COST Action CA18113

EuroMicropH

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

Open Meeting:

Understanding and exploiting the impacts of low pH on microorganisms

PROGRAMME AND ABSTRACTS

Vienna, Austria

18-19 September 2023

COST Action CA18113
EuroMicropH
Understanding and Exploiting the Impacts of Low pH on Micro-organisms

CONTENT

CONFERENCE ORGANISERS

SCIENTIFIC COMMITTEE

Merve Atasoy	WUR (NL)
Daniela De Biase	Sapienza University of Rome (IT)
Zeynep Cetecioglu	KTH (SE)
Aleksandra Djukić-Vuković	University of Belgrade (RS)
Sholeem Griffin	University of Malta (MT)
Peter Lund	University of Birmingham (UK)
Nuno Mira	IST (PT)
Sofia Pauleta	Nova University Lisbon (PT)
Aricia Possas	University of Cordoba (ES)
Ricardo Santos	University of Lisboa (PT)
Ott Scheler	TalTech (EE)
Jana Sedlakova	UCM Trnava (SK)
Matthias Steiger	TU Wien (AT)
Carmit Ziv	Volcani Center (IS)

LOCAL ORGANIZER

Matthias Steiger	TU Wien (AT)

COST Action CA18113

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ORAL PRESENTATIONS

TU WIEN, Gußhausstraße 27-29, 1040 Vienna, Austria Lecture hall: **EI 9** and **EI10**

Program

Monday, September 18, Lecture Hall EI 10

Session		Monday, September 18 Time			
MC Meeting	Daniela De Biase, Peter Lund	10:00 -12:00	**MC Members only**		
Registration for Open Meeting		**12:00-13:00**			
Session title	**Chairs**	**Time**	**Speaker**	**Affiliation**	**Title**
Opening remarks EuroMicropH Open Meeting		13:00-13:10	Daniela De Biase		
Molecular mechanisms underpinning acid stress responses in bacteria	Conor O`Byrne	13:10-13:40	Kirsten Jung	Ludwig-Maximilians-Universität München, Germany	The fine-tuned response of *Escherichia coli* to mild and severe acid stress
Panel discussion	Daniela De Biase	13:40-13:50	Peter Lund	University Birmingham, UK	Presentation of results of survey of all COST Action members
		13:50-14:30	Panel: Kirsten Jung, Peter Lund, Conor O`Byrne, Jana Sedlakova, Carmit Ziv		
Coffee Break		14:30-15:00			
Clinical applications of microbial responses to low pH	Nuno Mira	15:00-15:30	Stefano Pagliara	University of Essex, UK	Investigating the role of bacterial pH regulation in antibiotic resistance
		15:30-16:00	James A Mason	King's College London, UK	NMR metabolomic studies to understand relationships between clinically relevant bacteria and pH and their functional impacts
		16:00-16:15	Gizem Özlük	Hitit University, Çorum, Turkey	Effects of beverages taken with meal on some foodborne pathogens in simulated gastric fluid
		16:15-16:30	João Alves	Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal	Exploring the Role of Specific Amino Acid Residues of CexA from Aspergillus niger on Citrate Transport
Coffee Break		16:30-17:00			
Omics approaches to study microbial stress response	Carmit Ziv, Ott Scheler	17:00-17:30	Nicoletta Guaragnella	University of Bari, Italy	Acetic acid stress response in *Saccharomyces cerevisiae* : implications in fundamental and applied research.
		17:30-18:00	Pirjo Spuul	Tallinn University of Technology, Estonia	The interplay between novel *Lactobacillus salivarius* and *Helicobacter pylori* .
		18:00-18:15	Rozeta Hasalliu	Agricultural University of Tirana, Department of Food Science and Biotechnology, Albania	Identification of lactic acid bacteria from different traditional Albanian Yogurt with different pH
		18:15-18:30	Merve Atasoy	Wageningen University and Research, Wageningen, The Netherlands	Designing and Optimizing Metabolic Networks and Microbial Communities for Butyric Acid Production Under Acidic Conditions
Poster Flash presentations	Matthias Steiger	18:30-19:00	see flash poster list	reception with light food and drinks sponsored by	**Jungbunzlauer**
Poster Presentation Reception		19:00-20:30			

Tuesday, September 19, Lecture Hall EI 9

Session title	Chairs	Tuesday, September 19 Time	Speaker	Affiliation	Title
Biotechnological applications – exploitation of micro-organisms in low pH manufacturing processes	Jana Sedlakova, Zeynep Cetecioglu	9:00-9:30	David Barrie Johnson	Bangor & Coventry Universities and the Natural History Museum, UK	How the unique physiologies of extreme acidophiles allow them to be exploited in extracting and recovering metals from mineral ores and electronic wastes
		9:30-10:00	Mustafa Turker	Pak Gıda Üretim Pazarlama A.Ş., Türkiye	Fermentative Production of Propionic acid: Optimization and Process Intensification
		10:00-10:15	Zeynep Agirbasli	İzmir Institute of Technology, İzmir, Turkiye	Bioutilization of Whey with Acid Tolerant Lactic Acid Bacteria from Artisanal Fermented Foods
		10:15-10:30	Karen Trchounian	Department of Biochemistry, Yerevan State University, Armenia	Influence of acidic pH on the interaction between proton ATPase and enzymes responsible for molecular hydrogen generation
Break		10:30-11:00			
Applications in food and drink manufacture and processing	Merve Atasoy	11:00-11:30	Lisa Gödtke	Jungbunzlauer Ladenburg GmbH, Germany	Case study: Preservation of pea protein meat alternative with lactic acid and lactate blends
		11:30-11:45	Jean Costa	UCO, Córdoba, Spain	Impact of acidification and bacteriocin production on the antilisteria effect of Latilactobacillus sakei CTC494
		11:45-12:00	Hayriye Sebnem Harsa	İzmir Institute of Technology, Türkiye	Acid Tolerant Lactic Acid Bacteria: A Promising Probiotic Starter Culture Candidate
		12:00-12:15	Aline Reinfurt	Austrian Centre of Industrial Biotechnology, Austria	Manganese and its regulatory role on the citrate exporter CexA – exploring the citric acid production mechanism of Aspergillus niger
Break		12:15-13:30			
Opening Microbial Stress 2023	Stefan Pflügl	13:30-13:40	Matthias Steiger	TU Wien, Austria	Opening remarks
		13:40-14:00	Teuta Pilizota	University of Edinburgh, UK	Environmental conditions define the energetics of bacterial dormancy and its antibiotic susceptibility
Microbial Stress Responses to low pH - From mechanisms to applications	Aricia Possas, Aleksandra Djukić-Vuković	14:00-14:30	Peter Lund	University Birmingham, UK	Transposon-directed insertion sequencing (TraDIS) can deepen our understanding of microbial stress responses
		14:30-15:00	Conor P O'Byrne	University of Galway, Irland	New insights into mechanisms of acid resistance in *Listeria monocytogenes* using comparative genomics.
		15:00-15:15	Sofia R. Pauleta	Microbial Stress Lab, NOVA University Lisbon, Portugal	Effect of low pH in the denitrification pathway and nitrous oxide reductase from Marinobacter hydrocarbonoclasticus
		15:15-15:30	Aleksandra Djukić-Vuković	University of Belgrade, Serbia	Agri-food industry wastes as substrates for lactic acid production – overview of different strat
Break		15:30-16:30			
Understanding and exploiting the impacts of low pH on micro-organisms	Ricardo Santos	16:30-17:00	Hana Sychrova	Institute of Physiology of the Czech Academy of Sciences, Czech Republic	Transporters involved in yeast pH and cation homeostases
		17:00-17:15	Immanuel Sanka	Tallinn University of Technology, Tallinn, Estonia;	Landscape of Expertise and Collaborative Opportunities from EuroMicropH Network Dashboard
		17:15-17:30	Athira Venugopal	The Hebrew University of Jerusalem, Israel	The V-shaped structuring facilitates the biofilm developmental process during acid stress adaptation of L. plantarum
		17:30-18:00	Daniela De Biase	Sapienza University of Rome, Italy	Low pH responses in micro-organism: sharing knowledge and community building
End EuroMicropH Open Meeting		**18:00**			

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POSTER FLASH AND POSTER PRESENTATIONS

