)
- ▶ Urine: 114 ( 68.1%)
- ▶ Stool : 31 ( 15.2%)
- ▶ BAL 1/1: 2 ( 0.7%)
- ▶ Vaginal swab: 10 (5.2 %)
- ▶ Lungs tissue: 2 (0.7%)
- ▶ Throat swab: 5 (1.4%)
- ▶ Oral cavity swab 2 (0.7%)
- ▶ Sperm 2 (0.7%)
- ▶ Urethral swab 2 (1.4%)

## Enterotest 24 (Lachema, Czech Republic)

Isolated strains of *E. coli* with low discrimination - 16 (11.6%):

- 3 strains with rate of discrimination 36.35%
- 4 strains with rate of discrimination 52.23 – 55.88%
- 4 strains with rate of discrimination 70.17 – 73.77%
- 5 strains with rate of discrimination 82.18 – 87.48%



		H	G	F	E	D	C	B	A
		URE	ARG	ORN	IVS	H.S	SCI	MAI	DNF
1	+	Red	Blue	Blue	Blue	Black	Blue	Blue	Yellow
	-	Yellow	Green	Green	Green	White	Green	Green	White

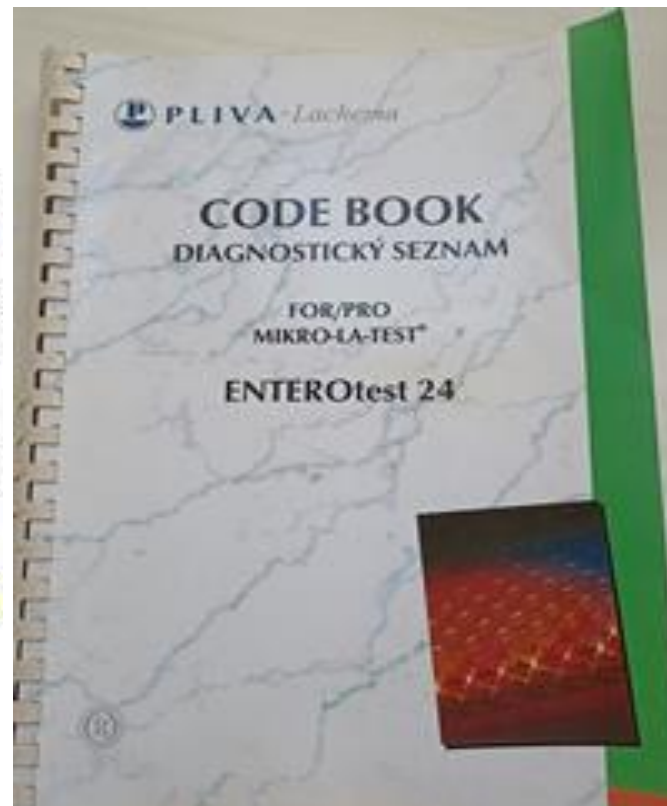
  

		H	G	F	E	D	C	B	A
		SAL	SOP	MIB	CEL	LAC	TRE	MAN	GIR
2	+	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
	-	Green	Green	Green	Green	Green	Green	Green	White

		H	G	F	E	D	C	B	A
		DUE	ADO	ABT	SUC	INO	BAF	ESI	LEY
3	+	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Black	Yellow
	-	Green	Green	Green	Green	Green	Green	White	White

