Campylobacter as an analytical target to improve food safety

BOOK OF ABSTRACTS

14 FEBRUARY 2023
Biotechnical Faculty

SYMPOSIUM

Campylobacter as an analytical target to improve food safety

ORGANISER: Anja Klančnik Department of Food Science and Technology
Biotechnical Faculty, University of Ljubljana

VENUE: Biotechnical Faculty / The Dep. of Food Science and Technology
Hall DJH / JAMNIKARJEVA 101, LJUBLJANA

EDITOR: Dr. Anja Klančnik

TECHNICAL EDITOR: Dr. Anja Klančnik

ISSUED BY: Biotechnical Faculty, University of Ljubljana

YEAR OF ISSUE: 2023

ONLINE EDITION: https://repozitorij.uni-lj.si/IzpisGradiva.php?id=146295

LJUBLJANA, 14 FEBRUARY 2023

CIP - KATALOŽNI ZAPIS O PUBLIKACIJI
NARODNA IN UNIVERZITETNA KNJIŽNICA, LJUBLJANA

KATALOŽNI ZAPIS O PUBLIKACIJI (CIP) PRIPRAVILI
V NARODNI IN UNIVERZITETNI KNJIŽNICI V LJUBLJANI
COBISS.SI-ID 153158915 ISBN 978-961-6379-77-9 (PDF)

PROGRAMME

14 FEBRUARY 2023

8:45 - 9:00 Sonja SMOLE MOŽINA: Campylobacter: old problem and novel approaches

9:00-9:15 Nicol JANECKO: Deep dive into MAGs: Can we rely on metagenomics to characterise bacterial genomes?

9:15-9:30 Ozan GUNDOGDU: Campylobacter pathogenesis and the chicken microbiome

9:30-9:45 Blaž STRES: Systems view of microbiome = The whole > the sum of its parts

9:45-10:00 Mojca MILAVEC: Quantitative analysis support of nucleic acids in health and food safety

10:00-10:15 Majda GOLOB: Veterinary microbiology: seeing the forest beyond the trees

Break and snack

11:00-11:15 Bilal DJEGHOUT: Is one colony enough: a genomic perspective on Campylobacter diversity in patients

11:15-11:30 Katarina ŠIMUNOVIĆ: Interactions between Campylobacter jejuni isolates resemble kin-discrimination like behaviour

11:30-11:45 Manca KOVAČ VIRŠEK: Microplastics as a carrier of bacterial communities

11:45-12:00 Anja KLANČNIK: Campylobacter as (good?) model

PROGRAMME

15 FEBRUARY 2023

9:00-12:00 Workshop on (meta)genome and biofilm practical session

9:00-10:00 Bilal DJEGHOUT: DNA sequencing of strains (Campylobacter as model pathogen)

10:00-11:00 Bilal DJEGHOUT: Metagenomes (WGS) – Sample processing to genomic interpretation

11:00-12:00 Manca Volk, Blaž Jug, Živa Kolenc: Laboratory research on biofilm

16 FEBRUARY 2023

9:00-12:00 Workshop protein, RNA and microbiome practical session

9:00-10:00 Blaž JUG: Protein profile of Campylobacter jejuni in different biofilm communities

10:00-11:00 Manca VOLK: Temporal transcriptomic analysis of Campylobacter jejuni biofilms

11:00-12:00 Živa KOLENC: Isolation of microplastics from poultry feces

INTRODUCTION

In this symposium, we will use the pathogenic bacterium Campylobacter as the leading pathogenic bacterium, as reported by EFSA. Dr. Sonja Smole Možina from the Biotechnical Faculty will present the problem of food safety and Dr. Nicol Janecko from Quadram Institute Bioscience will present the molecular methodology of methagenomics that could improve its research at multiple levels.

Dr. Gundogdu from the London School of Hygiene & Tropical Medicine and Dr. Majda Golob from the Faculty of Veterinary will make the case that we need to know what is happening at the farm and chicken microbiome level and their pathogenesis. Dr. Bilal Djeghout of the Quadram Institute Bioscience and dr. Katarina Šimunović from Biotechnical Faculty will present the characteristics of their strains, the mechanisms at the genomic level, and their interactions in complex forms such as the biofilm.

During this symposium, Dr. Mojca Milavec from National Institute of Biology will present new microbiological methods for quantitative analysis of nucleic acids. Dr. Blaž Stres from National Institute of Chemistry will address the systems approach to microbiome analysis and Dr. Manca Kovač Viršek from Institute for Water of the Republic of Slovenia will address the bacterial community that can change very rapidly when microplastics is involved as a potential microbial transport vector in the environment.