List of Posters

	Poster Number	Presenter	Affiliation	Title
18:30 - 19:00 Poster flash presentations in Room EI 10 followed by poster presentations from 19:00 - 20:30	P1	Hafidh Akkari	Laboratory of Parasitology, National Veterinary School of Sidithabet, Tunisia	Antimicrobial Activity of Organic Acids : Application in livestock production
	P2	Jialun Wu	University of Galway, Ireland	Manganese uptake mediated by the NRAMP-type transporter MntH is required for acid tolerance in *Listeria monocytogenes*
	P3	Valentina Veselinović	University of Banja Luka, Bosnia and Herzegovina	Antimicrobial effect of Gold Nanoparticles modified polymethyl methacrylate denture base material
	P4	Bazilė Ravoitytė	Laboratory of Genetics, Nature Research Centre, Vilnius, Lithuania	Interconnection between stress response and double-stranded RNA viruses in Saccharomyces yeasts
	P5	Miroslava Sincak	University of Ss. Cyril and Methodius in Trnava, Faculty of Natural Science, Slovakia	Impact of electromagnetic field on yeast Saccharomyces cerevisiae with potential applications in industry
	P6	Esther Mwangi	The Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Israel	Lactic acid modulates oxidative stress response to induce viable but nonculturable (VBNC) state in Listeria innocua challenged by nature-based antimicrobial formulation
	P7	Utku Avci	Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Türkiye	Algal Biodiversity in High Altitude Blanket Bogs under Low pH Conditions
	P8	Juliana Lukša	Nature Research Centre, Vilnius, Lithuania	Exploring the Interactions between ScV-LA Virus and Host Protein: Insights from Proteomics Analysis and RNA Sequencing
	P9	Mila Arapcheska	University "St. Kliment Ohridski" - Bitola, R. North Macedonia	Mechanisms of Survival of Salmonella enterica Typhimurium in Response to Environmental Stress
	P10	Arícia Possas	University of Córdoba, Córdoba, Spain	A statistical investigation on the association between EuroMicropH COST members' expertise and the areas of study.
	P11	Mustafa Kizilsimek	KSU, Kahramanmaras, Türkiye	An effective methof for isolating high lactic acid producer bacteria strains
	P12	Aleksandra Djukić-Vuković	University of Belgrade, Belgrade, Serbia	Lactic acid and probiotic biomass production on waste substrates from agri-food industry
	P13	Sibel Kucukyildirim	Hacettepe University, Ankara, Turkey	Low base-substitution mutation rate and predominance of insertion-deletion events in the acidophilic bacterium Acidobacterium capsulatum
	P14	Tamara Abaghyan	Yerevan State University, Yerevan, Armenia	The penetration of PPA is mediated with H+ efflux in gram-negative and gram-positive bacteria
	P15	Gabriela Rapeanu	"Dunărea de Jos" University of Galati, Faculty of Food Science and Engineering, Romania	The influence of pH on malolactic fermentation dynamics of red wines from Feteasca negra grapes
	P16	Refik Bozbuga	Eskişehir Osmangazi University, Eskişehir, Turkiye	The Influence of Soil pH on Nematodes
	P17	Violeta Nour	University of Craiova, Craiova, Romania	Dip wash treatments with organic acids and acidic electrolyzed water combined with UV-C treatment to improve the shelf life of some fresh fruits

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ORAL PRESENTATIONS[1]

[1] All abstracts in this book are in the original version as submitted by the authors. Some titles have been edited to ensure stylistic coherence in the book.

MOLECULAR MECHANISMS UNDERPINNING ACID STRESS RESPONSES IN BACTERIA

The fine-tuned response of *Escherichia coli* to mild and severe acid stress

Kirsten Jung

Faculty of Biology, Microbiology, Ludwig-Maximilians-Universität München, Germany

The ability to respond to acidic environments is crucial for neutralophilic bacteria. *Escherichia coli* has a well-characterized regulatory network that triggers a multitude of defense mechanisms to counteract excess of protons. We used ribosome profiling and mRNA sequencing to compare the response of *E. coli* (pH 7.6) to sudden mild (pH 5.8) and severe near-lethal acid stress (pH 4.4) conditions that mimic passage through the gastrointestinal tract. We uncovered new differentially regulated genes and pathways, key transcriptional regulators, and 18 novel acid-induced candidate ORFs.

CLINICAL APPLICATIONS OF MICROBIAL RESPONSES TO LOW PH

Understanding the role of pH regulation in antimicrobial resistance

Stefano Pagliara

University of Essex, UK

Persister and viable but non-culturable (VBNC) cells are two clonal subpopulations that can survive multidrug exposure via a plethora of putative molecular mechanisms. In this talk I will describe the combination of microfluidics, time-lapse microscopy, and a plasmid-encoded fluorescent pH reporter to measure the dynamics of the intracellular pH of individual persister, VBNC, and susceptible *Escherichia coli* cells in response to ampicillin treatment [1].

We found that even before antibiotic exposure, persisters have a lower intracellular pH than those of VBNC and susceptible cells. We then investigated the molecular mechanisms underlying the observed differential pH regulation in persister *E. coli* cells and found that this is linked to the activity of the enzyme tryptophanase, which is encoded by the gene *tnaA*. In fact, in a Δ*tnaA* strain, we found no difference in intracellular pH between persister, VBNC, and susceptible *E. coli* cells.

Whole-genome transcriptomic analysis revealed that, besides downregulating tryptophan metabolism, the Δ*tnaA* strain downregulated key pH homeostasis pathways, including the response to pH, oxidation reduction, and several carboxylic acid catabolism processes, compared to levels of expression in the parental strain.

Our study sheds light on pH homeostasis, proving that the regulation of intracellular pH is not homogeneous within a clonal population, with a subset of cells displaying a differential pH regulation to perform dedicated functions, including survival after antibiotic treatment.

[1] Goode *et al*. mBio 12:e00909, 2021

NMR metabolomic studies to understand relationships between clinically relevant bacteria and pH and their functional impacts

A. James Mason

Institute of Pharmaceutical Science, King's College London, UK

Although the metabolic strategies of many key species of bacteria have been understood for decades, NMR spectroscopy has emerged as a useful and relatively high-throughput and reproducible technique capable of characterising bacterial metabolism. I will describe how we have used NMR metabolomics to understand how different metabolic strategies effect environmental acidification to differing degrees, the variation in metabolism and acidification between and within species, how this is affected by symbiosis and how this may be related to vaginal health. I will also show how we have used a combination of liquid and semi-solid-state NMR to characterise bacterial responses to acid stress to help better understand the activity of membrane active antibiotics.

Effects of beverages taken with meal on some foodborne pathogens in simulated gastric fluid

Gizem Özlük

Hitit University, Çorum, Turkey

Although it is thought that human stomach acidity would inhibit microorganisms, when food poisoning cases are considered, it is seen that especially during consumption of meat and products, the pH in the stomach rises and the fat layer creates a protective effect on bacteria, allowing microorganisms to transfer to intestine, and subsequently cause foodborne illnesses. The aim of this study was to determine whether beverage intake with meals influences why some individuals get sick, while others do not in food poisoning outbreak cases. The effect of Coke, Ayran and Shalgam, which are popularly consumed beverages along with meals in Turkey, was examined on the survival of *Salmonella Enteritidis*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and cocktail of these pathogens in simulated gastric fluid (SGF). Doner, was used as food sample. It was observed that in vitro consumption of one serving (300 mL) of Shalgam, Coke, and Ayran along with contaminated doner, reduced the pathogenic bacteria count in SGF up to 2.6, 2.25 and 1.6 log CFU/g, respectively. The results showed that consuming those beverages alongside one portion (100 g) of low contaminated (~10^1 CFU/g) doner might eliminate the possibility of a healthy person having foodborne illness due to aforementioned bacteria. Shalgam was found to be noteworthy as it provided the greatest reduction in pathogen count. It can be concluded that, in food poisoning cases, not only personal immune system, but also the type and amount of beverage consumed along with food, as well as the amount of food intake are effective.

Exploring the role of specific amino acid residues of CexA from *Aspergillus niger* on citrate transport

João Alves [1)], Maria Sousa-Silva [1)2)], Pedro Soares [1)2)], Michael Sauer [3)4)], Margarida Casal [1)2)], Isabel Soares-Silva[1)2)]

[1)] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal; [2)] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; [3)] University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Microbiology and Microbial Biotechnology, Muthgasse 18, 1190 Vienna, Austria; [4)] Austrian Centre of Industrial Biotechnology (ACIB GmbH), Muthgasse 11, 1190 Vienna, Austria

The CexA transporter from *Aspergillus niger*, a member of the Drug-H^+antiporter (DHA) family (TC 2.A.1.2) of the Major Facilitator Superfamily, plays a crucial role in citric acid secretion. Disruption of the CexA gene in *A. niger* abolishes citric acid secretion, whereas its expression in *Saccharomyces cerevisiae* promotes citric acid secretion. This research aimed to investigate the activity and specificity of CexA by heterologous expression in *S. cerevisiae*. The CexA transporter exhibits a high degree of specificity for citric and isocitric acids. In addition to its role as a citrate exporter, it also has the ability to import citrate with low affinity and high capacity at pH 5.5 (K_m 43.7± 13.4 mM of citric acid and V_{max} 9.4 ± 4.0 nmol citric acid s^{-1} mg^{-1} dry wt.). To understand the structure-function relationships of CexA, this study employed rational site-directed mutagenesis, based on 3D structure prediction, molecular docking analysis, and conserved amino acid residues. Our results shed light on the structural characterization of CexA resulting in the generation of several mutants with modified activity and specificity. Mutations P200A, Y307A, S315A, and R461A resulted in an almost complete loss of citrate transport capacity, while substitutions at F188, T207, and P235 led to a decreased transport capacity. Interestingly, the S75A mutant allele exhibited increased affinity to citrate. Furthermore, the substitution R192A blocked citrate export while preserving the import capacity. These insights hold the potential for enhancing citrate bioproduction and contribute to understanding transport mechanisms within the DHA1 family.