RIDACOM



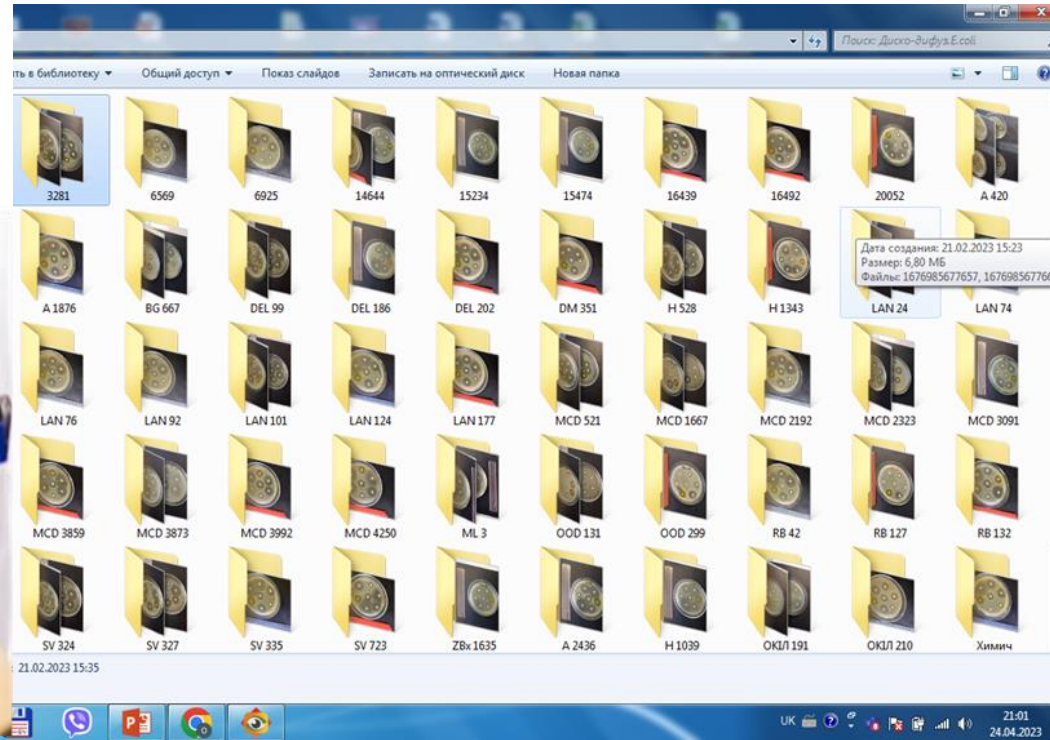
Antibiotics	Groups
amoxicillin	Penicillins
amoxicillin/clavulonic acid	Penicillins
tobramycin	Amynoglycosides
furazidinum	Nitrofurans
ofloxacin	Quinolones
ciprofloxacin	Quinolones
amoxicillin	Tetracyclines
tigecycline	Tetracyclines
polymyxin B	Polymixins
ceftazidime	3G Cefalosporins
cefotaxime	3G Cefalosporins
ceftriaxone	3G Cefalosporins
cefoperazone	3G Cefalosporins
cefepime	4G Cefalosporins
meropenem	Carbapenems
aztreonam	Monobactams
gentamicin	Amynoglycosides

## Strains of E. coli tested against 17 antibiotics

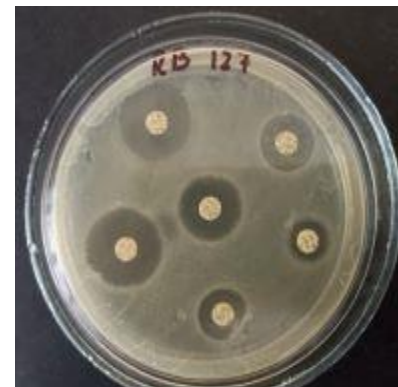
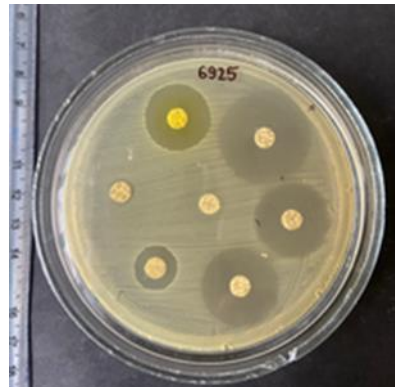
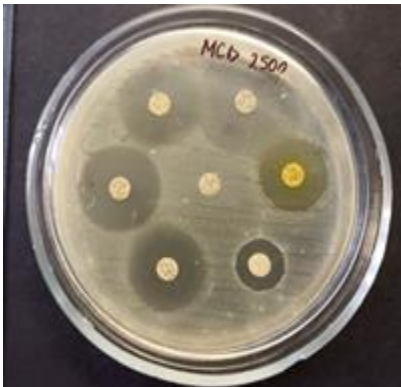
MDR - 36  
(19.6%)

NoDR - 147  
(80.4%)

# *Sensitivity to antimicrobial agents performed according to EUCAST recommendations*



## **Kirby-Bauer Disc Diffusion Method**



# Meetings supported by UUKi

## *Research/partnership development meeting*

Halle - 27.04.2023

Halle – 15.06.2023

London – 17.07.2023

## *Scientific training exchange*

Halle – 13.06. – 16.06.2023

London – 17.07.2023 – 21.07.23.



