COST Action CA18113

EuroMicropH

Understanding and Exploiting the Impacts of Low pH on Micro-organisms

1st Open Meeting

BOOK OF ABSTRACTS

12th-14th February, 2020, Lisbon, Portugal

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the Horizon 2020 Framework Programme of the European Union

TÉCNICO LISBOA

iBB Institute for Bioengineering and Biosciences

utad

BioISI Biosystems and Integrative Sciences Institute

Dear colleagues,

It is our great pleasure to host the 1st Open Meeting of the COST action EuroMicropH, devoted to the study of how low pH impacts microbial physiology and with that how it modulates many issues in our daily life ranging from biotechnology, to food and health. We hope that this might be a time of fruitful scientific discussions that might foster new interactions, new discoveries and new projects. You can follow all the aspects related with the action and with the meeting at the https://euromicroph.eu website and on social media of the action.

Welcome to Lisbon,

On behalf of the organizing committee,

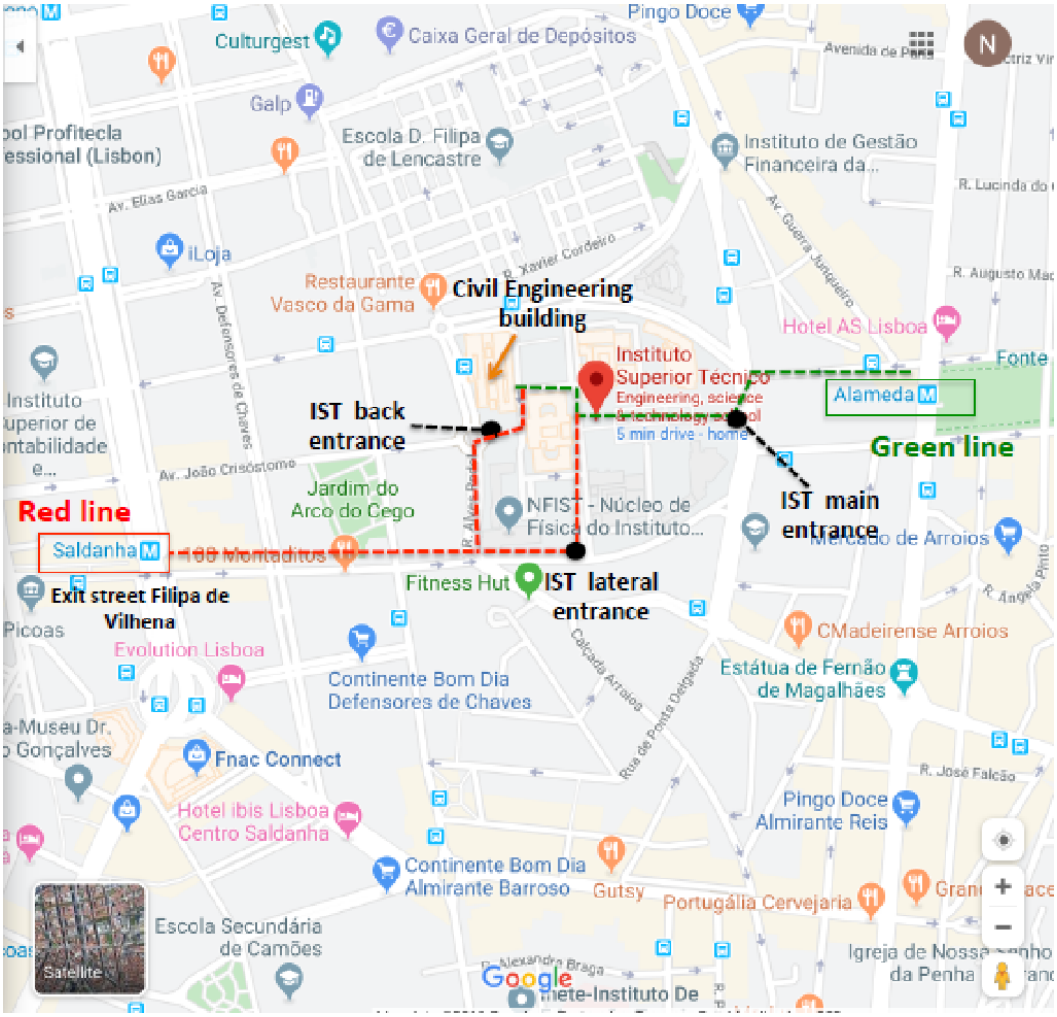
Nuno P Mira and Ana Mendes-Ferreira

Organizing Committee

Nuno P Mira, Instituto Superior Técnico, Chair of the Conference
Ana Alexandra Mendes-Ferreira, UTAD, Vice-Chair of the conference
Pete Lund, University of Birmingham, Chair of the EuroMicropHAction
Daniela De Biase, Sapienza University, Vice-chair of the action
Maria João Tavares, Instituto Superior Técnico
Patrícia Lage, UTAD
Isabel Seixas, UTAD
Maria Joana Pinheiro, Instituto Superior Técnico
Nuno Alexandre Pedro, Instituto Superior Técnico

Conference Venue

The meeting will take place at Instituto Superior Técnico in the Conference Center (located in the -2 floor of the Civil Engineering Building). Below you can find a map where it is highlighted the different ways by which you can reach the conference venue.



Food options

In the picture below you can find a list of options for food. These are all in walking distance of the conference venue. Below the picture you can find the direct links for directions via GoogleMaps.

Café Império (directions)
Gula´sfor Kitchen lovers (directions)
Vitaminas& Sabores (directions)
Alcaparra (directions)
Sena (directions)(inside IST Alameda campus)
TascaFit (directions)
Sushisan (directions)
UDON (directions)
KokoroRamen Bar (directions)
Don Costin (directions)
McDonald´s (directions)
AtriumShopping Center (directions)
FrankieHotDogs (directions)
ShreeRam (directions)

PROGRAMME:

Wednesday, February 12th 2020

For Management Committee members only

8:00-9:30 - Arrival and registration of Management Committee (MC) delegates

9:30-10:30 - MC meeting (part I): administration of the action

10:30-11:00 - Coffee-break

11:00-12:30 - MC meeting (part II): preparative meetings concerning organization of WG activities and preparation of GAPG2

Offered lunch

For all registered participants

14:00-15:10 - **Welcome notes** (Nuno P Mira, ***conference chair***; Professor Joaquim Sampaio Cabral, Full professor of IST and Director of Institute for Bioengineering and Biosciences; Prof João Pedro Conde, Full Professor of IST and Director of the Department of Bioengineering)

Low pH and the EuroMicropH action (Pete Lund, ***Action Chair***)

Opening conference.

Andreas Gombert, University of Campinas, Brazil
What can the industrial production of fuel ethanol from sugarcane teach us about tolerance to low pH in yeast?

15:15-15:45 - Coffee break

15:45-17:30: **Session 1: Integration of fundamental knowledge on impacts of low pH on micro- organisms and identification of knowledge and technical gaps.**

Session chairs: Conor O'Byrne, NUI Galway, Ireland; Oded Liran, Galilee Research Institute, Israel

Introduction to Working Group 1 - Connor O'Byrne, NUI Galway, Ireland; (WG1 leader)

Daniela De Biase (Sapienza University, Italy)
Die or survive under very low pH stress: when an amino acid can make the difference

Miguel Machuqueiro (Faculty of Sciences, University of Lisbon, Portugal)
Computational methods to evaluate the impact of low pH on the cellular water/membrane interface

Flash talks selected from the abstracts
– Karen Trchounian, Faculty of Biology, Armenia
Interaction of membrane-bound enzymes related to proton transport and hydrogen production in Escherichia coli at acidic pH

– Matthias G. Steiger, Technical University of Wien, Austria
Organic acid secretion by filamentous fungi

– Hana Sychrová, Institute of Physiology of the Czech Academy of Sciences, Czech Republic
Potassium transporters participate in yeast pH homeostasis and tolerance to low external pH

– Armands Vigants, University of Latvia, Latvia
The acetic acid tolerance of the yeast Kluveromyces marxianus

– Sholem Griffin, University of Malta, Malta
The Effect of Carbon Dioxide as a Climatic Parameter on Microbial Food Contaminants and Selective Isogenic Mutants

17:30-19:00 - **Poster session (at the ground floor of the civil engineering building)**

Thursday, February 13th 2020

9:30-10:30 – **Session 2: Methodologies and expertise in the study of micro-organisms at low pH.**

Session chairs: Gertien Smitts, University of Amsterdam; Ott Scheler, Galilee Research Institute, Israel

Introduction to Working Group 2 (Gertien Smits, University of Amsterdam, The Netherlands; WG2 leader)

Teuta Pilizota, University of Edinburgh, UK
The ability to maintain pH in E. coli is set by the maximum achievable proton motive force

Pete Lund, University of Birmingham, UK
High throughput methods for genetic analysis of acid stress

Flash talks selected from the abstracts
– Luís Ferraz, University of Milano-Bicocca, Italy
Membrane engineering to improve Saccharomyces cerevisiae robustness towards organic acids

– Mathew Milner, University of Birmingham, United Kingdom
Can Transposon Directed Insertion-site Sequencing (TraDIS) be used to explore multiple outcomes of evolution under stressful environments?

– Ott Scheler, Galilee Research Institute, Israel
Analysis of microbial growth and heterogeneity using droplet microfluidics

– Elia Tomás-Pejó, IMDEA Energy Institute, Spain
Evolutionary engineering of Lactobacillus pentosus improves lactic acid productivity from xylose-rich media at low pH

– Vasilis Valdramidis, University of Malta, Malta
Quantitative approaches to address the spore resistance in low pH food processing environments: the case of Alicyclobacillus acidoterrestris

10:30-11:00 - Coffee-break

11:00-12:30 – **Session 3: Low pH responses in medicine, biotech, and food: what can we learn and how can we help each other (part I)**

Session chairs: Karolina Rudnicka, University of Lodz, Poland; Rebecca Hall, University of Birmingham, UK; Jana Sedláková, Institute of Biology and Ecology, Slovakia

Introduction to Working groups 3, 4 and 5 (Nuno Mira, WG3 leader; Zeynep Gurol, WG4 leader; Estefania Noriega Fernandez, WG5 leader)

Jaroslav Michalko, Slovak Academy of Sciences
How systematic literature review and meta-analysis may provide a better understanding of the acidic stress response in highly pathogenic foodborne bacteria

12:30-14:00 –Free time for Lunch

14:00-15:30 – **Session 3: Low pH responses in medicine, biotech, and food: what can we learn and how can we help each other (part II)**

Session chairs: Nuno P Mira, Instituto Superior Técnico, Portugal; Jana Sedláková, Institute of Biology and Ecology, Slovakia

Karolina Rudnicka, University of Lodz, Poland
How Helicobacter pylori resists gastric acid and modulate hosts responses

Božidar Santek, University of Zagreb, Croatia
Yeast Trichospron oleaginosus: a producer of microbial lipids from lignocellulose containing feedstocks

Zeynep Gurol, KTH University, Sweden
Acidogenic bacteria as our co-workers for more sustainable production process

15:30-16:00 Coffee-break

16:00-17:30 **Poster session (on the ground floor of the civil engineering building)**

19:30 **Conference dinner** (at Museu da Cerveja, Terreiro do Paço)

Friday, February 14th 2020

9:30-10:30 – **Session 3: Low pH responses in medicine, biotech, and food: what can we learn and how can we help each other (part III)**
Session chairs: Estefania Noriega Fernandez, Nofima, Norway