OMICS APPROACHES TO STUDY MICROBIAL STRESS RESPONSE

Acetic acid stress response in the budding yeast *Saccharomyces cerevisiae*: implications in fundamental and applied research

Nicoletta Guaragnella [1)] Maurizio Bettiga [2),3)]

[1)]Department of Biosciences, Biotechnology and Biopharmaceutics, University of Bari A.Moro, Bari, Italy [2)]Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden [3)]Bioeconomy Division, EviKrets Biobased Processes Consultants, Landvetter, Sweden

Acetic acid stress represents a frequent challenge to counteract for yeast cells under several environmental conditions and industrial bioprocesses. The molecular mechanisms underlying its response have been mostly elucidated in the budding yeast *Saccharomyces cerevisiae*, where acetic acid can be either a physiological substrate or a stressor. We focused our attention on acetic acid stress and its response in the context of cellular transport, pH homeostasis, metabolism and stress-signalling pathways and how major cellular players involved in acetic acid tolerance have been identified. The improvement of acetic acid tolerance is also a key factor in the production of biofuels and renewable chemicals from lignocellulosic biomass. The knowledge coming from fundamental research can contribute to the development and competitiveness of yeast cell factories for sustainable applications. In our presentation, we will first give an overview of findings on the molecular mechanisms of acetic acid stress, with special focus on multi-omics, synthetic biology and metabolic engineering approaches and we will then elaborate on how this knowledge could be used to contribute to the development of cell factories for sustainable applications.

The interplay between novel *Lactobacillus salivarius* and *Helicobacter pylori*

Pirjo Spuul [1)] Lilian Ventsel [1)], Kaisa Roots [1)], Inga Sarand [1)], Külliki Suurmaa [2)]

[1)]Tallinn University of Technology, Department of Chemistry and Biotechnology, Estonia [2)] Department of Gastroenterology, the West Tallinn Central Hospital, Tallinn, Estonia

Lactobacillus salivarius is one of the few bacterial species capable of surviving in the hostile environment of the human stomach. Probiotic Lactobacilli, including *L. salivarius*, have been suggested to be useful for the eradication of the human pathogen Helicobacter pylori (H. pylori). We have isolated novel *L. salivarius* strain while cultivating *H. pylori* on Columbia blood agar (CBA) supplemented with vancomycin, trimethoprim, cefsulodin, and amphotericin B from a stomach biopsy sample. The new strain has been characterized, its genome assembled, pH tolerance tested and most importantly, its effect on *H. pylori* analysed. Interestingly, *L. salivarius* strain A1 suppresses but does not entirely inhibit the growth of *H. pylori*. In addition, the presence of strain A1 reduces the inflammatory response of *H. pylori*.

Identification of Lactic Acid Bacteria from different traditional Albanian yogurt with different pH

Rozeta Hasalliu[1)], Konrad Domig[2)], Johanna Burtscher[2)]

[1)]Agricultural University of Tirana, Department of Food Science and Biotechnology, Albania;

[2)]University of Natural Resources and Life Sciences, Department of Food Science and Technology, Vienna, Austria

The aim of this study is the identification of lactic acid bacteria (LAB) from different traditional Albanian yogurt, with different pH. The samples of traditional Albanian yogurts were collected from 34 regions of Albania. The yogurt is produced without the addition of starter cultures only with backslopping inoculation.

The isolation of LAB was conducted on microbial media MRS and M17 agar incubated at aerobic and anaerobic conditions at 30°C and 37°C. The total colony count was between 10^2 - 10^8 cfu/ml yogurt. The identification of the obtained isolates to species level was performed by MALDI-TOF MS (Matrix-assisted Laser Desorption/Ionization Time of flight Mass Spectrometry, (Bruker Biotyper) technique in the Laboratory of Food Microbiology and Hygiene, Institute of Food Science, Department of Food Science and Technology, BOKU - University of Natural Resources and Life Sciences Vienna, Austria. A total of 183 isolated colonies were stored in -80°C with 20% glycerol for further characterisation.

The pH of yogurt is between 3.75 (from sheep milk) to 4.76 (from cow milk). *Lactobacillus delbrueckii, Streptococcus salivarius* are the most species found in the yogurt samples with lower pH and in yogurt samples with higher pH are found more *Lactobacillus delbrueckii, Streptococcus salivarius* and other species like *Leuconstoc lactis, Lactococcus lactis, Lactococcus garviae, Candida kefyr, Enterococcus facium.*

Designing and optimizing metabolic metworks and microbial mommunities for butyric acid production under acidic conditions

Merve Atasoy [1)] William Scott [2)], Peter Schaap [3)], Hauke Smidt [1)]

[1)]Wageningen University and Research, Wageningen, the Netherlands;

[2)]UNLOCK, Wageningen University & Research and Delft University of Technology, Wageningen, the Netherlands;

[3)]Systems and Synthetic Biology, Wageningen University, the Netherlands;

Butyric acid is a versatile volatile fatty acid widely used in various industries, and its global market size is projected to increase significantly in the coming decade. However, the current reliance on petroleum-based chemical synthesis for butyric acid production raises environmental concerns and necessitates a greener alternative. Biobased production of butyric acid through fermentation is being explored to address this issue, but challenges in yield improvement and downstream processing hinder its industrial-scale implementation. Bioaugmentation and co-culture systems show promise in enhancing biobased butyric acid production by improving substrate utilization, product yield, and system robustness. Effective strategies for bioaugmentation and substrate selection are crucial for maximizing butyric acid production. This project aims to utilize the Design-Build-Test-Learn (DBTL) cycle, a synthetic biology approach, to develop a synthetic community that optimizes butyric acid production under acidic conditions. By employing metabolic pathway modeling, the project will predict the behavior of selected species under different conditions to optimize yield and productivity. Modeling tools will also identify potential bottlenecks in the production process and guide the development of strategies to overcome them. The main objectives of this project include designing an efficient co-cultivation system using different butyric acid producer species, constructing genome-scale models for simulation using constraint-based modeling techniques, experimentally validating the model predictions, and analyzing the results to determine optimal community interactions, metabolic pathways, and metabolic exchanges for high butyric acid yields. Special emphasis will be placed on selecting species that can convert by-products into butyric acid and enhancing system efficiency by reducing substrate selectivity.

BIOTECHNOLOGICAL APPLICATIONS – EXPLOITATION OF MICROORGANISMS IN LOW PH MANUFACTURING PROCESSES

How the unique physiologies of extreme acidophiles allow them to be exploited in extracting and recovering metals from mineral ores and electronic wastes

D. Barrie Johnson

Bangor & Coventry Universities and the Natural History Museum, UK

Over the past 60 or so years, full-scale world-wide operations have developed that exploit the unique abilities of acidophilic prokaryotic microorganisms to either solubilise metals from mineral ores or (as in the case of gold) to make them accessible for chemical extraction. More recently, this biotechnology has diversified into using the same or related microorganisms to liberate metals from spent batteries and electronic wastes. Many transition and other metals have greatly enhanced solubilities at low pH which is why acidophiles are particularly suited to bioleaching processes. Some species of acidophiles have also been demonstrated to facilitate the selective removal of metals from bioleach liquors and waste streams such as acid mine drainage waters. Expansion of biomining operations has paralleled knowledge and understanding of the biodiversity of extreme acidophiles, which are generally regarded as those that have pH growth optima at or below pH 3, and how are they are able to tolerate not only external hydronium ion concentrations that can be very many orders of magnitude greater than pH-neutral environments but also concentrations of soluble metals that are lethal to most life forms. One of the major differences between acidophilic and neutrophilic prokaryotes is that the former have (pH-dependent) positive membrane potentials. This endows them with a natural ability to repel the influx of cationic metals such as copper though does make them more susceptible to being inhibited by anionic metals (such as chromate) and non-metallic anions such as nitrate and chloride. The biodiversities, physiologies and industrial applications of acidophilic prokaryotes will be described and projected future developments of this area of biotechnology discussed.

Propionic acid production, optimization and process intensification

Mustafa Türker

Pak Gıda Üretim Pazarlama A.Ş., Türkiye

Propionic acid, also known as propanoic acid, is a short-chain fatty acid in “generally recognized as safe (GRAS)” status. It has various industrial uses such as mold inhibition and fruit flavouring in food industry, feed and grain preservation, as perfume bases in cosmetics, in cellulose based plastic manufacturing in plastics industry, in veterinary applications for wound infections, in pharmaceutical industry for conjunctivitis, anti-arthritic drugs and also for herbicide production. The major limitation of fermentative propionic acid production is its cost compared to petrochemical route. Due to low growth rates and productivity levels, some of microbial processes currently are not able to compete with the petrochemical routes in terms of production costs. Moreover, co-production of acetic acid and succinic acid leads to complicated and costly downstream processing when high degree of purity is needed. However, there is an upward trend in food industry to use molecules produced by fermentative routes as natural preservatives. This tendency provokes further investigations on fermentation strategies to achieve higher production rates from low-cost carbon sources, such as glucose, lactic acid, glycerol, flour and cheese whey. In this presentation, I will discuss pros and cons of alternative production strategies and how to intensify the process to increase the final titers and productivities.