## **Scientific training exchange: Halle (Saale) - 13.06. - 16.06.2023**

### **Schedule:**

#### **Tuesday 13.6.23**

- ▶ Safety instruction for working in NOMAD biotechnological laboratory
- ▶ Susceptibility screen of 4 strains for 4 purified Colicins
- ▶ Over night cultures of the 4 strains
- ▶ Over night culture of DH10B pICH 73311 used for soft agar overlay assay

#### **Wednesday 14.6.23**

- ▶ MIC determination for selected strains from susceptibility assay with selected Col in different media (MHB, caMHB, LB)
- ▶ Testing of influence of BSA in medium on MIC
- ▶ ColM extraction of *N.benthamiana* leaf material and seed using ball mill and 2 different buffer
- ▶ Softagar overlayer assay of prepared samples in comparison to purified ColMcontrol
- ▶ Optinal: Preparation of 12% SDS-gel





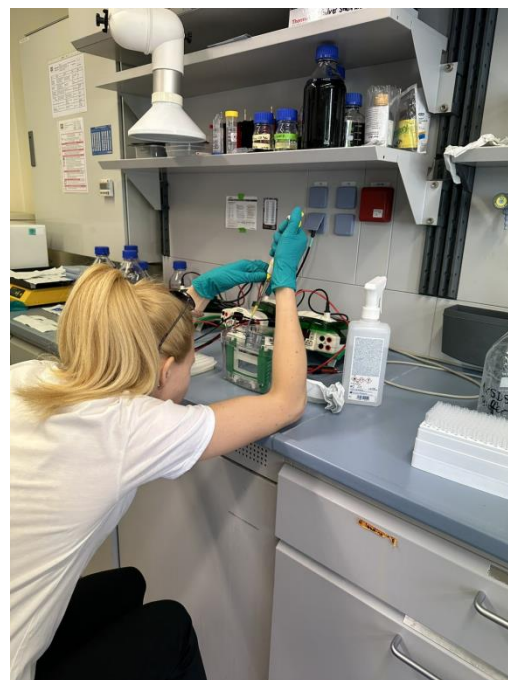
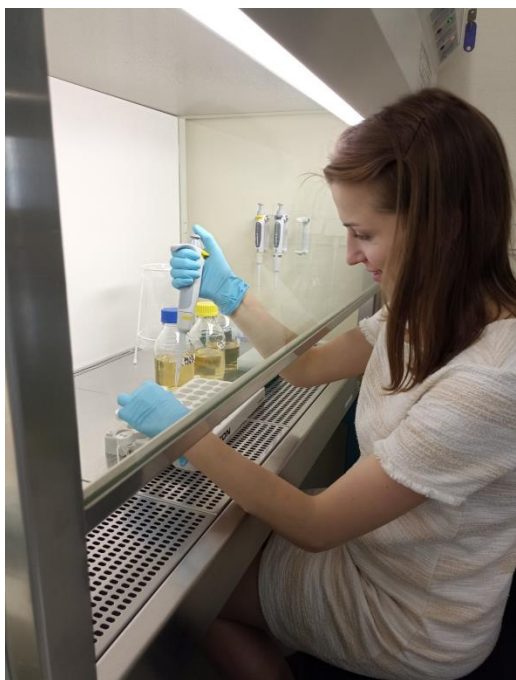
*Scientific training exchange:*  
Halle (Saale) - 13.06. - 16.06.2023