Nicholas Brian Johnson, Nestlé Research Center, Switzerland
Low pH and High Acid: a challenge or opportunity for the Food Industry

Mustafa Turker, Pakmaya, Turkey
Potential applications of low pH in Fermentation industry

Flash talks selected from the abstracts
– Jana Sedlakova-Kadukova, Faculty of Science, Slovakia
Acidophilic microorganisms – capable workers in metal-bearing waste treatment

– Ljupco Angelovski, University of "Ss. Cyril and Methods", Republic of North Macedonia
Effect of organic acids on pathogen microorganisms in meat

– Aleksandra Djukić-Vuković, Faculty of Technology and Metallurgy, Serbia
Low pH in production of lactic acid and probiotic biomass

– Lucian C. Staicu, University of Warsaw, Poland
Recovery and characterization of PbS using a novel strain of Bacillus

– Aysenur Ugurlu, Hacettepe University, Turkey
Acclimatization of anaerobic microorganisms to low pH conditions for enhancement of biogas production

10:30-11:00 - Coffee-break

11:00-12:30 – **Session 4: Dissemination at EuroMicropH**
Session chair: Matthias Steiger, Technical University of Wien, Austria

Michael Sauer, University of Natural Resources and Life Sciences, Austria (leader of WG6)
Communication and Dissemination in COST Actions

Final conference
Session chair: Daniela de Biase, University of Sapienza, Italy

Sara Bover Cid, Institute of Agriculture and Food Research and Technology, Spain

Predictive microbiology approaches to quantitatively assess the impact of pH on behavior of foodborne bacteria

12:30-14:00 Free time for Lunch

14:00-16:00 - **Open parallel sessions for all WGs (review work performed and establish goals for GP2)**

16:00-17h30 - **Core Group meeting**

Abstracts

What can the industrial production of fuel ethanol from sugarcane teach us about tolerance to low pH in yeast?

Andreas K. Gombert[1,2], Bianca E. Della-Bianca[2], Vijayendran Raghavendran[1], Felipe B. Beato[1], Bruno L. V. da Costa[1,2], Thiago O. Basso[2], Michael Desai[3]

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During industrial production of fuel ethanol from sugarcane, the yeast *Saccharomyces cerevisiae* converts sugars into ethanol in million liter-scale fermentors. This process runs without asepsis for ~8 months every year and low pH is one of the inherent conditions which ensures that contamination levels remain below an acceptable threshold. Low pH is not only important during fermentation per se, but also during cell recycling, which includes a sulphuric acid washing step at pH ~2 for 1.5 to 2 h, leading to severe loss of viability in contaminating bacteria, and to some extent also in yeast. By comparing yeast strains isolated from the industrial process to laboratory, baker's and wild strains using different experimental approaches, we gathered phenotypic data indicating that tolerance to low pH became one of the hallmarks of these industrial strains. In a more recent project, samples taken from two industrial units along two whole sugarcane crushing seasons were analyzed by genome sequencing, in order to track the molecular signatures in industrial strains. This might enable us to pinpoint which mutations occur in genes involved in tolerance to low pH.

Die or survive under very low pH stress: when an amino acid can make the difference

Daniela De Biase

[1]Department of medico-surgical Sciences and Biotechnologies – Laboratory affiliated to Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Sapienza University of Rome, Corso della Repubblica 79, 04100 Latina, Italy (daniela.debiase@uniroma1.it)

Bacteria that best grow at pH close to neutrality are referred as neutralophiles. However during their life cycle these microorganisms, which can be either beneficial or pathogenic, experience mild-to-harsh acidic pH in natural environments, such as those encountered in the digestive tract of the animal hosts, in the phagosome of macrophages, in fermented foods and in the soil. To contrast the harmful effects of intracellular acidification, these bacteria have developed molecular strategies which preserve the cellular activities and the membrane potential [1]. The amino acid-dependent acid resistance systems very effectively relieve the cells exposed to a very low pH [1]. These extremely powerful systems require enzymes acting primarily on amino acids such as glutamine, glutamate, arginine and lysine and the cognate antiporters [1-3].
Through structural and functional studies, we and others have shed light on the molecular determinants that in a very elegant way control the activity of the acid resistance systems in neutralophiles. I will provide an overview of the effectiveness and distribution of these systems and show how through a combination of biochemical and structural studies and transport assays it has been possible to understand the mechanism of action of these systems and finally, how this knowledge can be exploited for different applications.

References:

[1] Lund P, Tramonti A, De Biase D. (2014) *FEMS Microbiol Rev*. 38:1091-1125.
[2] Pennacchietti E, D'Alonzo C, Freddi L, Occhialini A, De Biase D. (2018) *Front. Microbiol*. 9:2869.
[3] Tsai M-F, McCarthy P, Miller C. (2013) *PNAS* 110: 5898-5902.

Computational methods to evaluate the impact of low pH on the cellular water/membrane interface

Miguel Machuqueiro

BioISI - Biosystems & Integrative Sciences Institute, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, (machuque@ciencias.ulisboa.pt)

pH is a crucial physicochemical property that affects most biomolecules. Changes in protonation equilibrium of susceptible sites will modify the electrostatic environment and, consequently, have an effect on the molecular structure, stability and catalysis.[1] A detailed description of a cell membrane has to take in consideration all important factors that affect the lipid bilayer behavior and stability. pH is recognizably one of those factors even though it is usually ignored due to its high complexity.

The pK_a values of the typical titrable amino acids can be significantly influenced by changes in solvent mixture or due to insertion in a lipid bilayer.[2-3] In this work, we present a methodology that allows us to calculate pK_a values of peptides, proteins, lipid headgroups, and even pharmaceutical drugs, at the water/membrane interface. We take advantage of a CpHMD method developed in our group[3-4] to estimate pK_a profiles along the membrane normal for several compounds, which should illustrate the high importance of including pH effects in atomistic simulations at the water/membrane interface.

References:

[1] J. Matthew, *Annu. Rev. Biophys. Biophys. Chem.* 14 (1985) 387.

[2] J. F. Hunt, P. Rath, K. J. Rothschild, D. M. Engelman, *Biochemistry* 36 (1997) 15177.

[3] Teixeira, V. H., Vila-Viçosa, D., Reis, P. B. P. S., Machuqueiro, M., *J. Chem. Theory Comput.*, 12 (2016) 930.

[4] D. Vila-Viçosa, V. H. Teixeira, A. M. Baptista, M. Machuqueiro, *J. Chem. Theory Comput.* 11 (2015) 2367.

Escherichia coli's pH homeostasis is set by the maximum achievable Proton Motive Force

Guillaume Terradot[1], Ekaterina Krasnopeeva[1], Peter Swain[1], Teuta Pilizota[1]

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Living cells can maintain intracellular composition different from the external environment, and often, properties of the inside are kept within a certain range, so called homeostaeses. For example, intracellular pH is maintained closed to neutral in neutralophilic bacteria, such as *Escherichia coli*, which have been reported to maintain cytoplasmic pH close to 7.5 in a range of external pH values [1]. More recently, this view has been challenged with reports on permanent changes in internal pH upon the environmental pH change [2]. Explanations offered for the discrepancy in the measurements include inaccuracies in the pH sensor calibration and usage, and strain to strain differences [2]. However, pH homeostasis has thus far not been looked at in the context of all the cellular physiological variables that are influenced by it: osmotic pressure, membrane voltage, the electrochemical gradient of protons, and the electrochemical gradients of all the other ions present in the cell. The intertwined nature of these physiological variables, suggest that we need to look at their maintenance in a coordinated fashion, in order to understand the homeostasis of even just one of them, for instance internal pH. To do so, we build a simplified mathematical model of cellular electrophysiology. We find that the cell's ability to maintain pH is set by the maximum achievable PMF. We employ assays for time-course, single-cell, concurrent measurements of PMF and pH [3,4], to measure *E. coli's* internal pH during external pH shifts at two different PMF values, and find agreement with the model prediction.

References:

[1] Joan L. Slonczewski, Robert M. Macnab, Jeffry R. Al-ger, and Anna M. Castle. Effects of pH and repellent tactic stimuli on protein methylation levels in *Escherichia coli*; Journal of Bacteriology, 152(1):384–399, 1982

[2] Smarajit Chakraborty, Ricksen S. Winardhi, Leslie K.Morgan, Jie Yan, and Linda J. Kenney. Non-canonical activation of OmpR drives acid and osmotic stress responses in single bacterial cells; Nature Communications, 8(1):1587, 2017

[3] Ekaterina Krasnopeeva, Chien-Jung Lo, and Teuta Pilizota. Single-cell bacterial electrophysiology reveals mechanisms of stress-induced damage. Biophysical Journal, 116(12):2390 – 2399, 2019

[4] Yao-Kuan Wang, Ekaterina Krasnopeeva, Ssu-YuanLin, Fan Bai, Teuta Pilizota, and Chien-Jung Lo. Comparison of *Escherichia coli* surface attachment methods for single-cell, in vivo microscopy. bioRxiv, 2019.

High-throughput methods for genetic analysis of acid stress

Mathew Milner[1], Francesca Bushell[1], Fatima Alatar[1], Hrishiraj Sen[1] and Peter A Lund[1,*]

[1]School of Biological Sciences & Institute of Microbiology and Infection, University of Birmingham, Birmingham B15 2TT, UK *lundpa@gmail.com

High throughput methods are usually based on the use of different 'omics techniques to collect data from organisms cultivated under specific conditions of interest, such as in the presence of a defined stress. They can be invaluable as a source of candidates (in the shape of gene lists) for more detailed study, but do not necessarily in themselves provide a direct measure of the importance of different genes under particular stresses. For example, a gene which is needed for survival or optimal growth in a stressful environment will not always show increased expression in such an environment, and so would not be detected by RNA-based methods. Recently, the sequencing of very dense transposon libraries before and after stress treatments (known as *traDIS* or *Tnseq*, [1,2]) has come to the fore as a way of directly measuring gene fitness, while laboratory-based evolution coupled with whole genome sequencing is also becoming popular as a way of detecting mutations that enable adaptations to different stresses [3]. We have used all three methods to study *E. coli* grown under low pH stress [4 ,5], and in this talk I will present some of the data from this analysis, including a comparison between the methods.

References:

[1] van Opijnen T and Camilli A. 2013. Transposon insertion sequencing: a new tool for systems-level analysis of microorganisms. Nature Rev Microbiol **11:** 435-442

[2] Chao MC *et al.* 2016. The design and analysis of transposon insertion sequencing experiments. Nature Rev Microbiol **14**: 119-128

[3] Dragosits M and Mattanovich D. 2013. Adaptive laboratory evolution: principles and applications for biotechnology. Microb Cell Fact. **12**: 64.