The research work on Campylobacter was funded under Slovenian Research Agency:

- research national projects J4-3088, J4-4548, J4-2542, J4-4550, MR 52659;
- international projects BI-IT-18-20-006, BI-US / 19-21-105, BI-US / 22-24-073;
- research national program P4-0116.

I would like to thank the Chair of Biotechnology, Microbiology and Food Safety for its contribution, especially Prof. Sonja Smole Možina and the young researchers Živa Kolenc, Blaž Jug and Manca Volk.

I would like to thank the Biotechnical Faculty and our department The Dep. of Food Science and Technology for their hospitality.

Dr. Anja Klančnik
Organizer

Campylobacter: OLD PROBLEM AND NOVEL APPROACHES

Sonja Smole Možina, Anja Klančnik

Chair of Biotechnology, Microbiology and Food Safety, Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Campylobacter has been the most frequently reported food-borne pathogen in the EU since 2005, causing human infections with costs exceeding 2.4 billion €/year (EFSA, 2022). With establishing a young research group at BF 20 years ago, we have introduced research work on pathogenic Campylobacter jejuni/coli transmission in the extra-intestinal environment – first its detection, identification and molecular typing methods to explore its fascinating diversity in the food chain and wider environment, following by studies of Campylobacter stress response and subsequent increasing resistance to environmental stresses like oxygen or starvation, with the impact on its survival and virulence. We continued with Campylobacter antibiotic resistance studies, mainly on ciprofloxacin-resistant C. jejuni prevalence and mechanisms of spreading, and possible solutions, such as efflux pump inhibitors involved in resistance modulation. The increasing prevalence and antimicrobial resistance are calling for novel alternative treatment options.

Our recent objective is to investigate novel biocontrol approaches for C. jejuni reduction, mainly in mixed biofilms as their important protective environment. Plant-based preparations targeting inter-cellular signalling, motility, adhesion and biofilm formation and persistence as well as inter-strain and inter-species microbial interactions are promising targets for control of this important pathogen in food safety management.

National and international research projects in the field of food safety (J4-2542, J4-3088, J4-4548, J4-4450, MR 138020, P4-0116, BI-USA-SLO, BioProMedFood) currently support this work and are acknowledged.