▶ **Thursday 15.6.23**

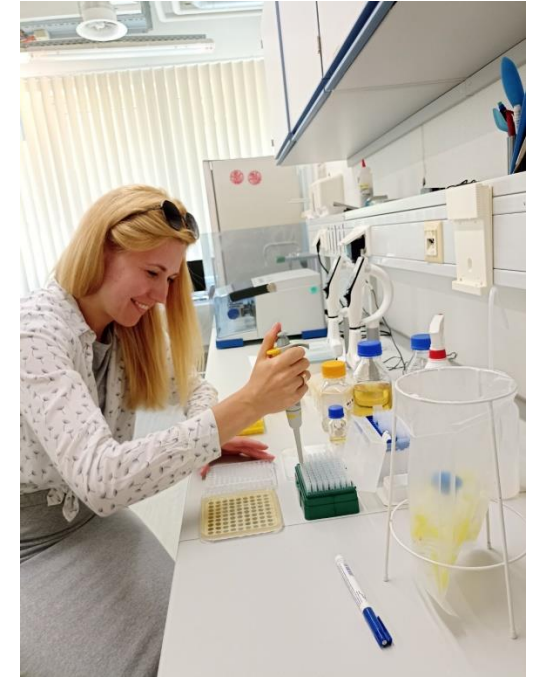
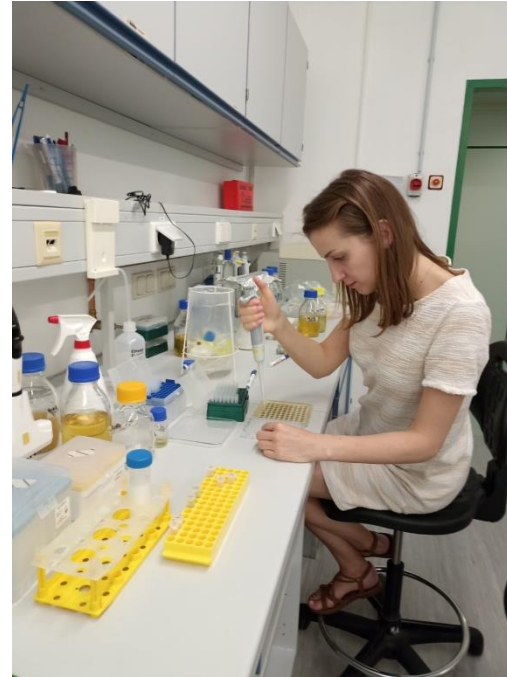
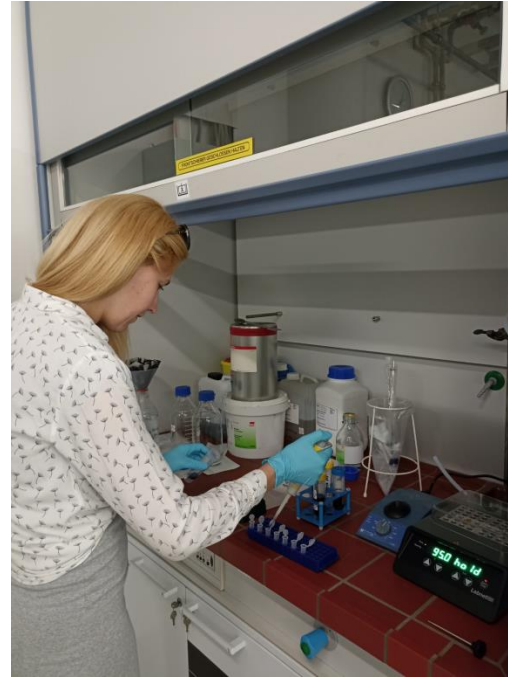
- ▶ Measurement of MICs
- ▶ Meeting with Julian Ma
- ▶ Loading samples on SDS-gel

▶ **Friday 16.6.23**

- ▶ Destaining of SDS gel
- ▶ Analysing of softagar-plate



Research/partnership development meetings: Halle (Saale) -  
27.04.2023; 15.06.2023



Research/partnership development meetings:  
Halle (Saale) - 27.04.2023; 15.06.2023

## Determination of antimicrobial activity of different Colicins

### Experimental procedures:

#### 1.) Susceptibility screen:

- Preparation of 6 well agar plates (5ml agar/well);
- Preparation of E.coli test culture and procedure;