[4] Stincone A *et al*. 2011. A systems biology approach sheds new light on *Escherichia coli* acid resistance. Nucleic Acids Res. **39**:7512-7528

[5] Johnson MD *et al*. 2014. Characterization of mutations in the PAS domain of the EvgS sensor kinase selected by laboratory evolution for acid resistance in *Escherichia coli*. Mol Microbiol. 93: 911-927

How systematic literature review and meta-analysis may provide a better understanding of the acidic stress response in highly pathogenic foodborne bacteria

Jaroslav Michalko[1*], Oded Liran[2], Dirk Hofreuter[3], David Attuy Vey da Silva[3], Sascha Al Dahouk[3]

[1]Mlynany Arboretum, Institute of Forest Ecology, Slovak Academy of Sciences, Slepcany, Slovakia; [2]MIGAL – Galilee Research Institute, Kiryat Shmona, Israel; [3]Department of Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany *jaroslav.michalko@gmail.com

Meta-analysis represents a method for quantitative synthesis of research results. It usually comprises a systematic literature review and statistical analysis of data extracted from the literature. Following a set of established guidelines, summarized in PRISMA [1], meta-analysis enables: (i) to merge knowledge on a particular research topic/question in a transparent and reproducible way by combining outcomes across different datasets, (ii) to examine patterns of response across available data, and (iii) to explore sources of heterogeneity in outcomes. Systematic reviews incorporating meta-analysis are therefore a valuable tool for *in silico* hypothesis testing based on already available literature data and, if done properly, can be a source of novel knowledge which is more informative than the lessons that can be learned from classical narrative reviews which are subject to a high degree of subjectivity [2]. In our presentation, we will demonstrate the suitability and general applicability of meta-analysis for synthesizing knowledge on the impact of low pH on microbes. We used *Brucella* spp. as a model for highly pathogenic foodborne bacteria, which have to evade acidic stress on their way from stable to table (environment, food hygiene measures, gastric acid, phagosome). The aim of our study was to analyze the growth response of brucellae inside macrophages where acidic stress plays a decisive role in the early phase of infection [3]. Based on this example, we will explain the individual steps in the process of a meta-analysis following standardized and generally accepted guidelines [1] and how we can address questions related to the aims and goals of the COST Action EuroMicropH in a more focused manner.

References:

[1] Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. et al. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Annals of internal medicine*, *151*(4), W-65.

[2] Gurevitch, J., Koricheva, J., Nakagawa, S., & Stewart, G. (2018). Meta-analysis and the science of research synthesis. *Nature*, *555*(7695), 175.

[3] Damiano, M. A., Bastianelli, D., Al Dahouk, S., Köhler, S., Cloeckaert, A., De Biase, D., & Occhialini, A. (2015). Glutamate decarboxylase-dependent acid resistance in Brucella spp.: distribution and contribution to fitness under extremely acidic conditions. *Appl. Environ. Microbiol.*, *81*(2), 578-586.

How *Helicobacter pylori* resists gastric acid and modulates hosts responses

Karolina Rudnicka[1], Agnieszka Matusiak[1], Małgorzata Siwińska[2], Weronika Gonciarz[1], Adrian Gajewski[1], Eliza Mnich[1], Marcin Włodarczyk[1,3], Maria Walencka[1], Agnieszka Krupa[1], Magdalena Chmiela[1]

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Helicobacter pylori (*H. pylori*) is a bacterium that successfully colonizes gastric mucus layer of more than half of human population. This bacterium inhabits the mucus-lined surface of the antrum of the human stomach where it induces a mild inflammation, but its presence is otherwise asymptomatic. However, mostly in 10-15% of cases, it leads to a development of gastric inflammation, gastric and duodenal ulcers or even gastric cancer. Although *H. pylori* is not an acidophile it possess mechanisms to survive and adopt to stomach acid that allow this bacterium to induce not only local but systemic consequences (1-4). The presence or absence of symptoms and their severity depend on multiple bacterial components, host susceptibility and environmental factors, which allow *H. pylori* to switch between pathogenicity and commensalism. A variety of virulence factors appear to play a role in pathogenesis. These include the vacuolating cytotoxin VacA, cytotoxin-associated proteins CagA, urease, flagellum and lipopolysaccharide. *H. pylori* accompany humans hundreds thousands of years - thus, it is no surprising that the bacterium "*learnt*" how to use its structures to manipulate the immune cells to remain unrecognized and avoid eradication. The mechanisms of these *H. pylori*-driven immune manipulations and their consequences on the local and systemic levels, in the light of our recent findings will be discussed (1-4).

The results presented during the lecture were financially supported by National Science Centre (Poland) - SONATA 12 no. 2016/23/D/NZ6/02553, PRELUDIUM 2013/09/N/NZ6/00805 and 2015/17/N/NZ6/03490 as well as N N401 015136 and N N303 451738.

References:

[1] Rudnicka K., Backert S., Chmiela M.: Genetic polymorphisms in inflammatory and other regulators in gastric cancer: Risks and clinical consequences. Current Topics in Microbiology and Immunology 2019, 421, 53-76. Book chapter in „Molecular mechanisms of inflammation: induction, resolution and escape by *Helicobacter pylori*". Springer, 2019.

[2] Chmiela M, Kupcinskas J. Pathogenesis of *Helicobacter pylori* infection. Helicobacter. 2019, 24 Suppl 1:e12638.

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[4] Chmiela M, Gonciarz W. Molecular mimicry in *Helicobacter pylori* infections. World J Gastroenterol. 2017 Jun 14;23(22):3964-3977. doi: 10.3748/wjg.v23.i22.3964.

Yeast *Trichosporon oleagninosus* - a producer of microbial lipids from lignocellulose containing feedstocks

Mirela Ivančić Šantek[1], Marina Grubišić[1], Božidar Šantek[1]*

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Microbial lipids are alternative feedstock for biodiesel or polyunsaturated fatty acids (PUFA) production. Yeast *Trichosporon oleaginosus* is one of the oleaginous microorganisms that has the capacity to grow and accumulate lipids on different lignocellulose containing feedstocks. In this study, corn cobs were used as lignocellulose containing feedstock which was pretreated by NaOH at loading from 0.08 to 1.6 g/g dry weight of raw corn cobs (g NaOH/g_{DW}) at 121 °C for 30 min. In followed step, alkaline pretreated corn cobs were hydrolyzed by using commercial lignocellulolytic multi-enzymes cocktails. The highest glucose and xylose yields were observed at NaOH loading rate of 0.16 g/g_{DW}. These enzymatic corn cobs hydrolysates were used for lipids production by *T. oleaginosus* in the following bioprocess configurations: separate hydrolysis and lipid production (SHLP) and simultaneous saccharification and lipids production (SSLP). Higher lipid yield (88.88 mg/g_{DW}) and productivity [2.4 g/(L day)] as well as considerable reduction of bioprocess time were observed in the SSLP compare to the SHLP configuration. After lipids extraction from yeast biomass, the fatty acids composition of these lipids was defined in order to evaluate their potential for biodiesel or PUFA production. Obtained results show that *T. oleaginosus* has great potential to be used as a lipids producer from lignocellulose containing feedstocks.

Acidogenic bacteria as our co-workers for more sustainable production process

Zeynep Cetecioglu[1]

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Environmental sustainability is a global goal today and to accomplish it, transition into a circular economy which includes resource recovery, reuse and recycling is essential. Therefore, renewability, degradability, and sustainability are the keywords of the bio-based production and they are key parameters to limit global temperature rise to well below 2°C and given the grave risks, to strive for 1.5°C which was addressed in The Paris Agreement (2016)[1]. Feedstock and energy recovery are also encouraged under the "*Resource Efficiency Roadmap*"[2]. This puts high demands on *the development of next generation production (NGP) technologies to turn carbon-rich substrate/waste streams into chemicals and materials* instead of petrochemical based production. One of the most promising bio-based products is volatile fatty acid (VFA) which can be produced by acidogenic bacteria as intermediate from waste-streams and has high market demand [3]. Nevertheless, to increase the bio-based VFA production yield is compulsory to make it competitive with the petrol-based production. The studies showed that pH and waste/substrate type in terms of content and matrix such as liquid, slurry, solid, etc. are main parameters which affect both bioproduction efficiency of VFAs and VFA composition in mixed fermentation and shifting of bacterial community in process[4–10].

References:

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Low pH and High Acid: a challenge or opportunity for the Food Industry?

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Nestlé, and the wider food industry, employ the acidity of many food and beverage products to assist in providing stability and safety. The ability to use lowered pH in food and beverage products has an enormous benefit in either reducing the need to deliver high amounts of heat or in needing an aseptic filling environment to prevent spoilage or both. Consequently, the costs of manufacture and wastage are greatly reduced. However, lowered pH as a sole hurdle would make most products unpalatable. Therefore, a range of product types with varying degrees of complexity have been developed within the food industry, to employ product recipe and process designs that combine antimicrobial hurdles. The combined use of hurdles enable a relatively higher pH whilst delivering reduced perceived sourness, but still providing a robust, safe and stable product. These approaches use the term multi-hurdle technology (MHT).

References to recent reported spoilage incidences in low pH food products will be used to demonstrate the impact these have on the industry, and how they have assisted in identifying and managing spoilage risk. Two case studies of low pH product designs (i.e. acidified sauces and ready-to-drink beverages) will be used as examples of the application, challenges and opportunities offered by MHT. Finally, we will briefly cover some of the current applications using 'novel' technologies in low pH food and beverage production, with attention on why these alternative non-thermal technologies are still often seen as niche by the food industry.

Potential Applications and Opportunities in Low pH in Food and Industrial Biotechnology

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Low pH, interfering with pH gradient and proton motive force across cellular membranes, influences a vast number of physiological functions and deleteriously affects bioprocesses involved in a variety of industrial applications. Thus, from the industrial point of view, it is a crucial necessity to learn how to cope with acid stress in cellular level. Although, there are a number of studies elucidating the acquired homeostatic mechanisms for relieving the disruptive effects of steep pH gradient across cell membrane (Baker-Austin and Dopson 2007; Lund, Tramonti, and Biase 2014), translation to industry is often interrupted.

In this presentation, potential opportunities of ameliorations in low pH response in terms of industrial needs and challenges will be discussed. Industrial demands upon economical and technical perspectives will be highlighted in the basis of direct examples from food and industrial biotechnology including dairy, food, beverage fermentations, control of pathogens/contaminants, biomining, bioremediation, generation of bioenergy and robust cell factories for production of various bio-products. Industrial exploitation of improvements in low pH response will eventually lead to advanced growth of world-wide bio-based economy.

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Predictive microbiology approaches to quantitatively assess the impact of pH on behavior of foodborne bacteria

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Predictive microbiology, also known as quantitative microbial ecology, aims to characterize the behavior of microorganisms as a function of the environmental factors, through the development of mathematical algorithms. In food, the environmental conditions include intrinsic (pH, water activity, type and concentration of antimicrobials, etc.), extrinsic properties (composition of atmosphere packaging, temperature, pressure, etc.) and intrinsic (interaction with other microorganisms). The mathematical algorithms contribute to understand the response of microorganisms to stresses associated with food production and preservation technologies, for instance in terms of growth, inactivation or metabolite (toxin) production. The quantitative approach allows the identification of the most relevant factors as well as their interactions. When properly validated, predictive models constitute key tools for developing quantitative microbial risk assessments (QMRA) and decision support systems for microbial risk management aiming to reduce food spoilage and improve food safety all along the food chain. The presentation will introduce the main features of the predictive microbiology discipline. Different approaches that can be useful to assess the impact of pH on the behavior of foodborne microorganisms will be addressed. Special attention will be paid on specific case-studies dealing with foodborne pathogens: (i) the application of available predictive models to understand the role of the pH on the growth inhibition in comparison with other factors; and (ii) modeling the impact of pH as an enhancing factor of high pressure processing within a food safety assurance strategy.