Campylobacter: OLD PROBLEM AND NOVEL APPROACHES

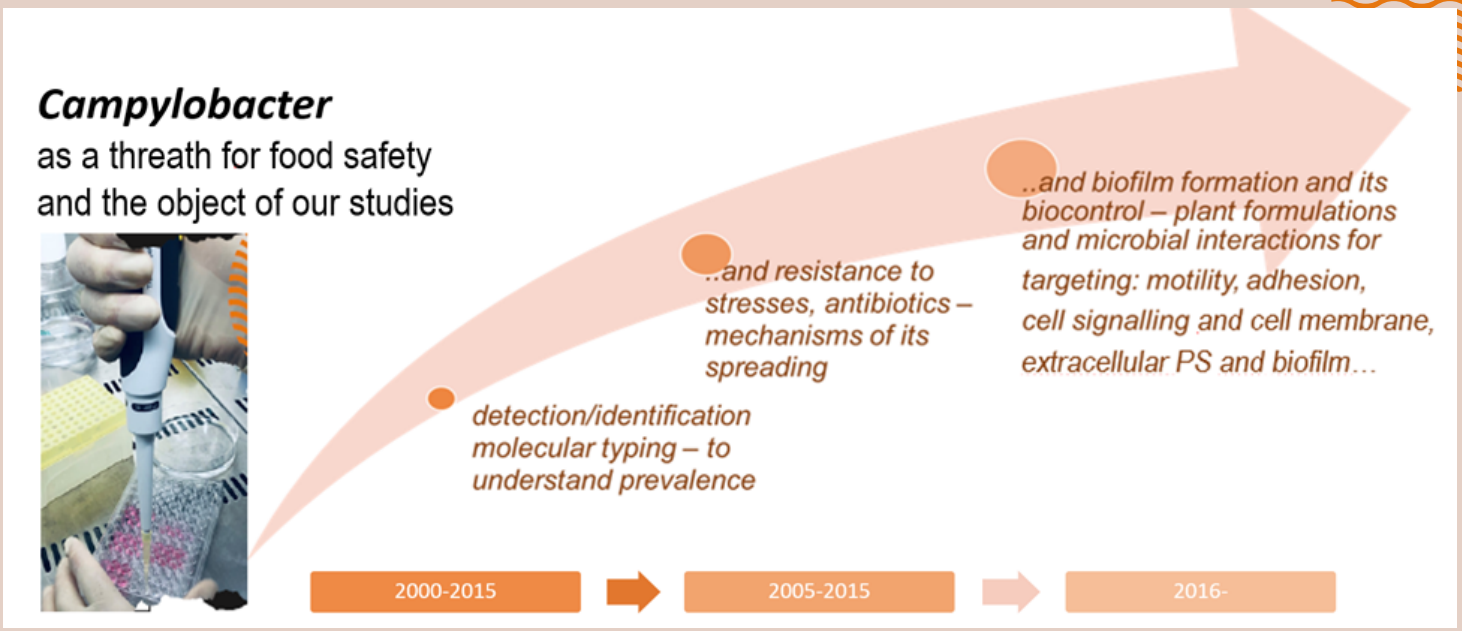


Fig. 1. Graphical presentation of the key steps in development of methodological approaches targeting pathogenic Campylobacter spp. at population, cellular and molecular level in the last 20 years in the Group for Food Microbiology at Biotechnical Faculty, Department of Food Science and Technology.

References:
https://cris.cobiss.net/ecris/si/sl/researcher/5981
https://cris.cobiss.net/ecris/si/sl/researcher/15503

DEEP DIVE INTO MAGs:CAN WE RELY ON METAGENOMICS TO CHARACTERISE BACTERIAL GENOMES?

Nicol Janecko

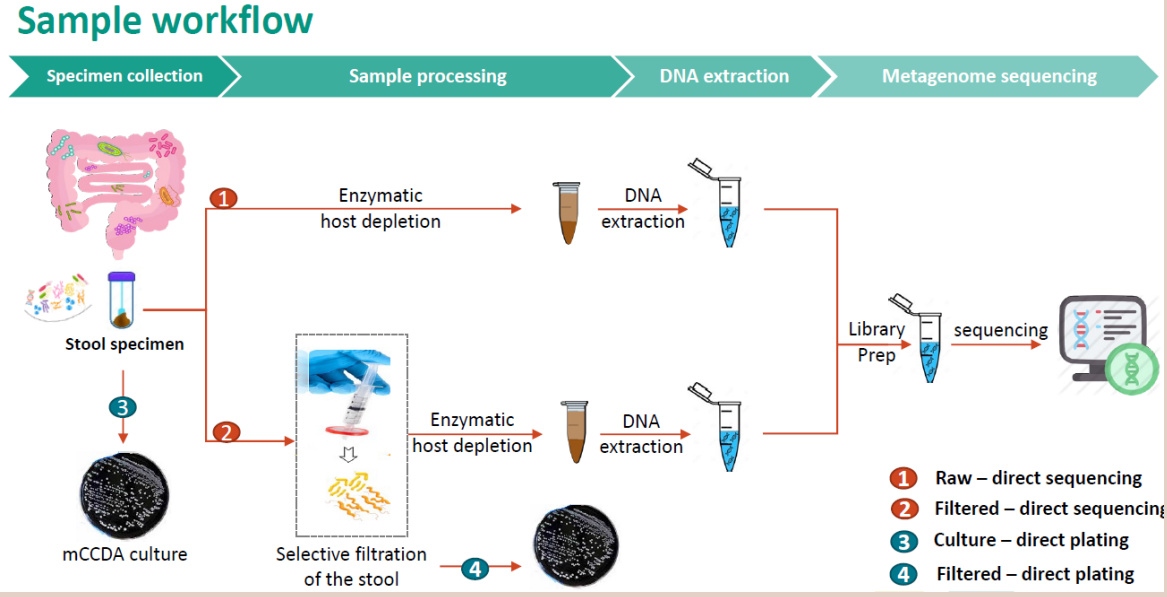
Quadram Institute Bioscience, Norwich, United Kingdom

Campylobacter continues to be the leading cause of bacterial gastrointestinal illness worldwide. There are over 40 different species to date, with more emerging. Due to the fastidious nature of this pathogen, culture-based testing has limits. Metagenomic analysis has in recent years has identify mixed community populations to the phylum, family and in some cases genus level, however, challenges remain to characterise low abundant strain level genomic traits.

The aim of our projects has been to develop and optimise methodological workflows from sample processing to informatic analysis to characterise key traits in Campylobacter genomes through targeted metagenomics.

We validated cultured Campylobacter against targeted metagenomics using short read paired-end sequencing an long read sequencing platforms by characterising key traits. Precision varied depending on the platform and processing techniques used, however Campylobacter species, antimicrobial resistance determinants and sequence type were attained by direct metagenome analysis to match culture genomes.