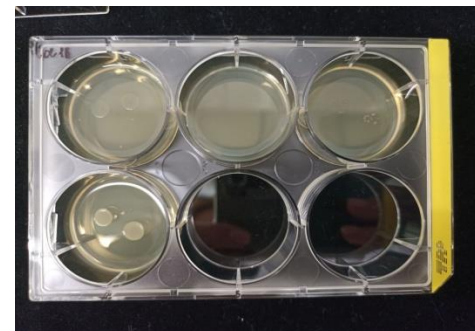
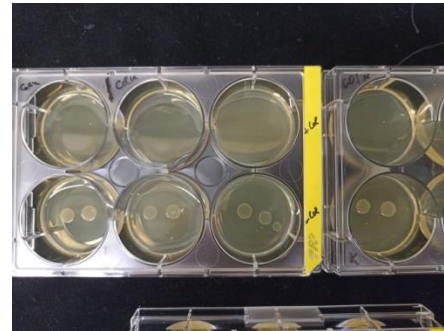
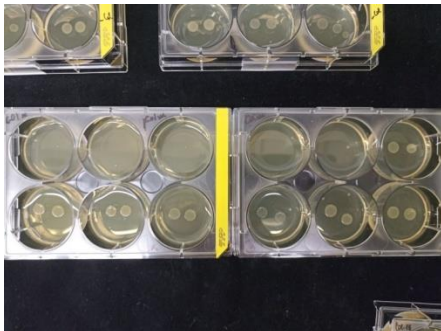
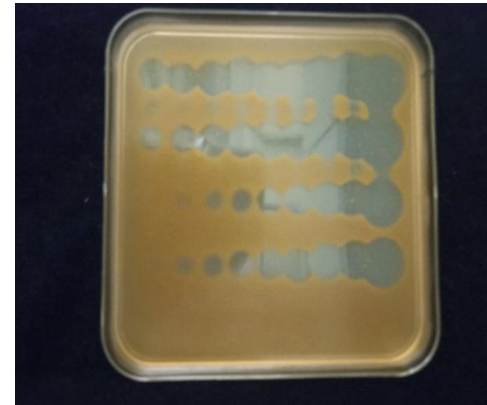
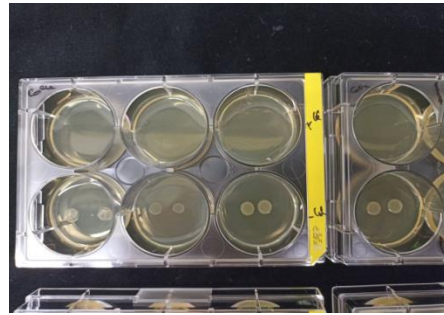
#### 2.) MIC determination:

- Preparation of colicin solution;
- Culture preparation;

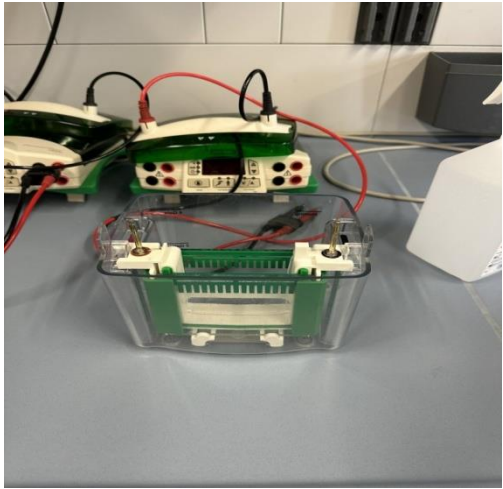
#### 3.) Softagar overlay assay:

- Preparation of ColM samples;
- Preparation of bacterial test medium.

- ▶ Colicins tested: ColU, ColM, ColK and Collb purified
- ▶ Tested strains: DH10B, DH5a, Nissle, NCTC 10538
- ▶ Comparison ColM expression in *N.benthamiana* vs. *N.tabacum* seeds by soft-agar overlay assay