Communication and Dissemination in COST Actions

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The declared aim of COST actions is to "help connect research initiatives across Europe and beyond and enable researchers and innovators to grow their ideas [...] by sharing them with their peers." The created COST networks "offer an open space for collaboration among scientists across Europe [...] and thereby give impetus to research advancements and innovation." COST funding "intends to complement national research funds, as they are exclusively dedicated to cover collaboration activities, such as workshops, conferences, working group meetings, training schools, short-term scientific missions, and dissemination and communication activities." Hence, connections and communication are the central aspects of all COST actions.
Dissemination is a task which can be addressed in a quite forward manner. The dissemination of her/his results is a central task for every researcher. Many routes for dissemination exist and success of dissemination is essentially a question of diligence and perseverance of the persons involved.
Communication to the contrary is a much more difficult and much less understood task. Building reliable and sustainable connections between researchers or – even more difficult - inside and between research fields bears many obstacles and various unsolved problems. While being in the lead of the respective work package, my own picture of how we can knit a sound communication network, is still kind of blurred. Here, I set out to summarize some doubts and questions to which I cannot give satisfying answers at the time being. I would like to kindly invite all participants to join my effort and clarify the needs of our communities and suggest useful measures how to meet them.

POSTERS

P1. Influence of acidic stress on biohydrogen production in dark fermentation

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Biohydrogen production technologies are nowadays widely investigated (see e.g. [Chaganti et al., 2012; Solowski et al. 2018] and references therein). This results from hydrogen wide application in chemistry, energetics (energy storage and fuel-cell CHP) or transport industry (hydrogen cars). The dark fermentation (DF) is related to methane fermentation process, with methanogenesis being inhibited e.g. by low pH value. It is known that methanogenic bacteria prefer pH values 7 – 8. Acidic conditions (pH < 6,5) although support substrates decomposition (with hydrogen generation) strongly inhibit methane generation. The optimal pH value for hydrogen generation (in DF) depends on substrates and technology but seems to be in the range 5.0 to 6.0 [Muñoz-Páez et al., 2019]. However, high hydrogen yields during DF of wheat straw are reported for pH 2.4 [Nasirian et al., 2011] but also 9.0 [Li et al., 2018]. Figure 1 presents results of cotton-waste fermentation under condition of controlled pH 6 value and without pH control (value rises above 7, favorite for methanogenesis). It is clearly seen that control of acidic conditions may significantly increase hydrogen production.

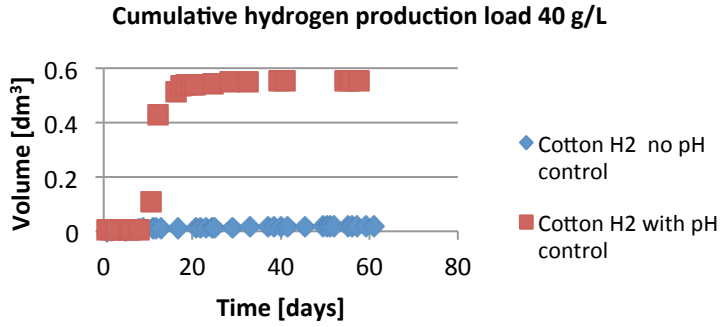

Fig. 1 Cumulative hydrogen production from cotton for VSS 40 g/L with (pH 6 or without pH control)

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P2. Bioactive composite coatings on Ti-6Al-4V

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Metals and their alloys have played a predominant role as structural biomaterials in reconstructive surgery. Nowadays, titanium alloys are biometals of particular interests, due to their good mechanical properties [1]. However, the contact of Ti with patient tissue may induce unspecific protein adsorption and bacterial adhesion causing poor implant osseointegration. Among the known materials used as an antifouling agent, a promising one is polyethylene glycol (PEG) [2].
Design and fabrication of materials with surface adjusted for a particular biological application is of essential importance to the synthesis of biocompatible materials. Taking into consideration the desired characteristics of the various biomaterials the great interest of the researchers focused on composite materials is well-justified [3].
The combination of antifouling polymer with bioactive substances can enhance cellular interaction of material with the host bone and reduce the risk of bacterial biofilm formation. Among all inorganic materials, hydroxyapatite (HA) has been considered as a proper candidate for enhancing regeneration-supportive properties of scaffold materials [4].

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This study was supported by grant from the National Science Centre in the frame of UMO-2016/21/D/ST8/01697.

P3. Wet method of synthesis calcium phosphate

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Osteoporosis is a disease of the 21st century that affects an enormous amount of people, both sexes but mainly women and the elderly. It is estimated that this problem currently affects as many as 200 million people worldwide, causing pain and lowering the comfort of life [1]. It is a skeletal system disease characterized by loss of bone density and deterioration of the microarchitecture of bone tissue, which leads to increased bone fragility and leads to fractures [2].

One of the basic materials of the mineral phase of bone is hydroxyapatite $C_{10}(PO_4)_6(OH)_2$ [3]. Its greatest advantages are bioactivity, biocompatibility and osteoconduction properties. Therefore, it is widely used in the regenerative medicine of the skeletal system, including bioactive coatings, bone filler, bone tissue engineering scaffolds as well as a component of composites [4].

Presented research is focused on synthesis and characterization of hydroxyapatite. In this work, HAp was obtained by two wet methods. The first one was carried out at room temperature, using ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and hydrated calcium nitrate ($Ca(NO_3)_2$ as reagents. Another one, at boiling point using calcium acetate ($(CH_3COO)_2Ca$) and disodium hydrogen phosphate (Na_2HPO_4). In both cases, the syntheses were performed under different conditions, changing pH and reagent concentrations.

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This study was supported by grant from the Foundation for Polish Science in the frame of TEAM NET POIR.04.04.00-00-16D7/18

P4. Low pH in production of lactic acid and probiotic biomass**

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Lactic acid (LA) is an important chemical with wide application range in food, cosmetic and pharmaceutical industries as acidifying agent and preservative. LA is also used for production of poly-lactides - thermostable, biocompatible polymers suitable for biomedical applications. Physicochemical properties of poly-lactides are determined by ratio of L- and D- LA. Some LA bacteria are producing L- or D- LA with great stereospecificity which makes biotechnological LA production preferred route today[1].

Decrease in pH during LA fermentation is a result of produced LA and can be favorable for prevention of contamination and enable open lactic acid fermentation [2], which is applied in silaging. But also, it heavily affects productivity of the process when high LA production is the main goal in process. $CaCO_3$ and NaOH were the most effective neutralizing agents in LA fermentation on stillage and similar agri-food industry substrates[3,4]. Control of pH and high LA production is even more challenging when LA producing strain has probiotic characteristics and remaining probiotic biomass should be valorized in feed.

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*** Poster selected for presentation in a flash talk*

P5. The acetic acid tolerance of yeasts *Kluveromyces marxianus***

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K. marxianus is non-conventional food grade yeast with great potential in biotechnology industry (1). Acetic acid is a by-product in fermentation processes of these yeasts. There is growing interest in studies of acetic acid tolerance of these yeasts (2,3). The inhibition of biomass growth by acetate of several *K. marxianus* strains was studied. It was found that increased concentration of acetate slows down yeast growth and this depends from medium pH and carbon source. Inhibition was more pronounced when *K.marxianus* growth on lactose as compare with glucose or glucose-galactose mix. The growth inhibition degree correlate with undissociated acetic acid concentration in medium. Increase of concentration of acetate results in lag-phase prolongation and decrease in biomass yield. The acetic acid tolerance significantly differs between strains. The correlation between acetic acid tolerance of K.marxianus grown on lactose and localization of b-galactosidase (cytoplasm or periplasm) was observed. The strain *K.marxianus CBS712* with b-galactosidase located only in cytoplasm had most pronounced inhibiting effects of acetate when grown on lactose. Increased tolerance of *K. marxianus* cells to elevated concentrations of acetate was observed through adaptation during an extended lag phase. The tested *K. marxianus* strains had different adapted cells amount in the culture population.

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*** Poster selected for presentation in a flash talk*

P6. Acclimatization of anaerobic microorganisms to low pH conditions for enhancement of biogas production**

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Anaerobic digestion is a mature technology that produces energy through biomass including wood, energy crops, wastes, and algae. It is an oxygen free biological process in which microorganisms work in consortia. Acidogenesis and methanogenesis are two important stages of anaerobic digestion that are prone to inhibition. Acidogenic and methanogenic microorganisms have different growth kinetics, physiology and pH tolerance [Ref1]. Methanogens are known as most sensitive microorganisms working with an optimum pH close to neutral. Imbalance between volatile fatty acids (VFAs) production and reduction by methanogens results in accumulation of VFAs. As the alkalinity is not sufficient, pH drop was observed and methanogenic activity is inhibited [Ref2]. NaOH or alkalinity addition may provide pH stability, however; operational costs and toxicity due to NaOH are the major concerns [Ref3]. So, recent investigations have focused on use of acclimated cultures to low pH as a cheaper and outstanding alternative. There are some studies proved use of acclimated cultures for biogas production from different types of wastes including food waste, cheese whey, vegetables fruit waste, dairy waste at low pH [Ref4]. So, detailed investigations should be carried out in the future to better comprehend the nature of the process applicability in large scale applications.

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*** Poster selected for presentation in a flash talk*

P7. Evolutionary engineering of *Lactobacillus pentosus* improves lactic acid productivity from xylose-rich media at low pH**

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Having in mind that xylose, the second most abundant sugar in lignocellulose, cannot be metabolized by most of wild type microorganisms, the use of this sugar for the production of chemicals is a challenging approach. In this context, lactic acid production from C5 has been reported in *Lactobacillus pentosus* (1-3). This acid is a rising platform chemical that can be used for the production of pharmaceuticals, chemicals and biopolymers (4). The pH drop produced during fermentation due to lactic and acetic acid accumulation can produce a detrimental effect on bacterial growth. In this study, *L. pentosus* CECT4023T was evolutionary engineered to improve its acid pH tolerance in xylose fermentation. After a long-term non-pH controlled sequential batch cultivation with increasing xylose concentration, a new strain was obtained (named MAX2). This strain showed between 1.5 and 2-fold increase in lactic acid production in xylose defined media independently of the initial pH. When the pH was controlled in bioreactor, 1.4-fold increase in productivity was attained both in xylose defined and in wheat straw hydrolysate. These results demonstrated the potential of MAX2 strain to produce lactic acid from hemicellulosic substrates at low pH, reducing the need of using neutralizing agents in the process.

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*** Poster selected for presentation in a flash talk*

P8. Efficient production of lactic acid from gardening residues: importance of pH control for the resistance of inhibitory compounds

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The expansion of urban green areas has boosted a new source of gardening residues. Although high volumes of these materials are generated annually, their valorization is still hindered due to its seasonality and heterogeneity (1). In this sense, both C6 and C5 sugars from gardening residues can be used to produce platform chemicals like lactic acid, as reported with other lignocellulosic materials (2-3). This acid is a versatile building block with plenty of industrial applications like food, pharma and biopolymers (4). In this context, an efficient lactic acid fermentation of hemicellulosic sugars from gardening hydrolysates with *Lactobacillus pentosus* CECT4023T is addressed. Together with sugars, some inhibitory degradation compounds are usually found in the hydrolysates due to the sugar and lignin degradation during pretreatment of lignocellulose. In this work, xylose consumption was found to be hampered due to a pH drop because of the presence of acids, both formed during pretreatment and fermentation. Automatic NaOH addition in bioreactor to control the pH resulted in 44 % increase in lactic acid production, reaching 95 % of the maximum theoretical yield. These results provide new insights for process optimization to valorise emerging lignocellulosic materials like gardening residues into high added-value products.