A specialised sample preparation method, sequencing and streamlined informatic approach offers a strain level characterisation of Campylobacter thereby enabling clinical treatment option and surveillance efforts for this fastidious microorganism.



Campylobacter PATHOGENESIS AND THE CHICKEN MICROBIOME

Ozan Gundogdu

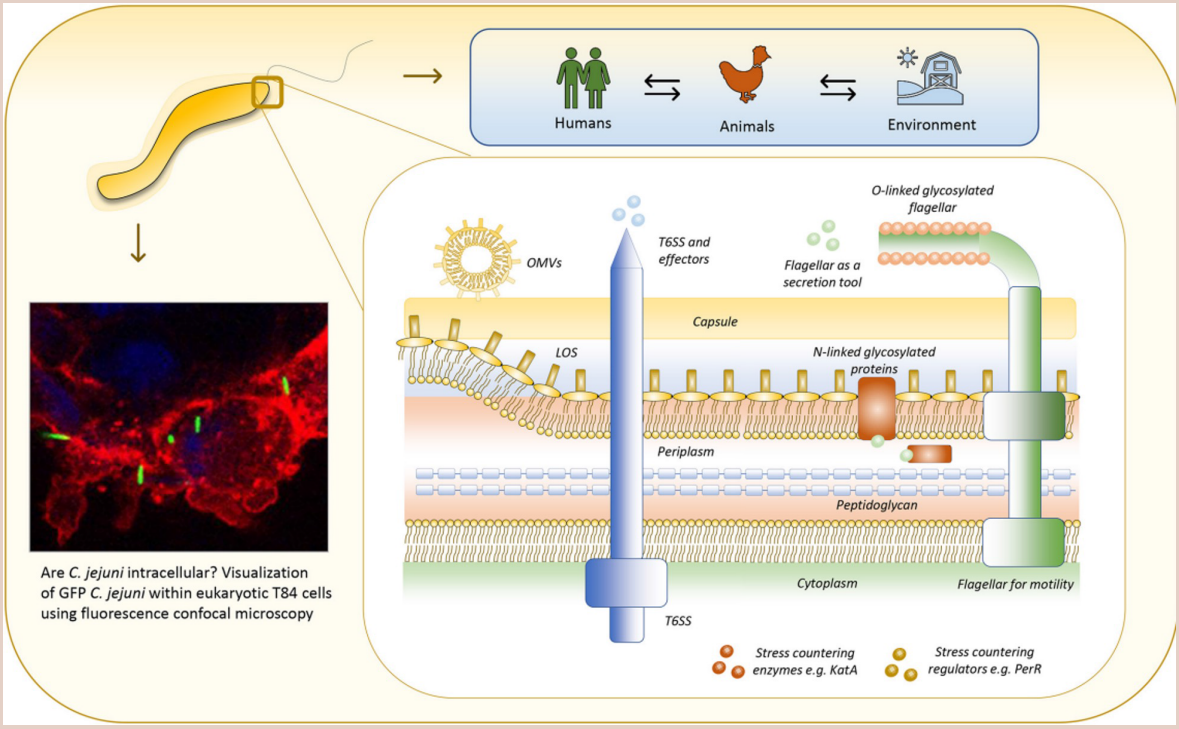
London School of Hygiene & Tropical Medicine; Faculty of Infectious & Tropical Diseases