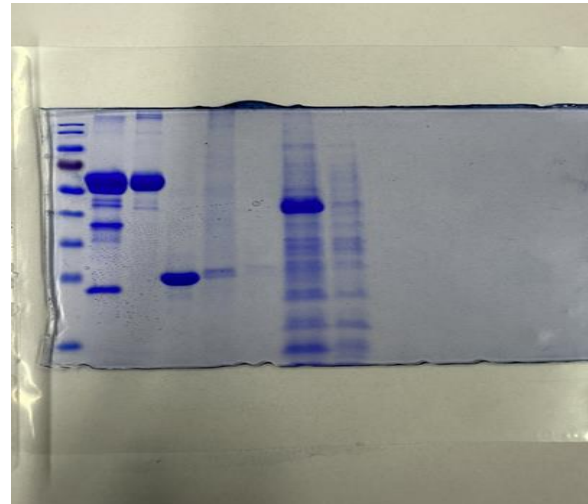


# Loading samples on SDR-gel



## Gel Electrophoresis Steps

- Preparing the samples for running;
- An agarose TAE gel solution is prepared;
- Casting the gel;
- Setting up the electrophoresis chamber;
- Loading the gel;
- Electrophoresis;
- Stopping electrophoresis and visualizing the proteins.





*Scientific training exchange:*

London - 17.07.23 - 22.07.23 p.

*Scientific training exchange:*

**London - 17.07.23 - 22.07.23 p.**

**Monday 17.07.2023:**

meeting with Chris Bakker to get acquainted with the work organization and technical capabilities of the simulation center for future paramedics training





## *Scientific training exchange:*

London - 17.07.23 - 22.07.23 p.

### **Monday 17.07.2023:**

- ▶ safety training before working in the biotechnological laboratory of SGUL;
- ▶ extraction of colicins from the leaves of genetically engineered *N. benthamiana* tobacco;



**Tuesday 18.07.2023:**

- ▶ Official meeting with representatives of St. George's University regarding the future cooperation between IFNMU and SGUL,
- ▶ Reports of leading specialists in the fields of microbiology, immunology, immunotherapy of oncological diseases and tuberculosis;
- ▶ Presentation of the results obtained in the project on isolation, identification and determination of sensitivity to antimicrobial drugs by classical microbiological methods of the following clinical strains of *Escherichia coli*,
- ▶ Analysis of the received data;



# St. George's University tour



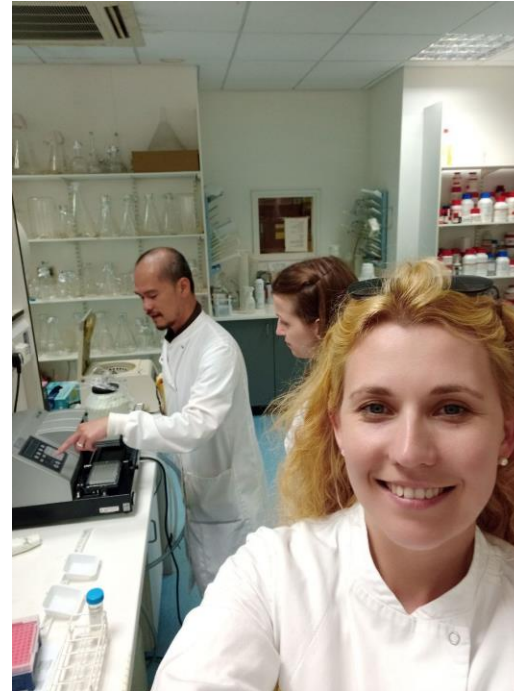
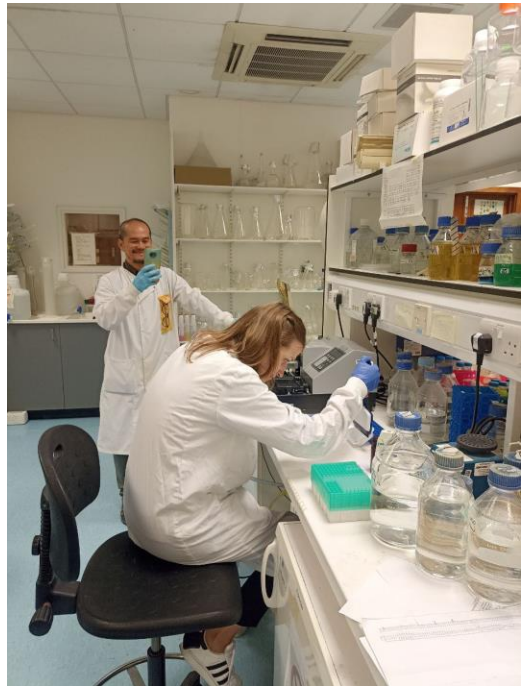
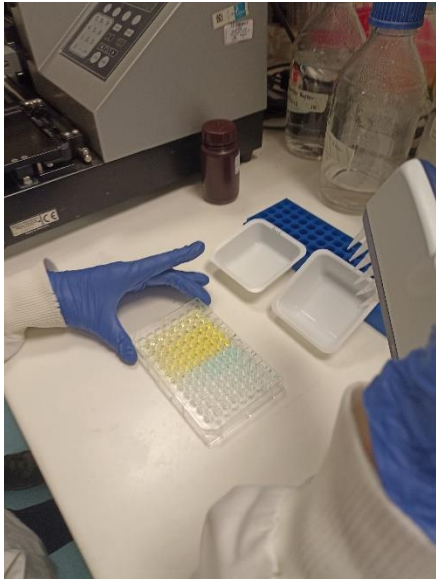
**Pharming for the Future**  
**The Hotung Molecular Immunology Unit**