Bioutilization of whey with acid tolerant Lactic Acid Bacteria from artisanal fermented foods

Zeynep Agirbasli, Hayriye Sebnem Harsa

İzmir Institute of Technology, İzmir, Turkiye

Lactic acid bacteria (LAB) have been exploited for biotechnological applications. They can be utilized in low pH manufacturing processes e.g. traditional and functional fermented foods. LAB are generally used in food/bio products to improve e.g. the flavor, increase the nutritional content, inhibit the spoilage and harmful substances, prolong the shelf life.

Environmental factors such as pH, temperature are quite important for LAB to obtain maximum growth conditions for biomass growth and lactic acid production etc. during fermentation. Mainly lactic acid is produced in fermentation broths when suitable nutrient medium is provided. Therefore, most of the LAB species have strong tolerance to low pH values e.g. below pH 5.0. Acid tolerant LAB may exert probiotic properties to promote health impact.

Acid tolerant strains of LAB isolated from artisanal fermented foods of Turkey have been investigated in this study. The genera *Lactiplantibacillus plantarum, Latilactobacillus curvatus, Levilactobacillus brevis, Streptococcus thermophilus* have been identified. Production patterns of important metabolites e.g. lactic acid, acetic acid, formic acid in whey medium and prospective by-products e.g. gamma amino butyric acid (GABA) have been followed for expanded application of LAB from the perspective of biotechnology. Fermentation kinetic parameters e.g. maximum specific growth rate, productivity and yield coefficients were assessed as well as growth of biomass,via colony-forming unit (CFU/ml) determination.

Glutathione (GSH) biosynthesis involved in bacterial acid stress resistance, improved tolerance to oxidative stress, better survival at low pH and high salt would be possible for the microorganisms having antioxidant properties, therefore antioxidant activities of LAB will also be presented.

Influence of acidic pH on the interaction between proton ATPase and enzymes responsible for molecular hydrogen generation

Karen Trchounian, Heghine Gevorgyan, Lilit Baghdasaryan, Anait Vassilian, Anna Poladyan

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Escherichia coli produces H_2 from formate via formate-hydrogen lyase complexes (FHL), which consist of formate dehydrogenase-H (Fdh-H) and hydrogenase-3 (Hyd-3) or hydrogenase-4 (Hyd-4). Four reversible Hyds form a H_2 cycle across the membrane. It is suggested that there is an interaction between the H_2 and H^+ cycles that is coordinated by the components of both FHL complexes together with F_OF_1-ATPase. To understand the impact of acidic pH on the interaction between these membrane-bound enzymes, Hyd and FDH activities were measured in wild type (wt) and DK8 mutant strain (deletion mutant lacking the complete *atp* operon) during fermentation of glucose, glycerol and formate. The data have shown that Hyd and FDH activities and H_2 generation rate were higher ~1.6, ~2.3 and ~2.0-fold, respectively, at pH 5.5, compared to pH 7.5 at 20 h of growth, when glucose is the main substrate being utilized. H_2 was not generated in DK8 and the Hyd and FDH activities were decreased. When glycerol was utilized (72 h) Hyd activity was higher in wt, meanwhile, H_2 generation was not observed. Therefore, Hyds exhibited higher H_2-oxidizing activity during glycerol utilization when H_2, generated during glucose utilization (20 h), was absorbed at a higher rate. Thus, a direct interaction of membrane-bound F_OF_1-ATPase, Hyd and FDH enzymes was observed only at the 20 hours of growth, when glucose is mainly utilized. It is suggested that the interplay between the H_2 and H^+cycles under acidic conditions aims to regulate ΔpH and maintain bacterial viability under acidic conditions.

CLINICAL APPLICATIONS OF MICROBIAL RESPONSES TO LOW PH

Case study: preservation of a pea protein-based meat alternative matrix with lactic acid and lactate blends

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Jungbunzlauer is one of the world's leading producers of biodegradable ingredients of natural origin. The products are manufactured utilising fermentation, a technology based on the ability of microorganisms to transform renewable raw material (carbohydrates from corn, grains or sugar beets) into useful biodegradable products such as lactic acid. Especially in the meat industry, lactic acid as well lactates and blends thereof are often used for their preserving properties, thus contributing to food safety. However, as the meat alternative segment grows globally, concerns about food safety arise, as knowledge about effective preservation systems for processed plant-based products is scarce.

In this study, lactic acid and lactate (blends) were tested in a pea protein-based meat alternative matrix for their preserving effects. For this purpose, both untreated and treated matrix samples were inoculated with *Staphylococcus aureus, Escherichia coli, Lactobacillus acidophilus* and *Candida albicans*, vacuum-packed and stored at 7°C. Microbiological growth was determined biweekly for eight to ten weeks. Water activity and pH were monitored accordingly throughout the storage period. In summary, lactic acid and lactate (blends) were able to prevent growth of inoculated microorganisms, except of *C. albicans*. No significant changes in pH and water activity were detected.

Impact of acidification and bacteriocin production on the antilisteria effect of *Latilactobacillus sakei* CTC494

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The study aimed to investigate the acidification impact on *Listeria monocytogenes* growth when co-cultured with *Latilactobacillus sakei* CTC494 (bacteriocin producer) or 23K strain at 5:3 ratio. The effect of dry-fermented sausages ingredients/additives (NaCl: 0.00-40.36 g/L, Mn: 0.08-0.27 g/L, glucose: 0.00-40.36 g/L) and temperature conditions (3-37 ºC) was evaluated through a central composite design. Growth potential (δ) of *L. monocytogenes* was calculated by the difference between maximum and initial counts. Lactic acid (LA), pH and bacteriocin production (BA) were measured periodically. The magnitude of δ depended on the *L. sakei* strain. For both LAB strains the lowest δ was observed under those conditions generating the lowest pH. Specifically, strain 23K showed reduced growth compared with other conditions (δ=1.59 log) and pH_{min}=3.7 (NaCl (8.18 g/L), Mn (0.27 g/L) and Glucose (32.18 g/L), while the bacteriocinogenic strain CTC494 showed enhanced inactivation (δ=-3.38 log) and pH_{min}=3.6 (NaCl (8.18 g/L), Mn (0.27 g/L) and Glucose (8.18 g/L)), indicating that the presence of bacteriocin promotes the inhibitory (lethal) effect of pH. The highest concentration of LA, the lowest pH tested and BA for *L. sakei* 23K and *L. sakei* CTC494 at the end of the experiment was 4.99 and 4.90 (g/L), 3.67 and 3.61, and 3.2 (AU/mL), respectively. The quantification of the *L. monocytogenes* growth potential as a function of the studied factors allows to optimize application conditions for bioprotective cultures to maximize its inhibitory effect in fermented meat products.

Acid tolerant Lactic Acid Bacteria: a promising probiotic starter culture candidate

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The main function of starter cultures used in fermented dairy manufacturing, mainly represented by lactic acid bacteria (LAB). LAB species cause the rapid production of lactic acid, which causes a reduction in pH, inhibiting the growth of spoilage and pathogenic microorganisms, increasing the shelf-life of fermented foods e.g. yoghurt. Other metabolites of LAB (e.g., lactic acid, propionic acid, benzoic acid, bacteriocins) improve the safety of foods. On the other hand, probiotics are bacteria that help to maintain the natural balance of microbiota in the intestine. The largest group of probiotic bacteria in the intestine is LAB, found in yoghurt with live culture.

In this study, novel starter strains were isolated from different artisanal yoghurt samples. These isolates were characterized by using biochemical, molecular and probiotic characterization techniques. Among 453 LAB strains in total, 5 *Streptococcus thermophilus* and 26 *Lactobacillus delbrueckii* ssp. bulgaricus isolates have been identified as probiotic candidates.

Probiotic features of strains were determined for low pH tolerance, bile salt tolerance, bile salt deconjugation, and cholesterol assimilation, transit tolerance to gastrointestinal tract, antibiotic resistances, autoaggregation, cell surface hydrophobicity, antimicrobial activities, adhesion abilities, growth capacity with prebiotics. All the LAB strains had different properties; but all of them were acid and bile resistant, had ability to adhesion Caco-2 cell lines, and grown very well with prebiotic sources. Further studies were conducted to determine the promising starter culture combinations using the two cocci and eight bacilli isolates; probiotic yoghurts were produced and evaluated according to physical, chemical, rheological, and organoleptic methods.

Manganese and its regulatory role on the citrate exporter CexA – exploring the citric acid production mechanism of *Aspergillus* niger

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Aspergillus niger is a biotechnologically important filamentous fungus used for the industrial production of citric acid. One of the most crucial factors that affect citric acid production is the amount of manganese present in the culture broth. Observations showed that the fungus develops a certain pellet-like morphology under manganese limitation conditions and that this limitation is decisive for high citric acid accumulation by *A. niger*. However, the exact mode of action of manganese in the cell is not clear. We show the impact of manganese ions on the citrate exporter encoding gene *cexA* and evaluate the citric acid production capability under wild-type and *cexA* overexpression conditions in the presence and absence of manganese. Additionally, further targets related to manganese and citric acid production are currently being investigated.