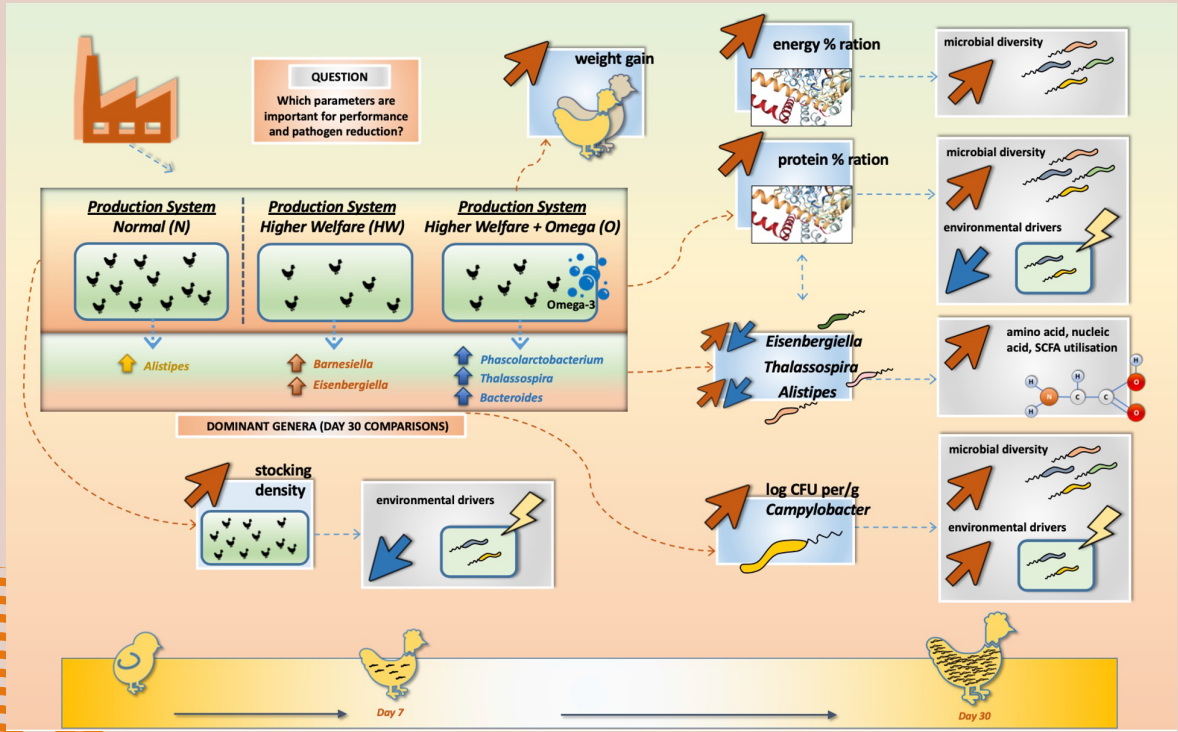
Campylobacter is the most common global cause of human gastroenteritis, with the species Campylobacter jejuni accounting for over 80% of human infections. Despite this, the molecular drivers of C. jejuni disease are still poorly understood, complicated by the lack of adequate infection models. In addition, the increasing frequency of infection and the relentless rise of antimicrobial resistance mean that development of new interventions to reduce Campylobacter in the food chain is a global imperative. Here we present a holistic approach to combat C. jejuni using classical molecular microbiology and omics-based approaches to investigate the physiology and pathogenesis of C. jejuni and translate this knowledge to real life settings i.e., implement control strategies.

We demonstrate the presence of complete Type VI Secretion System (T6SS) operons in several C. jejuni strains and species and provide evidence of its role in pathogenesis conferring C. jejuni a competitive advantage within the host. We investigate how C. jejuni interacts with the intrinsic defence machinery of human intestinal epithelial cells (IECs), differentially regulating intracellular and extracellular ROS production in human IECs.

C. jejuni are distributed in most warm-blooded animals, and therefore the main route of transmission is generally foodborne, via the consumption and handling of meat products (particularly poultry). Using our fundamental knowledge of C. jejuni we investigate how and when C. jejuni appears within poultry via a comprehensive day to day microbiome analysis of the chicken ceca. We also investigate external factors from industrial farms on host-pathogen ecology of the microbiome and C. jejuni. Our results show that microbial communities in different industrial production systems are deterministic in elucidating the underlying biological confounders, and these recommendations are transferable to farm practices and diet manipulation leading to improved intervention strategies against C. jejuni within the food chain.