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P9. Potassium transporters participate in yeast pH homeostasis and tolerance to low external pH**

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Potassium is a key monovalent cation necessary for multiple aspects of cell growth and survival, for example compensation of negative charges of macromolecules to maintain electroneutrality, cell turgor and volume, enzyme activity, and protein synthesis. Tightly regulated potassium fluxes across the plasma and organellar membranes are also indispensable for the maintenance of proper membrane potential and intracellular pH. In yeast cells, potassium is accumulated against its concentration gradient to relatively high amounts (usually, the accumulation ratio is more than 10^5; yeasts growing in the presence of micromolar KCl accumulate intracellularly 200-300 mM K^+). Yeast cells use three types of transporters to accumulate potassium in growing and dividing cells [1,2]. Analysis of transporters and corresponding knock-out mutants revealed that the main important is Trk1, a system present in all yeast species. The mal-function of Trk1 affects crucial physiological parameters and influences many processes including intracellular pH regulation and cell response to osmotic and pH stresses [1,2]. This mal-function also diminishes virulence of pathogenic yeast species [3,4].

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*** Poster selected for presentation in a flash talk*

P10. Acidophilic microorganisms – capable workers in metal-bearing waste treatment**

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Microorganisms are capable of transforming many metals by catalysing redox reactions. Many of them consequently promote the mobilisation or immobilisation of metals in ecosystems due to their dissolution or precipitation. Their capability to mobilise metals is successfully used in low-grade ore bioleaching, however, it can be applied in metal-bearing waste treatment. Bioleaching represents eco-friendly and cost effective alternative to conventional metallurgical process. In our work, we have isolated multiple sulphur oxidizing acidophilic bacteria, among them, *Acidithiobacillus albertensis,* for the first time reported from Europe territory. The isolate was applied for indium bioleaching from LCD monitors. Several other acidithiobacilli e.g. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* were tested for bioleaching abilities of various metals from metal bearing waste (spent alkaline batteries, Ni-Cd batteries, Li-accumulators, mother boards from computers etc.), bioleaching of Li from low grade ore lepidolite we have carried either in pure culture or in combination of acidophilic bacteria (*A. ferrooxidans, A. thiooxidans*) with acidophilic fungus *Aspergillus niger*, or yeast *Rhodotorula rubra*. According to obtained results, microorganisms have great potential in development of innovative processes of metal recovery from various waste materials.

The work was financially supported by grants VEGA 1/0229/17, SK-PL-18-0012 and COST CA 18113

*** Poster selected for presentation in a flash talk*

P11. The European Network for Optimization of Veterinary Antimicrobial Treatment (ENOVAT)

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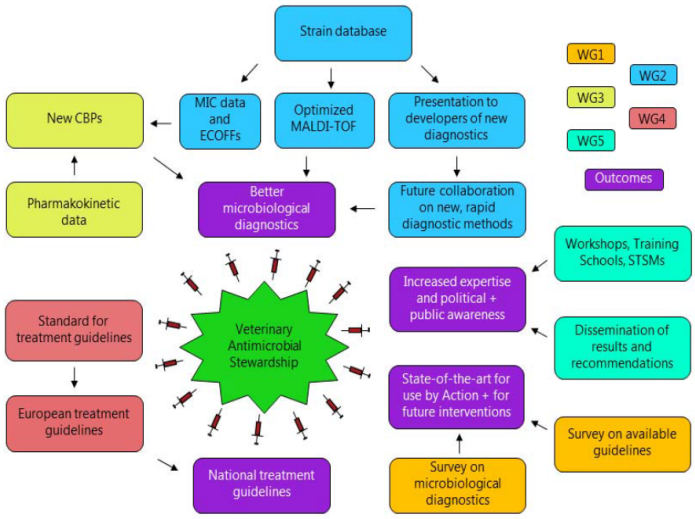
The global antimicrobial resistance crisis has been the driver of several international strategies on antimicrobial stewardship, that typically are only slowly being implemented into "real life". This is particularly unfortunate for veterinary medicine, which is challenged by (i) a shortage of experts in key disciplines related to antimicrobial stewardship, (ii) few antimicrobial treatment guidelines, and (iii) inferior diagnostic tests compared to human microbiology.



ENOVAT Action chart

The European Network for Optimization of Veterinary Antimicrobial Treatment (ENOVAT) COST Action network comprises participants from universities, knowledge institutes and companies across thirty-one European countries and represents the most comprehensive grouping in this particular field. The Action will 1) survey the state-of-the-art in microbiological diagnostic practices and veterinary treatment guidelines across Europe, 2) develop an extensive European strain database and a standard for making antimicrobial treatment guidelines, 3) refine microbiological diagnostic methods and European treatment guidelines. All will be disseminated to national and international stakeholders (Figure). Furthermore, the Action will recommend priority research areas for future optimization of antimicrobial treatment in animals, and develop a roadmap outlining how European countries can advance towards a common high level of veterinary antimicrobial stewardship.

P12. Response mechanisms of model organism *Saccharomyces cerevisiae* to yeast viruses

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Simple eukaryotic organism *Saccharomyces cerevisiae*, hosting dsRNA virus-originated killer systems, is used as an attractive model for understanding the molecular mechanisms of viral infection and pathways viruses exploit to overtake the host cellular machinery. In this study, we provide a transcriptome profiling of *Totiviridae* viruses (M-2 and L-A-lus) possessing strain M437 by applying RNA-seq. We demonstrate that elimination of both dsRNAs affected transcriptional changes of 715 genes, while M-2 free cells differentially expressed 486 genes. Most of the transcriptional responses induced by viral dsRNAs are moderate and do not exceed the limit of four-fold change. Enriched GO terms of positively regulated genes are related to stress response, namely cellular response to oxidative stress, oxidation-reduction process carbohydrate metabolic process, and ribosomal biogenesis. Negatively regulated genes in virus deficient baking yeast cells are related to cellular amino acid and lipid biosynthetic processes, RNA metabolism, and cellular respiration. Insights on the alteration of host gene expression will help to understand the biology of dsRNA mycoviruses, and their impact on the host cells.

P13. Interaction of membrane-bound enzymes related to proton transport and hydrogen production in *Escherichia coli* at acidic pH**

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Bacteria have strategies to survive and grow under acidic conditions by changing the cell structure, metabolism and transport systems [1,2]. *Escherichia coli* has four membrane reversible hydrogenases (Hyd), which catalyze the reversible reduction of protons to molecular hydrogen [3]. In this study interaction between proton ATPase, Hyd, formate dehydrogenase-H (FDH-H) and potassium transport system is first investigated in *E. coli* during fermentation of mixture of glucose, glycerol and formate at pH 5.5. The lack of FDH-H and/or Hyds reduced F_0F_1-ATPase activity. However, K^+ ions had a stimulating effect on proton ATPase activity. This effect has not found at slightly acidic pH 6.5 [3]. Accessible –SH groups were decreased in the membrane of mutant strain with defect of FDH-H, compared with wild type. Besides, significant changes of –SH groups were shown in the presence of DCCD, formate, ATP and K^+ ions. Taking together, the data obtained show that proton ATPase and FDH-H might interact together through dithiol-disulfide (-SH-HS to –S-S) interchange. Moreover, interaction between mentioned membrane-bound enzymes suggest a new survival mechanism for *E. coli* bacteria for maintaining intracellular pH and proton motive force generation at acidic pH.

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*** Poster selected for presentation in a flash talk*

P14. Natural Killler T-cells respond to *Helicobacter pylori* LPS Lewis^{XY} presented by CD1d-loaded dextramer

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The Natural Killer T (NKT) cells constitute a subpopulation of lymphocytes that share characteristics of NK cells and lymphocytes able to respond to lipid antigens. The involvement of unconventional NKT cells in response to *H.pylori* (*Hp*) glycolipids has not been well described. The aim of the study was to evaluate possible binding of *Hp* lipopolysaccharide (LPS) with CD1d receptor by flow cytometry. The peripheral blood mononuclear leukocytes were isolated from asymptomatic volunteers with defined *Hp* status, adjusted to the density of $2.5x10^7$/ml and stimulated overnight with *Hp* LPS (or standard LPS from *E.coli*). The percentage of NKT cell subpopulations and secretion of cytokines were evaluated by flow cytometry and ELISA, respectively. NKT cells isolated by a MidiMACS™ Separator (Milteny Biotec) were then co-incubated with FITC conjugated CD1d dextramer (Immudex) previously loaded with *Hp* LPS (or LPS of *E.coli*). The binding was visualized by flow cytometry. Stimulation with standard *E.coli* and *Hp* LPS led to a proliferation of classic $CD3^+CD56^+$ NKT cells, with no such impact on $CD3^+CD16^+$ subset. Exclusively *Hp* LPS induced the proliferation of $CD3^+Nkp46^+$ NKT subset only in cell cultures derived from *Hp*(+) donors, in comparison to nonstimulated cells. This effect was accompanied by the elevation of $CD25^+$ among $CD3^+Nkp46^+$ NKT cells able to produce IL-10. NKT cells responses to LPS of *Hp* depend on binding of lipid A with CD1d molecule and interaction of sugar moieties with NKT receptors.

This study was financially supported by National Science Centre (Poland) - SONATA 12 no. 2016/23/D/NZ6/02553.

P15. Effect of organic acids on pathogen microorganisms in meat**

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Interventions applied at slaughter in the U.S. and Canada comprise physical, chemical or biological treatments (1). In Europe interventions for carcass decontamination with substances other than potable water are not categorically banned (Regulation (EC) No. 853/2004) but EFSA approval is needed (2). Organic acids have considerable potential for industry use because they are quite inexpensive and generally recognized as safe (3).

In this study lactic, citric and acetic acid, each at 3% (v/v) concentration were used as two decontamination agents, and each acid was sprayed (30 seconds duration, 25°C) on ground beef meat (portions of 300 g) inoculated before with *Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Enterobacteriaceae* and coliform bacteria (in concentration from 8.3 to 8.5 log/cfu). During the 9 day storage at 4°C a sample was taken on 1, 3, 5, 7 and 9 day and analyzed for all of the mentioned microbiological parameters.

The results indicated a range of reduction in the bacterial populations from 0.6 log/cfu to the highest of 3.0 log/cfu. In the samples inoculated with *Salmonella typhimurium* the highest reduction of 2.3 log/cfu was noted in the samples treated with acetic acid. For *Listeria monocytogenes*, highest reduction (1.5 log/cfu) was noted in the samples treated with lactic acid.

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*** Poster selected for presentation in a flash talk*

P16. Recovery and characterization of PbS using a novel strain of *Bacillus***

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Lead (Pb) ranks as a major anthropogenic pollutant because it is used extensively by industry such as ore mining, lead-acid battery manufacturing and fossil fuel burning (CRC, 2018). Spent lead-acid battery wastewater is particularly challenging to treat as it contains high levels of sulfate (g's L-1), Pb (<5 mg L-1) and is characterized by low pH (1-3) (Vu et al., 2019). Most of the studies carried out to date on this industrial wastewater type focused on its treatment and marginally on resource recovery. Our work investigates novel metabolic pathways bacteria use to detoxify Pb and recover it in the form of valuable minerals. We study a novel *Bacillus* strain (Abq.) able to degrade cysteine to sulfide, accompanied by the biomineralization of Pb(II) to PbS (galena) (**Fig**). Within 48h of incubation the strain reduced 1 mM Pb(II) completely using a stoichiometric concentration of cysteine. The mineralogical identify of PbS was confirmed by XRD and further characterization is in progress. Its negative surface charge indicates the presence of a biopolymer layer, indicative of high colloidal stability. The biomineralization process occurs extracellularly, which opens the possibility to efficiently recover PbS.

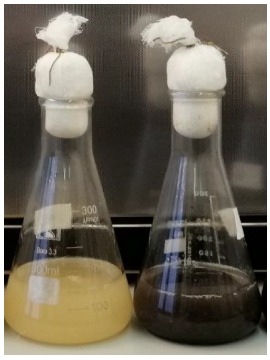

Fig. Control experiment and biogenic PbS produced by *Bacillus* sp. Abq.