MICROBIAL STRESS RESPONSES TO LOW PH- FROM MECHANISMS TO APPLICATIONS

Environmental conditions define the energetics of bacterial dormancy and its antibiotic susceptibility

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Bacterial cells that stop growing but maintain viability and the capability to regrow are termed dormant and have been shown to transiently tolerate high concentrations of antimicrobials. Links between tolerance and cellular energetics as a possible explanation for the tolerance, have been investigated and have produced mixed and seemingly contradictory results. Because dormancy merely indicates growth arrest, which can be induced by various stimuli, we hypothesize that dormant cells may exist in a range of energetic states that depend on the environment. To energetically characterize different dormancies, we first induce them in a way that results in dormant populations and subsequently measure both of their main energy sources, the proton motive force magnitude and the concentration of ATP. We find that different types of dormancy exhibit characteristic energetic profiles that vary in level and dynamics. The energetic makeup was associated with survival to some antibiotics but not others. Our findings portray dormancy as a state that is rich in phenotypes with various stress survival capabilities. Because environmental conditions outside of the lab often halt or limit microbial growth, a typologization of dormant states may yield relevant insights on the survival and evolutionary strategies of these organisms.

Transposon-directed insertion sequencing (TraDIS) can deepen our understanding of microbial stress responses

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Post-genomic methods such as RNA-seq and high-throughput proteomics enable us to describe, with high resolution, the responses of organisms to stress. Although these methods are powerful, they provide only an indirect measurement of the importance of individual genes in the stress response. A more direct approach to determining this would be to estimate the relative fitness of strains carrying mutations in different genes by competing them against each other under the stress condition of interest, and measuring how their relative proportions change in the population as a result of the stress. This can be done on a large scale by using dense transposon libraries. High throughput sequencing of these pre- and post-stress enables us to estimate, for all non-essential genes, their relative importance for the fitness of the organism. I'll explain how we have used this method (most often known as Tn-seq or TraDIS) to examine the contribution of all non-essential genes to the acid stress response in *Escherichia coli*, and how we can use this method to predict how *E. coli* will evolve to adapt to different stresses over time.

New insights into mechanisms of acid resistance in *Listeria monocytogenes* using comparative genomics.

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The ability to survive the acidic conditions found in the stomach is a key trait enabling the food-borne pathogen *Listeria monocytogenes* to gain access to mammalian gastrointestinal tract where it can initiate an infection. Little is currently known about how acid resistance is regulated in this pathogen and why this trait is highly variable between strains. Here we used genomic sequences from a collection of *L. monocytogenes* strains with known differences in acid survival to identify a novel transcriptional regulator controlling acid resistance, which we call GadR. The regulator belongs to a family of regulators previously found only in a small group of bacterial pathogens including the streptococci, where they are involved in regulating virulence properties. We show that GadR is the dominant regulator of acid resistance in *L. monocytogenes* and that variability in its gene sequence accounts for previously observed differences between strains in this trait. Together these findings significantly advance our understanding of how this important pathogen copes with acid stress and suggests a potential molecular target to better control it in the human food-chain.

Effect of low pH in the denitrification pathway and nitrous oxide reductase from *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) [3][4]. This study evidences an in vivo secondary level of regulation required to maintain N_2O reductase in an active state. In addition, it explains why acidic environments will have a negative impact in the atmospheric N_2O.

Agri-food industry wastes as substrates for lactic acid production – overview of different strategies

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Lactic acid (LA) is a platform chemical dominantly produced by microorganisms. LA is precursor of poly-lactides, advantageous polymers used in production of stents and grafts for medical applications. Also, it is preservative, acidulant and flavouring agent in food industry or in bulk, it is used as a cleaning agent, e.g. in marine industry. Market for lactic acid is steadily growing and it is challenging current bioprocesses for higher yields on more sustainable and affordable substrates.

Lactic acid bacteria, including former *Lactobacillus* sp. genus, were among the most often exploited microorganisms in fermentative lactic acid production on agri-food wastes - due to their high LA productivity on range of substrates, GRAS status and tolerance to lower pH. It is possible to achieve high LA and microbial biomass yield with many of these microorganisms and some also have probiotic properties. Therefore, residues after the fermentation on agri-food residues can be valorised as feed with added value.

This work will report on different techniques and strategies employed to decrease negative effects of low pH and other stressors often present in fermentations on waste substrates with aim to valorise both produced LA and biomass. Open fermentation and challenges related to media sterilization, immobilization and adaptation will be particularly highlighted.

UNDERSTANDING AND EXPLOITING THE IMPACTS OF LOW PH ON MICRO-ORGANISMS

Transporters involved in yeast pH and cation homeostasis

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Maintenance of optimal intracellular pH, an inward gradient of protons, and a proper intracellular concentration of monovalent cations are the prerequisites for yeast cell survival, mainly in an acidic environment. K^+ is required for many physiological (regulation of cell volume and intracellular pH, maintenance of stable plasma-membrane potential, compensation of negative charges of many macromolecules) and biochemical (e.g. protein synthesis, enzyme activation) functions. Still, a higher intracellular concentration of Na^+ is toxic. Yeast cells grow in a broad range of external pH (2.5 – 8) and wide external concentrations of K^+ (10 µM – 2.5 M) and Na+ (up to 1 M). To maintain an optimum content of protons and monovalent cations in the cytosol and organelles and a stable membrane potential for energisation of the uptake of necessary nutrients, yeast cells employ more than ten different alkali-metal-cation specific transporters and two H_+-ATPases. Except for cation extruding Ena ATPases, the activity of the other plasma-membrane monovalent-cation transporters depends on the activity of plasma-membrane Pma1 H^+-ATPase, and vice-versa, the activity of Pma1 relies on the rate of potassium influx and efflux. Upon malfunctioning of potassium uptake systems, yeast cells do not survive various stresses, including acidic stress.

Landscape of expertise and collaborative oOpportunities from EuroMicropH Network dashboard

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COST Action CA18113 EuroMicropH was initiated to enable interactions between different sectors, and to facilitate knowledge and experience transfer related to microbial response to low pH for the benefit of the research and industrial communities. The EuroMicropH community currently consists of 200+ members. With the aim of promoting this goal and to enable different stakeholders, researchers, practitioners, and academics to collaborate and work together on the impacts of low pH on micro-organisms, we have done a survey to map the community's methods and expertise. The survey consisted of six parts, including personal details, field of activity, methods and technologies, object of interest, different action, mechanism and behavior and additional background. From the survey we received 107 responses, and the data was analyzed and visualized with Python script and Tableau dashboard, respectively. Based on the dashboard, we can show the extent to which members spread over Europe and identify collaborators worldwide. Most of the members come from academia and the focus fields are basic research, food safety and environmental research. Moreover, the majority of the members conduct research on organic acids. From the results, we also found that there is a limited number of members who employ chemical production across the field of study. The dashboard of the results is available to all at the “Network” tab of EuroMicropH’s website (https://euromicroph.eu/network/) and can be easily filtered to facilitate and promote interactions between partners that may be interested in sharing knowledge and expertise. We hope this will promote collaborations and expertise exchange to the benefit of the field.

The V-shaped structuring facilitates the biofilm developmental process during acid stress adaptation of *L. plantarum*

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The probiotic bacterium *Lactiplantibacillus plantarum* thrives in diverse ecological niches through exhibiting effective adaptation strategies. Herein, we report on a developmental process characterized by unique V-shaped geometrical structuring of *L. plantarum* cells during their adaptation to acidic environment. Characterization of the V-shaped structuring was performed by confocal microscopy and high resolution SEM imaging. Global metabolic activity and cell cytokinesis of the geometrically rearranged cells were assessed through XTT assay, Bac-titer GloTM ATP assay and flow cytometry analysis, respectively. Biofilm formation was analyzed through crystal violet and spinning disk confocal microscopy. Additionally, relevant gene expression studies were also carried out. We find that this adaptation strategy is conserved in different strains of *L. plantarum* isolated from different ecological niches. Furthermore, it appears that elevating metabolic activity and altering cell cytokinesis facilitate adaptation of *L. plantarum* cells to an acidic pH environment. Additionally, we find developmental transition of V-shaped structures into robust 3D-biofilm during acid stress adaptation process. Finally, we provide transcriptional evidence for reinforcing involvement of the metabolic and cell division machinery in adaptation of *L. plantarum* cells to acidic environment. We propose accordingly that the self-generated V-shaped structuring, regulated by altering metabolism and cell cytokinesis, facilitates the developmental process during acid stress adaptation of the probiotic *L. plantarum* cells.

Low pH responses in micro-organisms: sharing knowledge and community building

Daniela De Biase

Chair COST Action CA18113 "EuroMicropH" - Department of medico-surgical Science and Biotechnologies, Sapienza University of Rome, Italy

That low pH is an important cue that allows to sense environmental and intracellular changes is a concept on which the scientific community undoubtedly agrees. We learn from textbooks how important pH, as a key parameter, is in controlling the activity of enzymes, so to make (bio)chemical reaction compatible with life.