We are one of the leading Molecular Pharming groups in the world. Our research group is unique in having plant biotechnology embedded within a medical research institute. This allows us to work closely with medical researchers for drug discovery and clinical trials.

<ul style="list-style-type: none"> <li> Julian Ma</li> <li> Thais Guerra (lab manager)</li> <li> Rajko Reljic (Senior Lecturer)</li> <li> Pascal Drake (Hon. Lecturer)</li> <li><u>Post-doctoral scientists</u></li> <li> Craig van Dolleweerd</li> <li> Mathew Paul</li> <li> Harry Thangaraj</li> <li> Audrey Teh</li> <li> Tim Szeto</li> </ul>	<ul style="list-style-type: none"> <li><u>PhD students</u></li> <li> Verena Hellie</li> <li> Luísa Madeira</li> <li> Leonard Both</li> <li> Sean Collison</li> <li> Szymon Steller</li> <li> Gí Reynolds Diogo</li> <li> Ahmad Ibrahim</li> <li> Sandy Patel</li> </ul>
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## Tuesday 18.07.2023: working in the lab. with Tim Szeto

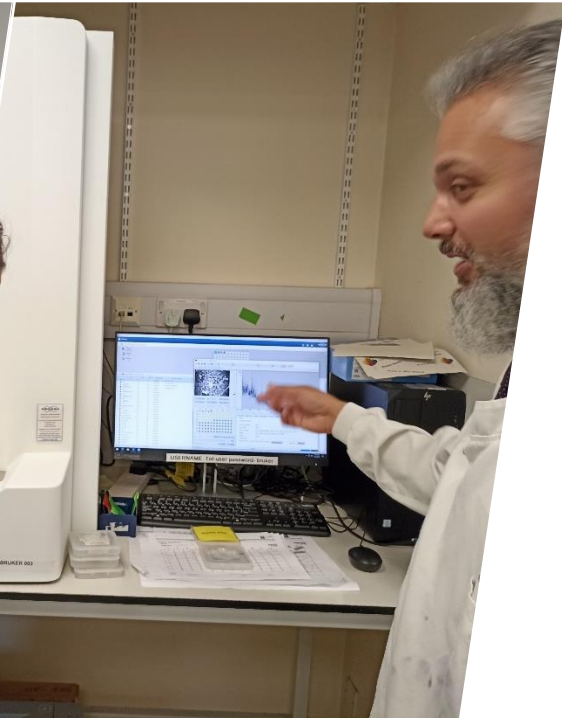
- Identification of secretory immunoglobulin A proteins by ELISA, evaluation of the results





# Wednesday 19.07.23

- ▶ Identification and determination of molecular weight of secretory immunoglobulin A proteins by Western blot method;
- ▶ SGUL microbiological laboratory tour;
- ▶ Infectious department of St. George's university tour



# Thursday 20.07.2023

- ▶ Evaluation of conducted research results;
- ▶ Discussion of current problems and prospects for further collaboration;
- ▶ London tour



Thanks a lot people of UK for help and support!