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Acknowledgments: This work is supported by the National Science Center (Poland) research grant 2017/26/D/NZ1/00408.

*** Poster selected for presentation in a flash talk*

P17. Membrane engineering to improve *Saccharomyces cerevisiae* robustness towards organic acids**

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During bio based industrial processes yeasts are challenged to perform in adverse conditions, such as low pH, high temperatures and in the presence of inhibitors derived from biomass. Organic acids (OAs) play a unique role in these processes since they are often released from biomass treatments and, when in the fermentative broth they can compromise the cell metabolism and cause growth arrest. OAs can also be products of interest obtained from microbial factories, with applications in several industrial sectors. Therefore, controlling the cellular inward/outward flux of OAs can improve fermentation performances.

Plasma membrane plays a pivotal role in this process, acting as a selective gate and changing its composition in response to the presence of OAs. Thus, its engineering has been envisaged as a strategy to increase yeast performance. We are evoking a membrane rewiring focusing on the modulation of the transcription factor *ECM*22, involved in the regulation of ergosterol biosynthesis.

Here we show how changes in the cellular content of ergosterol can have a positive or negative effect on yeast robustness depending on the molecular structure of the OAs. Furthermore, these effects can vary according to other parameters relevant for industrial processes, such as temperature.

*** Poster selected for presentation in a flash talk*

P18. Can Transposon Directed Insertion-site Sequencing (TraDIS) be used to explore multiple outcomes of evolution under stressful environments?**

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Laboratory-based evolution has become a widely used method to explore fundamental questions about evolution as a process, and is also a powerful tool to study the link between genotype and phenotype. We are interested in understanding short and long terms adaptations of bacteria to stressful environments, and to study this we have evolved six populations of E. coli MG1655 for in a dynamic pH environment, by iterative growth and daily dilution over 5 months in unbuffered LB, starting at pH 4.5. Whole genome sequencing of the evolved populations and of individual clones from each population revealed many striking similarities in the evolutionary trajectories in the evolved strains. We are interested in exploring the impact of multiple different parameters on evolutionary trajectories, but as evolution experiments take a long time, we are currently investigating whether traDIS experiments can partially replicate evolution experiments in a relatively short time frame. Since TraDIS provides a measure of relative contributions to fitness of each gene (by comparison of read counts after growth in two conditions), in principle it should be possible to use TraDIS to identify genes whose loss of function provides a fitness benefit. Here we present our latest results.

*** Poster selected for presentation in a flash talk*

P19. Organic acid secretion by filamentous fungi**

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Filamentous fungi are excellent producers of organic acids under extremely low pH conditions. *Aspergillus niger* is capable of producing citric acid and is used for large scale fermentations for more than a century. We identified the main gene for citric acid secretion in *Aspergillus niger*, cexA, which belongs to belongs to the major facilitator superfamily subclass DHA1. Members of this family work as drug-H+ antiporter. The disruption of this gene completely abolishes citric acid secretion, Overexpression of cexA leads to a significant increase in secreted citric acid in *A. niger*. New evidence how the gene expression is regulated in *A. niger* is presented.

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*** Poster selected for presentation in a flash talk*

P20. Effects of lactic acid bacteria inoculation in pre-harvesting period on alfalfa (*Medicago sativa* L.) silage

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The aim of the current study was to determine the effect of lactic acid bacteria (LAB) as microbial additive on fermentation features and feed quality parameters of alfalfa silage.

Alfalfa forage was inoculated with different LAB bacteria on the day before cutting. Bacterial inoculation significantly affected the fermentation properties and some feed quality parameters of alfalfa silage. The use of the bacterial inoculant in alfalfa silage increased dry matter recovery, decreased silage pH more effectively and prevented proliferation of some unwanted microorganisms in silages compared to control. The LAB isolates belonging to Lactobacillus bifermentans species gave the best result all silage and quality characters was taken into consider.

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P21. Influence of low pH food ingredients and sport on the human microbiome and health, monitored by mobile application and taste

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We propose a new nutrition concept using available food databases and own experience of low pH food quality ingredients on the microbiome on human surfaces. Every day average 700 kcal sport using aerobic exercising like swimming and bike riding can essentially improve human health through activating the lymphatic system on the gut skin axis. Proper selection of acidic food ingredients, elimination and/or consumption of certain microbiome containing living foods the weight balance and the personal mood can be controlled using simple mobile applications, and coupling the available food ingredient components in the available databases and its tastes. The acid containing foods were selected from pickles, yoghurt, citrus fruits and berries, wines and coffees; and certain yeast containing food like beer and bread were eliminated. By this new method a significant, adjustable and sustainable body weight, mood and health control were achieved. During the study the skin was cleaned only by food quality acids, water and various infra-red radiations which drastically decreasing the previous skin problems like acne and dandruff. Our future plan is to widen our study on a clinical group trial with available serum test protocols for vitamins and certain acids and developing spectral tasting as a biochemical sensing method for the human body which can be the key triggers of the digestion and immune processes.

P22. Analysis of microbial growth and heterogeneity using droplet microfluidics**

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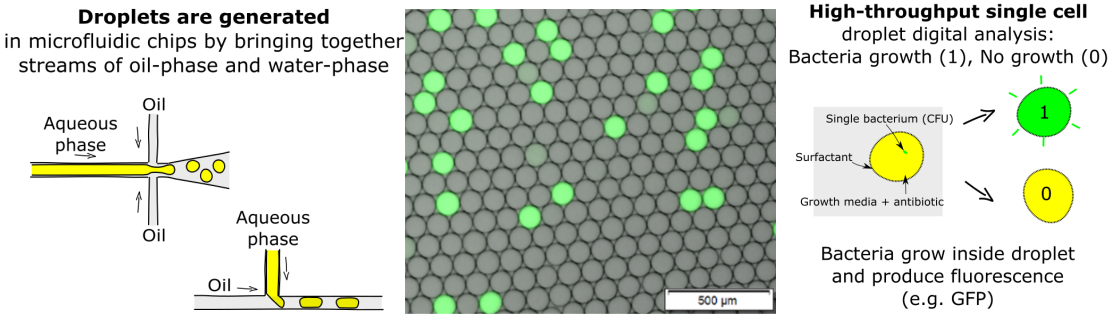
Droplet microfluidics deals with generation, manipulation and analysis of small (pL-nL sized) droplets that act as tiny test tubes, where biological and chemical reactions take place. Typical droplet microfluidic experiment can involve generating and analyzing hundreds of thousands (or even millions) of droplets.[1]



The detection and analysis of (bio)chemical reactions or cell growth in droplets is usually achieved via fluorescence. Droplet fluorescence can be analyzed with user-friendly freeware, for example CellProfiler that is often used in fluorescent microscopy.[2] Droplets are very suitable platform for the analysis of single cell growth patterns. For example, they enable precise analysis of antibiotic susceptibility at single cell (CFU) level, that can reveal the phenotypic heterogeneity in growth.[3]

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*** Poster selected for presentation in a flash talk*

P23. Effect of bacteriocins on pathogen microorganisms in cheese

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Due to the decreasing of pH value and the formation of organic acids and their preservative effect, lactic acid bacteria (LAB) for centuries have been used in food and feed conservation (1, 2). By preventing the increase of non-pathogen and pathogen microflora bacteriocins can inhibit food deterioration. Because they are harmless to eukaryotic cells the bacteriocins or bacteriocin producing LAB except cytolysin can be used as safe alternatives to chemical preservatives in foods (3).
The aims of this study were to identify bacteriocin producing enteroccoci isolated from North Macedonian artisanal cheeses (200 samples) and to determine the existence of bacteriocin structural genes (4,5). Furthermore, the following cultures were tested for sensitivity to enterocins: *L. monocytogenes* (NCTC 11994), *L. innocua* (cheese isolate), *S. aureus* (cheese isolate), *P. aeruginosa* (NCTC 10662), *B. cereus* (NCTC 7464), *S. enteritidis* (meat isolate), *E. coli* (NCTC 9001) and *Y. enterocolitica* (ATCC 11303). Bacteriocin producing isolates were identified as *E. faecalis* (n=8), *E. faecium* (n=2) and *E. hirae* (n=2). The 13 isolates were screened for antibacterial activity, since they may produce enterocins that can control the growth of the tested indicator bacteria. All of the isolates expressed their antibacterial activity predominantly against *L. monocytogenes* in comparison to *L. innocua*. None of the isolates were found to be inhibitory against *S. enteritidis, Y. enterocolitica* and *E. coli*.

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P.24 Regulatory networks involved in Jen1 lactate transporter endocytosis triggered by alkali stress

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Saccharomyces cerevisiae cells modulate extracellular pH, being able to alkalinize its surroundings when grown in the presence of alternative carbon sources. We have recently shown that, in *S. cerevisiae*, prolonged growth in lactate triggers Jen1 lactate transporter endocytosis. This phenomenon is associated with a pH-dependent effect and requires a functional Jen1 transporter. Proteins of the alpha-arrestin family are key players in linking signalling events and nutrient transporters endocytosis. We found that a member of the conserved alpha-arrestin family, Bul1p, mediates Jen1 endocytosis in response to alkali stress[1]. This downregulation involves the Npr1p kinase and the Sit4p phosphatase and it is only operating in a rich nitrogen source, as replacing ammonium with L-proline leads to reduced Jen1p turnover in prolonged growth in lactate. How an increase in pH leads to the turnover of Jen1p and possibly other transporters, especially H^+ symporters, is still unknown.

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P25. The diffusion of the viral-derived membrane-active peptide pepR along *Staphylococcus aureus* biofilms causes a graded spatiotemporal profile of bacterial disruption

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Infections caused by bacterial biofilms are a major healthcare problem given their reduced susceptibility to conventional antibiotics. Development of effective antibiofilm agents is urgent. In this work, we show that pepR, a multifunctional peptide derived from the Dengue virus capsid protein, is able to both prevent the formation and act on preformed *Staphylococcus aureus* biofilms. Thorough and detailed mechanistic studies using flow cytometry and confocal microscopy reveal that pepR targets the bacterial membrane. More importantly, the peptide is able to diffuse through a preformed biofilm and rapidly kill biofilm-embedded bacteria through the same mechanism. However, the diffusion of the peptide along the matrix of *S. aureus* biofilms shaped a spatiotemporal gradient of pepR which was responsible for its reduced activity at the inner layers of the biofilm. As shown recently [1] the inner layer of the biofilm corresponds to areas of low pH (~5.1) and the areas where the peptide is more active corresponds to an increase in the pH value towards a neutral pH. Overall, our work demonstrates the potential of pepR towards the development of novel membrane-active antibiofilm peptides and the importance of diffusion/biofilm structure for the biological activity of antimicrobial molecules.