Aware of the centrality of low pH as an environmental cue for living organisms, the COST Action CA18113 (EuroMicropH), broad in its technical and scientific scope, aimed to gather a scientific community around the understanding and exploitation of the microbial responses of micro-organisms to low pH. These organisms include bacteria, yeasts, and other fungi: all are affected by a low extracellular pH and change their metabolism to adapt to it. While this is evolutionary relevant, because it ensures their survival, i.e. they will not succumb, it has also important implications on many human activities, in disparate sectors that traditionally do not communicate well with each other, such as microbiology of food and drink, industrial biotechnology and bio processing, clinical and veterinary treatment of infections, bioremediation of sites contaminated by the anthropogenic activities.

Our Action has created a new forum for communication and sharing of scientific expertise that turned out to be very beneficial to harmonize the scientific approaches and, importantly, to stimulate people to think more in a cross–disciplinary way, i.e. how some findings in one field could be exploited in other fields. In the last four years the Action worked to i) increase the understanding of the fundamental mechanisms that micro-organisms use to detect and respond to low pH ii) leverage the many different areas of expertise that exist across the Action members, iii) promote communication so that technical developments being made in one field could be used also by other fields by putting in direct contact different expertise, iv) ensure, through participation and dissemination, that these developments reach as a wide audience as possible, including pure and applied scientists in the Inclusiveness Target Countries, and finally v) increase the awareness of the next generation of scientists on the importance of low pH when understanding biological phenomena and exploiting the new knowledge.

Through my presentation, a look back, but most of all a look at what we have achieved, and will achieve, in this field and in our community will be offered.

POSTER PRESENTATIONS

POSTER #1: Antimicrobial activity of organic acids : application in livestock production

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Organic acids have long been used both for preserving foods and in livestock production for disease prevention or as growth-promoting feed additives. It has thus been demonstrated that organic acids have an antimicrobial activity which is pH dependentand commonly used for improving the quality of poultry feed. In this context, the present work evaluated the inhibitory effect of four organic acids, namely, acetic acid, citric acid, lactic acid, and tartaric acid, at different levels of contamination by *Salmonella typhimurium*. The neutralization of these organic acids in vitro and in the presence of one-day-old chick's organs was also investigated during the search for *Salmonella* serovars in birds. The effect of four organic acids on *Salmonella typhimurium* was tested in vitro and in the presence of chick's organs at different concentrations set of strain and organic acids tested. The MIC results demonstrated that tartaric acid, citric acid, and acetic acid inhibited *Salmonella typhimurium* at concentrations of 0.312%, 0.625%, and 1.25% for the three levels of strain: 10, 100, and 10^3 CFU/ml, respectively, while lactic acid and depending on the amount of the strain introduced acts differently: 0.088% for 10 CFU/ml and 0.176% for 100 and 10^3 CFU/ml. The concentration of 0.05M of Na_2HPO_4 solution has proved, in vitro, in caecums and organs of chicks (in presence of organic acids) that strain introduced, even at low concentrations, can be recovered. The use of additives has beneficial effects in *Salmonella* control program.

POSTER #2: Manganese uptake mediated by the NRAMP-type transporter MntH is required for acid tolerance in Listeria monocytogenes

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Listeria monocytogenes is a foodborne pathogen that can withstand mild stresses (e.g. cold, acid, salt) often encountered in food-related environments. From a collection of food and clinical *L. monocytogenes* isolates, we observed reduced survival at pH 2.3 and no growth at pH 4.9 (which supports the growth of most strains) in a strain designated 1381. In this work, we investigated the cause of inability to grow at low pH (acid intolerance) in strain 1381 by analysing reversion mutants that fully restored acid tolerance. Whole genome sequencing showed that a truncation in *mntH*, which encodes a putative NRAMP (Natural Resistance-Associated Macrophage Protein) type Mn^{2+} transporter, is responsible for the acid intolerance phenotype of strain 1381. However, the *mntH* truncation alone cannot explain the poor survival of strain 1381 at lethal pH. Further growth experiments demonstrated that Mn^{2+} (but not $Fe2^{+}$, Zn^{2+}, Cu^{2+}, Ca^{2+}, or Mg^{2+}) supplementation fully rescues the growth of strain 1381 under low pH conditions, suggesting that Mn^{2+} limitation is likely the cause of growth arrest. Moreover, the transcription of Mn^{2+} transporters (*mntH* and *mntB*) was induced by mild acid stress (pH 5). Taken together, these results provide evidence that MntH-mediated Mn^{2+} uptake is essential for the growth of *L. monocytogenes* under low pH conditions. Moreover, strain 1381 was recommended for conducting food challenge studies, the use of this strain in evaluating the growth potential of *L. monocytogenes* in low pH environments where Mn^{2+} is scarce should be reconsidered.

POSTER #3: Antimicrobial effect of gold nanoparticles modified polymethyl methacrylate denture base materials

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Introduction: The World Health Organization has identified antimicrobial resistance as one of the biggest problems facing humanity today. Preventing the development of microbial biofilm, resistant to conventional drugs, on the surface of prosthetic restorations made of polymethyl methacrylate (PMMA), represents a great therapeutic challenge. The aim of the study is to investigate the antimicrobial effect of PMMA modified by the addition of gold nanoparticles.

Material and methods. AuNPs sintetized by Ultrasonical spray pyrolisis were added to heat-polymerized denture base PMMA material in two different concentration (0,12 and 0,43 %) and PMMA and PMMA/AuNPs specimens were prepared according to test specification. Surface properties evaluation - roughness, surface free energy, hydrophobicity and contact angle were performed. Antimicrobial activity of newly formed PMMA/AuNPs were evaluated for three different microbial species (*Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*) in monomicrobial biofilm form. Colony forming units (CFUs), cell metabolic activity (MTT) and disc diffusion test for each tested strain were performed.

Results: The surface roughness, surface free energy increase in PMMA/AuNPs samples. There is no statistically differences between tested groups. Hydrophobicity and contact angle measurement shows differences compared to unmodified PMMA. PMMA/AuNPs showed a significant reduction in the monomicrobial biofilms of all tested species compare to control group and it is in direct correlation with percentage of added AuNPs. Released AuNPs did not recorded around the PMMA/AuNPs samples.

Conclusion: Incorporation of AuNPs into heat-polymerised denture base PMMA led to surface properties change and shows significant antimicrobial effect. Newly formed nanocomposite PMMA/AuNPs represent promising antimicrobial material.

POSTER #4: Interconnection between stress response and double-stranded RNA viruses in *Saccharomyces* yeasts

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Yeasts are widespread in natural habitats where they encounter competitors, especially in acidic conditions. *Saccharomyces* genus yeasts are widely used in genetic and microbiological research and employed in industrial processes. Yeasts have evolved to maintain the capacity to harbour double-stranded RNA viruses. These viruses can act as a system encoding killer toxin, than can be produced in yeasts and secreted into the growth medium. Maintenance of the killer toxin can be beneficial in acidic environment and can aid to outcompete other yeast strains that are sensitive to the toxin. Viruses and the yeast host closely interact to form a functional unit. Viruses can impact the host gene transcription, and transcription factors are also main regulators of cell response to stress. Environmental stress, including pH, can affect the toxin bioactivity, maintenance of double-stranded RNA (ds RNA) viruses, and toxin production.

In this work, we described the stress-response connection to dsRNA maintenance. We have analyzed RNA-seq data of several *Saccharomyces* strains that are dsRNA-free and have L-A dsRNA. Transcriptional responses to L-A maintenance were similar to those induced upon stress. Our work has identified different transcription factors, altering the expression of genes, asociated with dsRNA levels in yeasts. We examined the killing phenotype of *Saccharomyces paradoxus* killer strain that possess two types of dsRNA viruses L-A and satellite M. The killing properties of *S. paradoxus* killer against other yeast strains grown at acidic pH were described. The stability of killing phenotype in growth media with different pH levels were investigated.

POSTER #5: Impact of electromagnetic field on yeast *Saccharomyces cerevisiae* with potential applications in industry

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Nowadays, organisms face challenges from anthropogenic electromagnetic fields, the overall impact of which remains poorly understood. While research on electromagnetism primarily focuses on human health risks, evidence is emerging that electromagnetic fields could be another tool capable of influencing biological processes.

Saccharomyces cerevisiae yeast is extensively used in the food industry for ethanol production, fermentation, and bakery product manufacturing. However, the utilization of yeast in the industry has limitations concerning efficient process control. In our preliminary experiments, we focused on the effect of magnetic fields on the growth of *S. cerevisiae* with different exposure times (24-48 hours). We observed that electromagnetic fields either inhibited yeast growth by almost 30 % (in the absence of a magnetic field) or had no noticeable effect on growth (at a weak electromagnetic field of 0.15 mT), while stronger electromagnetic fields stimulated yeast growth by 5-30%. Additionally, we tested an immersed coil in the experiment, which could be directly inserted into the cultivation media and promote biomass production in a 3L industrial fermenter using electromagnetic fields ranging from 2.5 to 10 mT.