Campylobacter PATHOGENESIS AND THE CHICKEN MICROBIOME





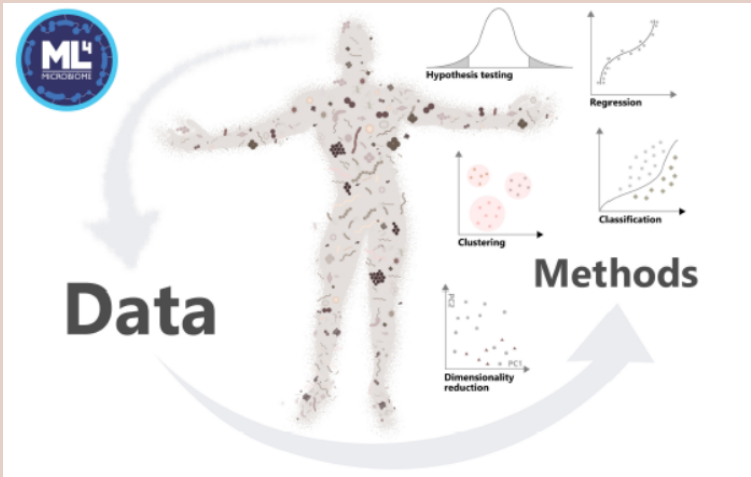
SYSTEMS VIEW OF MICROBIOME = THE WHOLE > THE SUM OF ITS PARTS

Blaž STRES

National Institute of Chemistry, Ljubljana, Slovenia

We are surrounded by complex systems consisting of various individual components that exhibit their own properties and interact with each other nonlinearly through space and time. Due to the complexity of the interactions, these interactions result is emergence of novel properties that none of the individual components had before. One such property, for example, is life. Each piece of information changes over time due to imperfect replication, and is also subject to selection based on environmental properties and interactions with other microbes and hosts. The observed changes through space and time yield the complex data on microbiomes that lead us to iterrogate large data sets using advanced multivariate statistics and machine learning to identify biomarkers by which the properties of our observed systems can be reliably distinguished from other types of the system (e.g. sick - healthy) and to connect them into dynamic models of systems that can be used for assessment and prediction future states, for rapid classification of new samples, and understanding the interdependence of a multitude of microbes that respond to specific environmental parameters. Such approaches have contributed crucially to important breakthroughs in various disciplines in the last decade, from pharmacy, ecology, nutrition, medicine, biology, microbiology, biochemistry: from sources of drinking water, food production, animal husbandry, to sewage treatment plants, reactor technology, and animal and human syndromes and diseases, communicable and non-communicable.

Today, we are talking about the analysis of microbiomes with 'omic technologies and combining mega data with evironmental information, which allows us to discover the complexity of their functioning across different size and time scales. And this is again a new feature of the complex system, which none of the components had before.



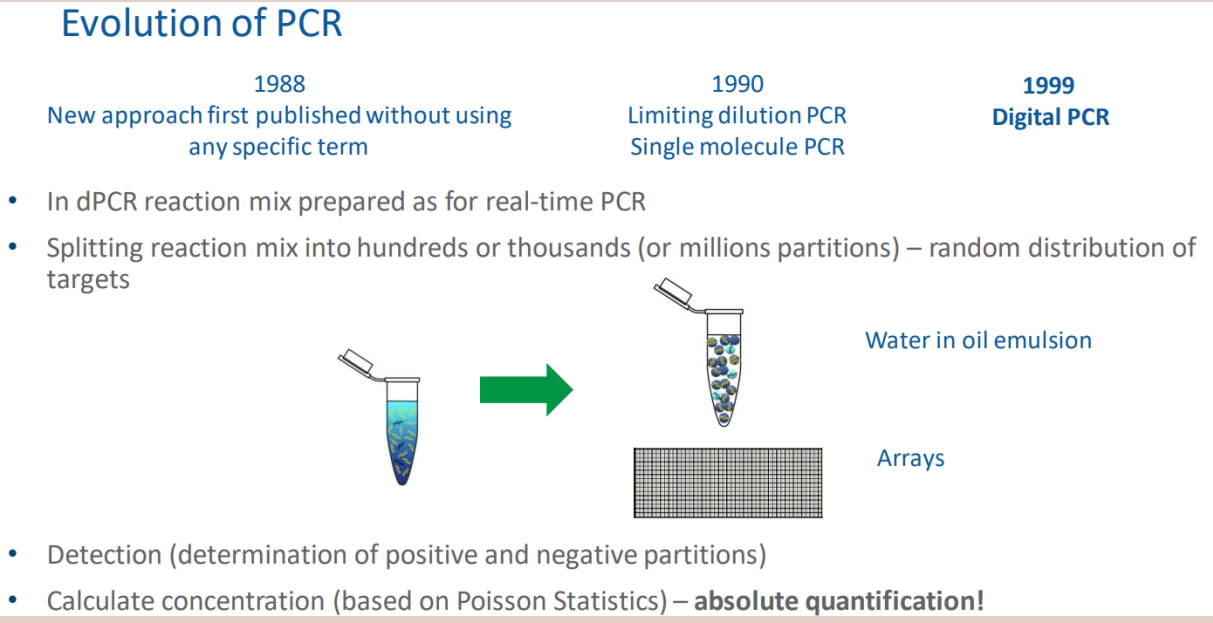
Statistical and Machine Learning Techniques in Human Microbiome Studies: Contemporary Challenges and Solutions10.3389/fmicb.2021.635781

QUANTITATIVE ANALYSIS SUPPORT OF NUCLEIC ACIDS IN HEALTH AND FOOD SAFETY

Mojca MILAVEC

National Institute of Biology, Večna pot 111, 1000 Ljubljana

Nucleic acid analysis is one of the most important measurements in various fields of basic life sciences, including molecular and cellular biology, genetics, and microbiology, as well as in biotechnology, food safety, medicine, veterinary medicine, and environmental monitoring. Nucleic acid analysis enables detection, identification and quantification of DNA and RNA from different organisms in diverse backgrounds. Accurate, precise, and comparable measurements are critical for providing reliable results not only in basic research, but also to support decision makers such as inspectors and competent authorities. In recent years, significant progress has been made in quantification of DNA and RNA in terms of absolute copy number concentrations (e.g., viral and bacterial counts) or ratios (e.g., % of genetically modified organism in a product and mutation/wild type). Advances in nucleic acid quantification will be presented with examples from national and international research in support of nucleic acid measurements.