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P26. The Effect of Carbon Dioxide as a Climatic Parameter on Microbial Food Contaminants and Selective Isogenic Mutants**

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Carbon dioxide dissolves in aqueous solutions existing in a chemical equilibrium as carbonic acid and bicarbonate, lowering its pH. An increase in accumulation of atmospheric CO_2 levels can lead to the lowering of pH, which may provide a selective pressure causing adaptational changes to some organisms. In particular, pathogenic microorganisms which have a high replicative index; such as *Escherichia coli*, may stimulate their growth. The glutamate decarboxylase (GAD) system maintains the pH in *E. coli* under acidic conditions. Mutations in the GAD system can lead to changes in the growth of *E. coli* leading to its enhanced growth under acidic conditions. To evaluate the effect of increasing CO_2 concentration on bacterial growth and assess its mechanism of action, wild-type *E. coli* and its metabolic or stress response mutants Δ*dnaK,* Δ*gadB,* Δ*gadC,* Δ*gadD* and Δ*rpoS,* were grown in a plate reader in tryptic soya broth until stationary phase at atmospheric gas conditions, 2.5, 5 or 10% CO_2. The optical density was recorded at 600 nm for 24 h and used to calculate the growth response and the time of detection (TTD) to assess growth. Increase in CO_2 to 5% significantly increased the TTD of *E. coli* Δ*dnaK,* Δ*gadC,* Δ*gadD* and Δ*rpoS* by 1 h, and significantly decreased the growth of *E. coli* Δ*dnaK,* Δ*gadB* and Δ*gadC* compared to wildtype *E. coli* ($p < 0.05$). This highlights the importance of the GAD system and metabolic stress response genes in the environmental adaptation to increasing CO_2.

*** Poster selected for presentation in a flash talk*

P27. Genome-wide mutational profile of acidophilic bacterium *Acidobacterium capsulatum*

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Analyses of spontaneous mutation have shown that total genome-wide mutation rates are qualitatively similar for most prokaryotic organisms (Lynch et al. 2016; Long et al. 2018). However, this view is mainly based on organisms that grow best around neutral pH values (6.0-8.0). However, some results for archaea adapted to life in extreme habitats have suggested relatively a lower genomic mutation rate and predominance of insertion-deletion events (Grogan et al. 2001; Mackwan et al. 2007; Drake 2009). Therefore, in order to understand how environmental factors (temperature, pH, etc.) and intrinsic mechanisms (DNA replication and repair) cooperate and determine the genome-wide mutation rate and spectrum across the tree of life, it is necessary to expand experimental assays to species living in extreme environments. Here, we have determined the genome-wide rate of spontaneous mutations in the acidophilic *Acidobacterium capsulatum* using a direct and unbiased method: mutation-accumulation experiments and whole-genome sequencing. Whole-genome sequencing of 70 mutation accumulation lines of A. capsulatum after an average of ~2900 cell divisions yielded a base-substitution mutation rate of 1.22×10^{-10} per site per generation or 5×10^{-4} per genome per generation, which is significantly lower than the consensus value of mesophilic (approx. 15-40C) and neutrophilic (pH 6-8) prokaryotic organisms with DNA genomes. However, the insertion-deletion rate is high relative to the base-substitution mutation rate. Organisms with a similar effective population size and a similar expected effect of genetic drift should have similar mutation rates. Since selection operates on the total mutation rate, it may be suggested that relatively high insertion-deletion rate may compensate low base-substitution rate in *A. capsulatum*.

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P28. Quantitative approaches to address the spore resistance in low pH food processing environments: the case of *Alicyclobacillus acidoterrestris***

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The elimination and control of sporulating microorganisms, such as *Alicyclobacillus acidoterrestris*, from the processing environment is of great importance, as its endospores are difficult to detect and are able to survive the pasteurization processes. Their growth is associated with off-flavours as well as the reduction of the shelf-life of acidic fruit juices and acidic beverages (pH<4) during distribution and storage. To control its development and optimize the application of heat treatments, in such food products, it is necessary to characterize its heat resistance and growth dynamics, particularly its recovery following heat treatments in relation to environmental pH. The current study presents quantitative approaches to assess the impact of the heating temperature and the pH of the products. Concurrently, the impact of the recovery medium on the apparent heat resistance is assessed. For that purpose, the thermosensitivity parameter of *A. acidoterrestris* is estimated by the use of Bigelow type model, while the impact of the heating medium on the heat resistance is also evaluated. Quantitative approaches on assessing the effect of the acidic environment in relation to the recovery media are also presented. For that purpose, a Rosso type of model that incorporates the estimation of cardinal value parameters is used to present quantitatively the impact of the recovery media.

*** Poster selected for presentation in a flash talk*

P29. Impact of gastric pathogen *H. pylori* on MUC5AC production and development of chronic infection

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Helicobacter pylori survive in stomach in acidic and colonize gastric epithelial cells of more than half of the human population, causing chronic inflammatory response, peptic ulcer or gastric cancer. We asked whether *H.pylori* and their soluble components may promote or maintain gastric colonization due to enhancement of mucin 5 (MUC5AC) production. We used *Cavia porcellus* experimentally infected with *H.pylori* 7/28 days after inoculation. Furthermore primary gastric epithelial cells of *Caviae porcellus* were propagated *in vitro* for 24h in the culture medium alone or with *H.pylori* components: glycine acid extract-(GE), 10µg/ml; cytotoxin-associated gene A-(CagA) protein, 1µl/ml; UreA urease subunits, 5µg/ml; *H.pylori* or *Escherichia coli* lipopolysaccharide-(LPS) 25ng/ml or with the live *H.pylori* CCUG17874 (2h, 2×10^7 CFU/ml). Primary gastric cells or gastric tissue were stained with anti-MUC5AC antibodies and secondary FITC antibodies to assess MUC5AC production or with anti-*H.pylori* FITC antibodies to show adhesion. *H.pylori* components or live bacteria enhanced the production of MUC5AC by gastric epithelial cells, *in vivo* and *in vitro,* which was related to increased adhesion of *H.pylori.* MUC5AC was produced more intensively during acute (7 days) than chronic (28 days) phase of infection. Upregulation of MUC5AC production in response to *H.pylori* is an important mechanism the maintenance of infection.

P30. Physico-chemical and microbiological characterization of typical Bulgarian sourdoughs

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In the past 15 years, consumer's interest in Bulgaria to sourdough (SD) baked goods is growing fast because of their distinct flavor, texture and healthy attributes. As a result, there is a high industrial demand for adequate sourdough starter cultures with good technological performance. Yeasts and lactic acid bacteria form stable mixed populations in artisanal sourdoughs, with composition mainly defined by the raw materials used, geographical factors, preparation methods, and the production environment. The aim of this study was to study typical artisanal sourdoughs from various locations in Bulgaria with focus on the lactic acid bacteria (LAB) and yeast species represented in their microbiota. Some physico-chemical characteristics were also assessed. Physico-chemical analyses of the 16 SD samples showed dry matter content from 25.17 to 53.6%, pH values within 3.1 and 5.3, and total titratable acidity from 6.8 to 32.6. The total LAB counts varied from $5x10^5$ to $4x10^9$ cfu/g, and yeast counts were between $6x10^4$ and $4x10^8$ cfu/g. Among the 11 LAB species identified, the most commonly found isolates were *Lactobacillus brevis* (31%), *Lactobacillus plantarum* (24%), and *Pediococcus pentosaceus* (14%). *Saccharomyces cerevisiae* was the most abundant yeast species found in all 16 sourdoughs (86%). *Pichia fermentans*, *Kluyveromyces marxianus*, *Yarrowia lipolytica*, *Kazachstania humilis*, *K. barnettii* and *Candida glabrata* were also identified.

P31. Influence of soy milk fermentation with *Lactobacillus* and *Bifidobacterium* strains on the protein immunoreactivity and mineral availability

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The aim of the study was to investigate the ability of 26 *Lactobacillus* and 26 *Bifidobacterium* strains to grow in the soymilk and acidify the medium. Moreover, their ability to decrease the immunoreactivity of β-conglycinin and glycinin – two main soy allergens and to influence the mineral availability were investigated. Among 52 investigated strains tested, 8 *Lactobacillus* and 6 *Bifidobacterium* strains were standing out in terms of decreasing soy protein immunoreactivity. Among the selected strains, lactobacilli lowered the pH to the level of 4.3-4.9, whereas bifidobacteria to the level of 4.6 to 6.4. *Bifidobacterium* strains characterized with the lowest acidifying abilities released the lowest amounts of magnesium, calcium, manganese and zinc and the highest amounts of copper. All tested bifidobacteria released manganese, which was at the lowest amounts in most of *Lactobacillus*-fermented soymilk. The concentration of iron was lower than in a control in all samples what indicates on the utilization of this element by growing bacteria. To sum up, the influence of *Lactobacillus* and *Bifidobacterium* strains on the immunoreactive properties of soy proteins was pH-independent, whereas their ability to release micro- and macroelements from the matrix was pH-dependent. The investigated strains will be further characterized for their technological properties useful in the production of fermented soymilk.

P32. High content analysis of fruit and berry fungal microbiota

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Carposphere is a unique and dynamic habitat, with microbial communities subjected to relatively large environmental changes. Microorganisms flourishing on fruit and berry surface are exposed to ripeningconditioned acidity variations affecting their survival and shifting the pattern of microbiota. In the present study, the abundance, structure, and diversity of the fungal microbiota associated with carposphere of sea buckthorn berries were investigated. High-throughput amplicon-based sequencing approach was used to reveal the fungal community alterations. Analysis of the most abundant representative OTUs showed a clear separation among microorganisms inhabiting the unripe and mature berries. In parallel, culture-dependent methods were used to isolate and characterize yeasts and yeast-like fungi distributed on the surface of sea buckthorn and cherry at different maturation stages. Potentially beneficial, inducing resistance in the hosting plant, or pathogenic, responsible for disease development, microorganisms were detected. The information on the prevalence of fungal microorganisms on the carposphere of tested plants is highly relevant for the development of strategies for plant cultivation and disease management as well as the quality of berries with potential in food production.

P33. Molecular approaches to understand the effect of acetic acid in uropathogenic *E. coli*

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Acetic acid has long been known for its antibacterial activity. We are using traDIS to investigate the molecular mechanisms by which acetic acid acts as an antibacterial agent.

To do this, we grew a high-density transposon library in uropathogenic *E. coli* EO499 serotype 131 in M9 media at pH 7 and pH 5.5 with acetic acid concentrations of 40mM and 4mM, respectively, or without added acetic acid. Sequencing libraries were generated from total bacterial populations after growth, and sequenced using a transposon-specific primer to generate positions and frequencies for each transposon. By comparing numbers of reads before and after the stress, we identified candidate genes where transposon inserts led to a decrease of fitness under acetic acid stress. Eight of these were chosen for further study: *nuoM, nuoG, sucA, sthA, pitA, apaH, rssB* and *ytfP.*

Because of the difficulties of constructing gene deletions in the uropathogenic strains for validating TraDIS results, we tested the relative fitnesses of the corresponding gene deletion mutants from the Keio library (in strain BW25113), with the growth conditions used for EO499. Interestingly, only a few knockouts showed a reduced in relative fitness in time course competition at pH with acetic acid, while TraDIS identified relatively higher number of genes. This may due to the differences between strains used in TraDIS and competition. To overcome this issue, we have also isolated transposon mutants from *E. coli* EO499 transposon library for the determination of relative fitness. The results will be presented.

P34. Physicochemical and biological characterization of pyomelanin isolated from *P. aeruginosa* in regard of antimicrobial and pro-regenerative activity

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Numerous studies report on the biomedical potential of melanines, however little is known on the chemical structure and bioactivity of pyomelanin from *Pseudomonas aeruginosa*. The aim of the study was to determine the physicochemical and biological properties of pyomelanin isolated from *P.aeruginosa* Mel+ strain by acid precipitation. Chemical properties were assessed with standard oxidizers and reducers. Solubility was tested in polar, polar aprotic and non-polar solvents and thermostability in the temperature range of 4-500°C. The cytotoxicity was evaluated in the MTT and resazurin reduction assays using mouse L929 fibroblasts. Cell migration and pro-regenerative properties were assessed in the *in vitro* wound healing assay. Antibacterial activity towards Gram-positive cocci and Gram-negative rods was assessed in broth microdilution method and resazurin reduction assay. The *in vitro* studies revealed that pyomelanin (in non-toxic concentrations) exhibit the highest antimicrobial potential towards *H.pylori* (MIC=64 µg/ml), and that it stimulates (1-32 µg/ml) the regeneration of previously damaged fibroblasts. Pyomelanin manifests thermostabile, reducing and oxidizing properties. The polarity of the solvent do not affect the solubility of the pigment. Low cytotoxicity and the ability to support cell migration and anti-*H.pylori* activity allows considering pyomelanin as a potential natural agent supporting gastric ulcers healing and limiting microbial expansion.