Our results indicate that the effect of the electromagnetic field depends on field parameters and exposure time. Furthermore, our findings suggest that the magnetic field, or its absence, not only impacts yeast growth but also holds the potential for controlling biotechnological processes in the food industry in the future. The work was supported by the Slovak Grant Agency (project No. VEGA 1/0018/2).

POSTER #6: Lactic acid modulates oxidative stress response to induce viable but nonculturable (VBNC) state in *Listeria innocua* challenged by nature-based antimicrobial formulation

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Using natural antimicrobials as "green" alternatives to synthetic sanitizers is of great promise to the industry. Nevertheless, individual efficacy of such compounds is insufficient for bacterial inactivation. We have found that combining sub-lethal concentrations of 8 mM natural polyphenol gallic acid with 1 mM GRAS hydrogen peroxide caused abundant generation of ROS and reduced the culturability of Gram-negative *Escherichia coli* by >8 log CFU mL^{-1} within 30 minutes. This effect was bactericidal according to the ATP-based viability assay. At the same time, this formulation had limited efficacy against Gram-positive *Listeria innocua* demonstrating 1 log CFU mL^{-1} count reduction with proportional 90% viability decrease. Amending the formulation with 20 mM of natural GRAS lactic acid did not change the ROS generation but achieved a complete growth inhibition of *L. innocua*. However, this proliferation arrest was accompanied by enhanced bacterial survival when 70-87% of the treated *L. innocua* cells maintained viability according to ATP-based and membrane integrity assays. Apparently, Lactic acid modulated the stress response of the ROS-challenged *L. innocua* cells resulting in viable but nonculturable (VBNC) state. Our current activities are concentrated on mechanistic investigation of this phenomenon by flow cytometry, testing membrane permeability, membrane potential, electron transport, redox stress, and efflux pump activity. The analysis indicates the predisposition of late stationary phase cells to VBNC development. Further transcriptomic analysis will unravel the molecular mechanisms of the microbial stress adaptation and allow their overcoming for developing efficient "green" sanitizers.

POSTER #7: Algal Biodiversity in High Altitude Blanket Bogs under Low pH Conditions

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Blanket bogs are peatland ecosystems found at high altitudes, characterized by unique environmental conditions and crucial roles in carbon sequestration and water regulation. These habitats are known to host diverse microbial communities, including algae, which contribute significantly to ecosystem productivity. However, the impact of low pH conditions on algal biodiversity in blanket bogs at high altitudes remains relatively understudied. Low pH conditions in high altitude blanket bogs result from various factors, including the accumulation of organic acids, atmospheric deposition, and mineral weathering. These acidic conditions pose challenges to algal communities, affecting their abundance, diversity, and composition.

Algal biodiversity in low pH blanket bogs at high altitudes comprises a range of taxonomic groups, including diatoms, green algae, cyanobacteria, and eukaryotic microalgae. These taxa exhibit distinct ecological strategies and physiological adaptations to overcome the constraints imposed by low pH conditions. For example, diatoms often dominate the algal community due to their high tolerance to acidic environments and their ability to withstand fluctuations in pH and nutrient availability. Here, we present our findings for the algal microorganisms living in the blanket bogs of Türkiye at high altitudes.

Understanding the responses of algal biodiversity to low pH conditions in high altitude blanket bogs is crucial for assessing the vulnerability of these ecosystems to environmental changes, such as acid rain deposition and climate change. Advancing our understanding of algal responses to low pH conditions will contribute to informed conservation strategies, restoration practices, and sustainable management approaches in high altitude peatland environments.

POSTER #8: Exploring the interactions between ScV-LA virus and host protein: insights from proteomics analysis and RNA sequencing

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Saccharomyces yeasts are widely distributed in the environment and microbiota of various organisms. Yeasts also are a profitable host as a protein factory. Notably, a killing phenotype has been discovered in yeast strains, rendering it a desirable trait in industrial applications. This biocidal feature enables effective control of spoilage microorganisms, ensuring the preservation of food products and beverages' quality. The yeast killer property is characterized by the production of killer toxins. These killer toxins, typically active in a pH range of around 3 to 6, exhibit their effectiveness across acidic conditions. The synthesis of killer toxins is often facilitated by two persistent Totiviridae viruses, LA and M, which work in conjunction within the host cell.

Our research focused on three isogenic strains of *Saccharomyces cerevisiae* M437, each harboring varying amounts of dsRNA viruses LA-lus and M2. Through equilibrium centrifugation and quantitative proteomics, we identified virus-associated host factors in selected fractions. Additionally, we conducted sequencing analysis to investigate the viral-linked cellular RNAs. Our findings were largely in agreement with transcriptomics data, revealing a moderate response of the host cell to the viral content.

Virus-linked subsets showed significant enrichment of major protein metabolism pathways, including ribosome biogenesis, folding, and proteasomal degradation. LA-lus virus was inherently associated with ribosomal RNAs and other essential RNAs for ribosome biogenesis. These findings highlight the close integration of the virus with vital host cell pathways, providing insight into the persistence of ScV-LA viral infections.

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POSTER #9: Mechanisms of survival of *Salmonella enterica Typhimurium* in response to environmentals

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Salmonella enterica Typhimurium is etiological agent in bacterial food-borne diseases, and are one of the most challenging bacteria for food manufacturers. As a result of its life cycle S. Typhimurium occupies and survives in wide ranges of host and non-host environmental niches in which it encounters numerous stressful factors. Exposed to acid pH, high osmolarity, temperature variations, toxic oxidative products etc), bacterial cells undergo various adaptations in the phenotypic and genotypic level.

In this study hyperosmotic stress induced changes in *S. enterica* Typhimurium SL1344 bacterial cells were analyzed at the level of gene expression. Subject of the study was the set of 369 genes directly or indirectly involved in regulatory mechanisms, and in virulence, and some unknown genes putatively involved in virulence or in regulatory mechanisms. PCR-based microarray technique was used for analysis of differences in gene expression profiles between bacterial cells in exponential growth phase (OD_{600}≈0.3) and cells subjected to hyperosmotic stress (0.56M NaCl - 10 minutes).

Performed transcriptome analysis did not indicate significant differences in genes expressions between bacterial cells subjected to hyperosmotic stress and cells in exponential growth phase. In response to hyperosmotic stress, higher ratio of expression (stress /control condition) was noted in genes involved in general stress response.

Expression of global stress response genes provided bacterial cells with ability to survive the forthcoming stress challenges, and made them broadly stress resistant by inducing expression of genes encoding proteins with a relatively general role in stress resistance.

POSTER #10: A statistical investigation on the association between EuroMicropH COST members' expertise and the areas of study.

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The COST Action CA18113 EuroMicropH aimed to foster collaboration and knowledge transfer on microbial responses to low pH. The goal of Working Group 2 (WG2) was to gather comprehensive information on members' expertise, to identify methods not covered by the network and to highlight methods which are needed but not yet developed, or methods needed in industrial sectors that can be transferred from academia. To achieve this goal, we have launched a survey to map the community's methods and expertise. The survey consisted of 25 questions across six categories, including personal details, field of activity, and methods and technologies employed by members. In collaboration with Working Group 1 (WG1), a statistical analysis was carried out using R to identify associations between the answers to different questions of the survey and to identify methodological gaps within the COST field. Prior to the statistical analysis, the survey data were processed for annotation standardization and redundancy elimination. Then the Fisher's exact test ($p \leq 0.05$) was applied to determine whether there were non-random associations between the answers of two questions of the survey (dealt as categorical variables).

A total of 107 members participated in the survey. The results revealed seven statistically significant associations out of 264 tested cases. It is important here to remind that EuroMicropH reflects European and Middle-Eastern interests in low pH microbial research. Findings indicated regional preferences in research fields among EuroMicropH members based on their countries of origin. The survey revealed connections between research fields, evaluated organisms, and fermentation methods. Bacteria were commonly assessed for food safety and bioreactions. Associations were found between evaluated organisms and industrial processes, highlighting underutilization of non-bacterial microorganisms in industry. A link between organisms and host-pathogen interactions, especially human pathogens, was noted. Core research fields include food safety, basic research, environment, agriculture, biotechnology, and medical/healthcare, focusing on bacteria, fungi, LAB, and food pathogens. Fermentations showed associations with microorganisms, suggesting their impact on microbial chemical production warrants further exploration. While bacteria dominate, limited involvement in our Action of experts in Archea, Microalgae, and Protozoa raises questions

about interest and expertise sharing. The correlation between evaluated organisms and industrial processes emphasizes the need to explore non-bacterial microorganisms like fungi and algae in various industrial applications.

Overall, this analysis underscores the ability of EuroMicropH to unite members from different countries and different fields of study offering collaborative research and knowledge exchange opportunities, especially in the realm of fermentations and practical applications. More members are encouraged to respond to the WG2 survey (https://forms.gle/PhseVUD6ydgq5doz7) to strengthen our analysis and make even more impactful the cross-feeding of different sectors and fields of study. The database of WG1 (https://cost-euromicroph.web.app/) is another useful tool for knowledge sharing.