VETERINARY MICROBIOLOGY: SEEING THE FOREST BEYOND THE TREES

Majda GOLOB, Bojan PAPIĆ, Jana AVBERŠEK, Darja KUŠAR

University of Ljubljana, Veterinary Faculty, National Veterinary Institute, Institute of Microbiology and Parasitology, Department of Bacteriology and Mycology, Gerbičeva 60, Ljubljana, Slovenia

Contact: majda.golob@vf.uni-lj.si, bojan.papic@vf.uni-lj.si, jana.avbersek@vf.uni-lj.si, darja.kusar@vf.uni-lj.si

Overview of IMP. The Institute of Microbiology and Parasitology (IMP) is part of the Veterinary Faculty (VF) and also the National Veterinary Institute (NVI), which is a special internal organizational unit that performs tasks of public veterinary services. In total, 35 national reference laboratories (NRLs) are operating under NVI, of which 18 NRLs for various infectious diseases are located at IMP. VF is accredited by the Slovenian Accreditation in the field of testing (SIST EN ISO/IEC 17025). IMP is divided into three departments: Department of Bacteriology and Mycology, Department of Virology, and Department of Parasitology. Our laboratories have state-of-the-art technical equipment enabling us to perform more than 280 microbiological methods. Through bacteriological, mycological, virological, parasitological, serological, and molecular investigations, the institute provides diagnostics of most contagious animal diseases under regulatory control as well as diagnostics of other economically important diseases of individual animal species. In addition to investigations for the needs of the state or official controls, it also carries out investigations for various clients (e.g. veterinary practices, animal owners, livestock breeders, processing and pharmaceutical industries).

Research at IMP. Researchers at IMP are involved in the national research programme Animal Health, Environment and Food Safety (P4-0092), which follows the concept of 'One Health' and aims to introduce new, rapid, comprehensive diagnostic approaches and to expand the usefulness of modern molecular-epidemiological methods for identifying pathogens to ensure animal health and well-being, expedient use of medication, and reducing economic damage. In addition, the institute is involved in several core projects, targeted research programmes and projects in the framework of bilateral and multilateral cooperation.

VETERINARY MICROBIOLOGY: SEEING THE FOREST BEYOND THE TREES

Identification and typing of bacterial pathogens at IMP. We perform identification of bacterial pathogens by conventional and molecular methods: (i) general bacteriological examination (e.g. skin, mucous membranes, urine and feces), (ii) targeted bacteriological tests (e.g. Salmonella spp., campylobacters, Listeria monocytogenes, Shiga toxin-producing E. coli [STEC], methicillin-resistant staphylococci, Taylorella equigenitalis causing contagious equine metritis [CEM], detection of bacterial pathogens of bees and fish), (iii) mycological examinations, including dermatophytes, aspergilli and yeasts, (iv) tests for BSL-3 priority pathogens (e.g. anthrax, brucellosis, tuberculosis and tularemia), and (v) molecular diagnostic tests (conventional PCR, real-time PCR [qPCR], digital PCR in singleplex or multiplex formats; metagenomic sequencing) and typing (including whole-genome sequencing [WGS]). An important part is dedicated also to determination of antimicrobial resistance (AMR), both by conventional methods and WGS.

Our institute has large strain collections of Salmonella spp., Campylobacter spp., Helicobacter spp., Listeria monocytogenes, enterococci (e.g. E. faecalis and E. faecium), MRSA, methicillin-resistant Staphylococcus pseudintermedius (MRSP), Escherichia coli including multidrug resistant (MDR) strains, Paenibacillus larvae, Mycobacterium spp. and others.

AMR monitoring. Resistant bacteria from the food producing animals and/or food of animal origin represent reservoirs of antimicrobial resistance genes (ARGs), which can be transferred to humans through the food chain. Moreover, the food-producing animals and the retail meat might be important vehicles for community dissemination of MDR bacteria.