These studies were performed within the project "Multifunctional composites biologically active for applications in regenerative medicine of bone system (POIR.04.04.00-00-16D7/18)" carried out within the TEAM NET programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

P35. General Stress Response of *Salmonella enterica* Typhimurium to Environmental Stress

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Salmonella enterica Typhimurium is an enteric pathogen and etiological agent in bacterial food-borne diseases. The ability of *Salmonella* to survive in the food chain is due, in part, to its ability to respond effectively to environmental changes. *S. enterica* Typhimurium response to wide variety of environmental stresses is a result of highly complex and tightly regulated process of gene expression. The stress-induced changes in *S. enterica* Typhimurium bacterial cells were analyzed at the level of gene expression. For that purpose, the simultaneous analysis of gene expression of a set of 369 genes directly or indirectly involved in regulatory mechanisms and in virulence was performed by PCR-based microarray technique. Expression profile of bacterial cells submitted to oligotrophic stress (24 hours incubation in distilled water) was compared with expression profile of bacterial cells in exponential growth phase, considered as control condition. A number of induced genes under studied stress conditions, which ratio of gene expression (stress condition/control condition) had a value above 1.05 were genes that mediate in a general stress response. In fact, general stress response renders bacterial cells broadly stress resistant in a way in which damage is rather to be avoided than to be repaired.

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P36. Regulation of endothelial cells activation / apoptosis by *Helicobacter pylori* antigen components – link to cardiovascular heart disease

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Coronary heart disease (CHD) usually associates with hyperlipidemia coexisting with obesity, hypertension and diabetes. Involvement of microbiological components in the pathogenesis of atherosclerosis is also considered. *Helicobacter pylori* (HP) colonizes gastric epithelium causing acute local inflammation and gastric epithelial damage. HP infection may also stay asymptomatic and develop chronic inflammatory reaction in infected organism. Interestingly, HP antigenic components can influence endothelial cells causing disorder of endothelial homeostasis and dysregulation of its activity and apoptosis. The aim of our research was focused on investigating mechanisms initiated in vascular endothelial cells during HP infection. Experiments were performed using primary endothelial cells developed from *Caviae porcellus* aortic tissue. Cells were stimulated with HP bacterial components: lipopolysaccharide and acid glycine extract – GE, containing surface components. To date, analysis consist of expression of pErk and pro- or anti-apoptotic proteins: Bax, Bcl-2, Bcl-xL, caspase 3 in primary vascular endothelial cells. Additionally, cell DNA was tested for any signs of damage (DAPI staining) and endothelial cells were examined for metabolic activity change (MTT assay) upon stimulation with HP components. Obtained results suggest that HP components activate endothelial cells via Erk-dependent pathway and regulate their survival. Endothelial dysfunction caused by HP may significantly impact the development of CHD.

These studies were performed within the project "Multifunctional composites biologically active for applications in regenerative medicine of bone system (POIR.04.04.00-00-16D7/18)" carried out within the TEAM NET programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

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P37. Adaptation of Crohn's disease related bacteria AIEC LF82 to protect intracellular microcolonies from phagolysosomes attacks

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Patients with Crohn's disease present an abnormal colonization of the intestine by proteobacteria. Among them the family of Adherent Invasive *E. coli* (AIEC) are found associated with intestinal lesions in patients with inflammatory bowel disease. AIEC are predominant in the mucus, adhere to epithelial cells, colonize them and survive inside macrophages. We are interested in the adaptation of the AIEC allowing survival and growth in macrophages. The AIEC resides inside mature phagolysosomes where the environment is highly stressful (acid pH, oxidative stress, nutrient starvation, etc.). This environment dramatically limits the growth of other *E. coli* strains. We have recently demonstrated that stringent response and SOS induction are critical for AIEC survival and multiplication within macrophages (1). The aim of this project is to decipher acid stress interplay with virulence response of AIEC in phagolysosomes. We observed that intracellular LF82 produce an extra-bacterial matrix presenting traits of a biofilm that governs the formation of LF82 intracellular bacterial communities (IBC) inside phagolysosomes. The expression of the High Pathogenicity Island (HPI), allowing iron capture by the Yersiniabactin siderophore, is essential for the formation of the IBC and LF82 survival within macrophages. We explore HPI role in acidic pH growth.

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P38. Postharvest antimicrobial treatments with organic acids to improve the shelf life of fresh blueberries

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Fresh highbush blueberries (*Vaccinium corymbosum* L.) are one of the most popular soft fruits characterised by attractive sensorial attributes and by high antioxidant potential due to their richness in phenolics, especially anthocyanins [1, 2]. They are highly perishable as they are susceptible to various microbial infections, both pre- and postharvest, their shelf life being estimated between 35 and 40 days [3]. The present research was conducted to investigate the effects of postharvest treatments with citric (2%), benzoic (0.2%) and sorbic (0.2%) acids on physicochemical, biochemical and microbiological evolution in fresh blueberries under cold storage conditions. Samples were evaluated initially and at 7-day interval for dry matter, total soluble solids, titratable acidity, total phenolic content, total flavonoid content, antioxidant activity and microbial load for six weeks storage time. Chemical treatments significantly reduced the development of microorganisms on the surface of the fruits throughout the storage period as compared to the control samples, but they caused a significant increase in moisture loss (sorbic acid > benzoic acid > citric acid > water), probably due to the partial damage of the natural cuticular wax layer covering the fruit. Antimicrobial effects of chemical treatments were more noticeable than their biochemical effects. The results showed that chemical treatments did not affect the total phenolics, total flavonoids and antioxidant activity since no significant differences were observed among treatments and control.

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P39. Role of mitochondrial phospholipids in acetic acid-induced cell death

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Yeast cell death induced by acetic acid stress has been correlated with a plethora of cell death effectors whose mammalian orthologues have been themselves linked to the apoptotic process [1]. Most recently, we identified the endoplasmic reticulum-mitochondria encounter structure (ERMES) components as players in acetic acid-induced apoptosis, with deletion mutants displaying a prominent delay in the appearance of several apoptotic markers [2]. Additionally, we found that absence of ERMES induces changes in mitochondrial phospholipid profile, and that acetic acid treatment in both wild-type and mutant strains was accompanied by mitochondrial phospholipid remodeling. Indeed, mitochondrial phospholipids such as cardiolipin and phosphatidylserine have already been linked to mammalian apoptosis [3,4], but so far there are no studies regarding the role of these lipids in acetic acid-induced cell death. Hence, we used yeast strains deficient in either the cardiolipin synthase Crd1p, the lyso-phosphatidylcholine acyltransferase Taz1p, the phosphatidic acid transfer protein Ups1p or the phosphatidylserine synthase Cho1p. Results show a significant delay in loss of cell survival and plasma membrane integrity, as well as in mitochondrial depolarization and degradation, and in superoxide anion accumulation, supporting a role for phospholipid metabolism in yeast cell death induced by acetic acid.

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P40. Adaptive response and tolerance of the yeast *Saccharomyces cerevisiae* to stress induced by sulfur dioxide under low pH: the role of the transcription factor Com2

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During vinification *Saccharomyces cerevisiae* cells are frequently exposed to high concentrations of sulfur dioxide (SO_2) that is used to avoid overgrowth of unwanted bacteria or fungi present in the must. Up to now the characterization of the molecular mechanisms by which *S. cerevisiae* respond and tolerate SO_2 was focused on the role of the sulfite efflux pump Ssu1 and investigation on the involvement of other players has been scarce, especially at a genome-wide level. In this work, we uncovered the essential role of the poorly characterized transcription factor Com2 in tolerance and response of *S. cerevisiae* to stress induced by SO_2 at the enologically relevant pH of 3.5. Transcriptomic analysis revealed that Com2 controls, directly or indirectly, the expression of more than 80% of the genes activated by SO_2, a percentage much higher than the one that could be attributed to any other stress-responsive transcription factor. Large-scale phenotyping of the yeast haploid mutant collection led to the identification of 50 Com2-targets contributing for protection against SO_2 including all the genes that compose the sulfate reduction pathway (*MET3*, *MET14*, *MET16*, *MET5*, *MET10*) and the majority of the genes required for biosynthesis of lysine (*LYS2, LYS21, LYS20, LYS14, LYS4, LYS5, LYS1* and *LYS9*) or arginine (*ARG5,6*, *ARG4*, *ARG2*, *ARG3*, *ARG7*, *ARG8*, *ORT1* and *CPA1*). Other uncovered determinants of resistance to SO_2 (not under the control of Com2) included genes required for function and assembly of the vacuolar proton pump and enzymes of the antioxidant defense, consistent with the observed cytosolic and mitochondrial accumulation of reaction oxygen species in SO_2-stressed yeast cells.

P41. Effect of low pH in the denitrification pathway and nitrous oxide reductase of *Marinobacter hydrocarbonoclasticus*

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Increasing atmospheric concentration of N_2O has been a concern, as it is a potent greenhouse gas and promotes ozone layer destruction [1]. Release of N_2O from soils increases at low pH. Here, *Marinobacter hydrocarbonoclasticus* was grown in batch mode in the presence of nitrate, to study the pH effect in the denitrification pathway by gene expression profiling, quantification of nitrate and nitrite, and evaluate the ability of whole-cells to reduce NO and N_2O [2]. At pH 6.5, nitrite accumulates in the media and the cells were unable to reduce N_2O. The amount of N_2O reductase at acidic pH was lower than that at pH 7.5 and 8.5, pinpointing a post-transcriptional regulation, though pH did not affect gene expression of N_2O reductase accessory genes. The enzyme isolated from acidic growth has its catalytic center as CuZ*(4Cu1S), while the enzyme from pH 7.5 or 8.5 has it as CuZ(4Cu2S). This study evidences an *in vivo* secondary level of regulation required to maintain N_2O reductase in an active state.

Acknowledgments: We thank Fundação para a Ciência e Tecnologia for the financial support through the project PTDC/BIA-PRO/098882/2008 (SRP) and PTDC/BBB-BQB/0129/2014 (IM), and the scholarship SFRH/BD/87898/2012 (CC).

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P42. Pathway prospection for the implementation of microbe-based production of levulinic acid: a computational approach

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Levulinic Acid (LA) is a C5 carboxylic acid with a large range of industrial applications, from cosmetic products to fuel additives[1]. This commodity chemical may be produced through a petrochemical route or via chemical catalysis using pure sugars or lignocellulosic residues as substrates, while the biological production of LA in a biorefinery context has yet to be explored [2]. In this work we use pathway prediction and retrobiosynthetic methodologies to pinpoint new-to-nature biochemical routes that can be imported into the model organisms Escherichia coli and Saccharomyces cerevisiae leading to LA production. The candidate pathways are selected and further evaluated in silico on the basis of parameters such as the availability of enzymes to catalyze the required synthetic reaction steps, thermodynamics, pathway yield and possibility to couple chemical production to growth. With the described methodology four candidate new-to-nature LA producing pathways are reported here.

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