POSTER #11: An effective method for isolating high lactic acid producer bacteria strains

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Lactic acid bacteria (LAB) strains are widely used for many kinds of lacto-fermented foods such as milks, yogurts, meats, sourdough bread, olives, kimchi, and pickled vegetables. Silage is an another lacto-fermented products for feeding for ruminants. LAB inoculation to such food and feed has generally positive effects on their fermentation profile. However, procedure of isolating and finding out successful LAB strain from natural sources is time consuming and inconvenient.

Fermented silage feed may be a good natural source for isolating and selecting successful LAB isolates.

The objective of this study is comparing sources for selecting high lactic acid producer bacteria strains by using their natural ecology and fermented silage feeds as main source.

Poster #12: Lactic acid and probiotic biomass production on waste substrates from agri-food industry

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Lactic acid (LA) is a platform chemical dominantly produced by microorganisms. LA is precursor of poly-lactides, advantageous polymers used in production of stents and grafts for medical applications. Also, it is preservative, acidulant and flavouring agent in food industry or in bulk, it is used as a cleaning agent, e.g. in marine industry. Market for lactic acid is steadily growing and it is challenging current bioprocesses for higher yields on more sustainable and affordable substrates.

Lactic acid bacteria, including former *Lactobacillus* sp. genus, were among the most often exploited microorganisms in fermentative lactic acid production on agri-food wastes - due to their high LA productivity on range of substrates, GRAS status and tolerance to lower pH. It is possible to achieve high LA and microbial biomass yield with many of these microorganisms and some also have probiotic properties. Therefore, residues after the fermentation on agri-food residues can be valorised as feed with added value.

This work will report on different techniques and strategies employed to decrease negative effects of low pH and other stressors often present in fermentations on waste substrates with aim to valorise both produced LA and biomass. Open fermentation and challenges related to media sterilization, immobilization and adaptation will be particularly highlighted.

Poster #13: Low base-substitution mutation rate and predominance of insertion-deletion events in the acidophilic bacterium *Acidobacterium capsulatum*

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Analyses of spontaneous mutation have shown that total genome-wide mutation rates are quantitatively similar for most prokaryotic organisms. However, this view is mainly based on organisms that grow best around neutral pH values (6.0–8.0).

In particular, the whole-genome mutation rate has not been determined for an acidophilic organism. Here, we have determined the genome-wide rate of spontaneous mutation in the acidophilic *Acidobacterium capsulatum* using a direct and unbiased method: a mutation-accumulation experiment followed by whole-genome sequencing. Evaluation of 69 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 4×10^{-4} per genome per generation, which is significantly lower than the consensus value ($2.5–4.6 \times 10^{-3}$) of mesothermophilic (~15–40°C) and neutrophilic (pH 6–8) prokaryotic organisms. However, the insertion-deletion rate (0.43×10^{-10} per site per generation) 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. Because selection operates on the total mutation rate, it is suggested that the relatively high insertion-deletion rate may be balanced by a low base-substitution rate in *A. capsulatum*, with selection operating on the total mutation rate.

Poster #14: The penetration of PPA is mediated with H^+efflux in Gram-negative and Gram-positive bacteria

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Organic acids such as short-chain fatty acids have a cytotoxic effect on bacteria and can be entered into the cell by the possible release of H^+. The impact of propionic acid (PPA) on the bacteria H^+flux (JH^+) depending on various concentrations of PPA (11.7 and 33.4 mM) was investigated in gram-negative *Escherichia coli* K12 and gram-positive *Enterococcus hirae* ATCC9790 at pH 7.5. Changes in JH^+in the external medium were registered using a selective pH electrode.

The $JH^+$was ~1.86 and ~1.63 mmol min^{-1} during glucose utilization in *E. coli* and *E. hirae*, accordingly. $JH^+$was ~0.24 mmol min-1 when PPA was added with 11.7 mM in both *E. coli* and *E. hirae*. Meanwhile, 33.4 mM PPA increased JH^+~0.32 mmol min^{-1} and was unchanged in *E. hirae*. Suggested that PPA penetrated into the cells in its undissociated form, which dissociated in the cytoplasm increasing the intracellular level of H^+. As a result, the transmembrane pH gradient could be dissipated affecting the proton motive force. As the pKa of PPA is 4.86-4.87 and its dissociation is higher at a slightly alkaline pH 7.5, the extracellular H^+ level is higher. Thus, the addition of PPA decreases the proton efflux from the cell, compared to glucose utilization. Taken together, in the presence of PPA bacterial cells try to regulate ΔpH and H^+concentration gradient to save viability by releasing H^+ to the external environment. Moreover, the penetration of PPA might be an antiporter with H^+ efflux, regulating pH gradient.

Poster 15: The influence of pH on malolactic fermentation dynamics of red wines from Fetească neagră grapes

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Malolactic fermentation (MLF) takes place immediately after the alcoholic fermentation and represent a key stage in red winemaking, because leads, therefore, to profound changes in the composition and quality of red wines.

Made under the action of lactic acid bacteria, of which the majority species is *Oenococcus oeni*, the process mainly consists in the transformation of malic acid into lactic acid and carbon dioxide. This transformation, accompanied by a more or less important metabolism of citric acid, leads to a decrease in total acidity and a slight increase in the volatile acidity of wines. At the same time, there are important changes in their aromatic and taste characteristics.

The main objective of the present study was to investigate the effect of pH on malolactic fermentation (MLF) in red wines (adjusted to pH 3.0, 3.2, 3.4, 3.6 and 3.8, respectively) from Fetească neagră grapes. The wines were inoculated with *Oenococcus oeni* commercially available starter culture. The inoculation (about 10^6 CFU/mL) of diffferent bacteria in wines, was carried out at the end of the alcoholic fermentation after racking. Inoculation was performed according to the method of rehydrating active dry bacteria included in producer protocol. The wines were evaluated by analysing the evolution of *L*-malic, *L*-lactic, citric and acetic acids during MLF by using enzymatic methods. The alcohol content, pH, free and total sulphur dioxide, reducing sugar, total and volatile acidity of the wines were performed according to the OIV methods. Colour of red wine samples was measured before and after MLF, using a tri-stimulus colorimeter.

The results revealed that the duration of MLF was influenced by pH and LAB. The time required to complete MLF in wines from red Fetească neagră grapes was completed within 30 days and was highly depending on the value of pH in wine. MLF started after 18 days, at pH = 3.0, after 15 days, at pH = 3.2, after 12 days, at pH = 3.4 and after 9 days, at pH = 3.6.

The results demonstrate that, throughout the elaboration of the wine, the pH represents a selective factor on the starter culture of bacteria according to acid resistance, orients their metabolism and determines, above all, their rate of multiplication.

Poster 16: The Influence of soil pH on nematodes

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The pH of soil may affect the nutrient content in the soil, thereby influencing plant growth and development. Several researchers have indicated that the ideal pH for plants ranges from 5.5 to 6.5. In addition to plants, soil pH may also influence the activity, presence, and abundance of microorganisms in the soil. As microscopic organisms, nematodes can live in soil as entomopathogenic, free-living organisms, or plant parasites. Nematodes can be found in various locations worldwide, including freshwater, oceans, land areas, forests, and mountains. The ability of nematodes to inhabit different environments suggests their exposure to different pH conditions. This situation indicates that nematodes may exhibit adaptation and/or tolerance to different pH conditions. This work aims to review the relationship between nematodes and pH by compiling studies conducted by various researchers on the effects of soil pH on nematodes. Previous studies have shown that nematodes interact with soil pH and that pH can influence nematodes. Some earlier studies done by researchers has demonstrated that certain nematode species may exhibit a higher survival range in ammonium-acetate pH compared to other nematode species. These nematode-pH interactions, which are believed to shed light on future studies, can be utilized in new nematode control strategies. Investigating the molecular and genetic basis of pH tolerance differences among nematode species in future studies is crucial for understanding the nematode-pH relationship.

Poster 17: Dip wash treatments with organic acids and acidic electrolyzed water combined with UV-C treatment to improve the shelf life of some fresh fruits

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The objective of our studies was to investigate the effects of post-harvest treatments with organic acids (citric, sorbic, benzoic, ascorbic) and acid-electrolyzed water, alone or in combination with UV-C irradiation, on the physicochemical, biochemical and microbiological changes of fresh blueberries and strawberries during refrigerated storage. Weight loss, firmness, soluble solids content, titratable acidity, total phenolics content, total anthocyanins content, antioxidant activity, decay incidence, and surface microbial load of control and treated fruits were investigated during cold storage. Dip wash treatments of fresh strawberries with 2% citric acid, 0.2% benzoic acid, 0.2% sorbic acid, and acidic electrolyzed water delayed the physiological collapse of fruits during storage, slowing down the water losses. Dipping strawberries in 0.2% sorbic acid or 0.2% benzoic acid aqueous solutions were the most effective treatments for maintaining firmness and phytochemical content and for delaying the decay of strawberries during cold storage. Dip wash treatment with organic acids followed by UV-C irradiation was significantly more effective than UV-C treatment alone in reducing fruit decay and weight loss and in maintaining at higher levels titratable acidity, total anthocyanins content, total phenolic content, and antioxidant activity of strawberries during refrigerated storage. On blueberries, the chemical treatments significantly reduced the microbial growth on the fruit surface 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.