In Slovenia, AMR in bacteria from food-producing animals and food of animal origin has been regularly monitored for more than 20 years as part of the National control program of monitoring zoonoses and zoonotic agents. Salmonella spp., Campylobacter jejuni and indicator commensal bacteria E. coli were included in the national AMR testing. In 2012, the European Food Safety Authority (EFSA) published scientific reports on technical specifications regarding harmonized monitoring and reporting of AMR for Salmonella, Campylobacter, indicator commensal E. coli and Enterococcus transmitted through food as well as MRSA and E. coli with transferable genes encoding extended-spectrum beta-lactamases (ESBL or AmpC) and carbapenemases (E. coli CP) in food-producing animals and food.

VETERINARY MICROBIOLOGY: SEEING THE FOREST ABOVE THE TREES

Slovenia is included in the harmonized AMR monitoring since 2013 as a EU member state, and reports Salmonella spp., C. jejuni and C. coli, indicator commensal E. coli, E. faecalis and E. faecium, and specific monitoring of ESBL/AmpC and E. coli CP. Our institute operates the NRL for AMR, which is a part of the EU Reference Laboratory for antimicrobial resistance (EURL-AR) network.

WGS. In addition to comprehensive isolate characterization, outbreak investigation and investigation of transmission routes has been greatly improved by whole-genome sequencing (WGS) of microbial pathogens. Another important application of next-generation sequencing (NGS) also includes metagenomics. Since IMP has a contract with the Ministry of Defense of the Republic of Slovenia for providing professional advice and taking measures in the event of a biological attack, our team participated in the world-wide inter-laboratory control to review the current state of countries' preparedness for the detection of bioterrorist agents using NGS. For the purpose, the participating laboratories could use any bioinformatic tools and their own professional judgment. The test involved public health, veterinary and general microbiology laboratories, and laboratories specializing in biological defense. A total of 60 laboratories participated, and our laboratory performed best with a success rate of 94.3 %. The results show how studying many trees in the forest, despite being challenging, brings many advantages in the sense of seeing things clearly from a broader perspective, but also keeping in mind the details.

In the WGS-based epidemiological surveillance of bacterial pathogens and our research projects, we are mostly focused on L. monocytogenes, Salmonella enterica (serovar Infantis), STEC, honeybee pathogen P. larvae, Clostridioides difficile and Helicobacter/Campylobacter spp. (determination of novel species). For example, we described the population structure of L. monocytogenes in the natural environment and in animal clinical cases. We showed that **L. monocytogenes** clones (clonal complexes, CCs) differ in terms of their virulence and tropism. We also collaborate with the French agency for food, environmental and occupational health and safety (Anses) to implement their qPCR method (for > 30 different CCs in 13 duplex/triplex qPCR reactions) for L. monocytogenes, which can serve as a screening method prior to WGS.

VETERINARY MICROBIOLOGY: SEEING THE FOREST BEYOND THE TREES

We are studying if the method is suitable for CC detection in complex samples like brain and placenta samples. A lot of our research and diagnostics is also dedicated to P. larvae, which is the most important bacterial pathogen of honeybees, causing a disease called American foulbrood (AFB). Only honeybee larvae are susceptible to infection, but once the honeybee colony is infected, this gradually leads to its collapse. Therefore, monitoring of P. larvae in apiaries is of high importance to enable timely preventive measurements.

Using WGS, we were able to resolve AFB outbreaks and identify several transmission routes, which could be explained by the activities of beekeepers. In addition, we constructed a TaqMan-based qPCR assay for reliable quantification of P. larvae in bee-related samples (honey, hive debris and honeybees), which was calibrated using dPCR to allow for absolute quantification. In a pilot study, we analyzed a large number of hive debris samples collected in the winter and spring of 2022, and were able to classify honeybee colonies into categories according to the number of P. larvae spores; categories differed in their probability of developing AFB clinical symptoms in the near future. Finally, WGS enables identification and characterization of novel taxons. For example, it enabled us to describe several **novel bacterial species**: five novel Helicobacter species (H. labacensis, H. mehlei and H. vulpis from gastric mucosa of red foxes [in 2020], H. colisuis sp. nov. from cecal contents of domestic pigs [in 2022], and H. passerinus sp. nov. from cloacal swabs of migratory birds [in preparation]), one novel Campylobacter species (from pig caeca, in preparation), and two novel Mycobacterium species (from mussels, in preparation).

IS ONE COLONY ENOUGH: A GENOMIC PERSPECTIVE ON Campylobacter DIVERSITY IN PATIENTS

Bilal DJEGHOUT (a), Samuel J. BLOOMFIELD (a), Steven RUDDER (a), Ngozi ELUMOGO (a,b), John WAIN (a,c), Alison E. MATHER (a,c), Nicol JANECKO (a)

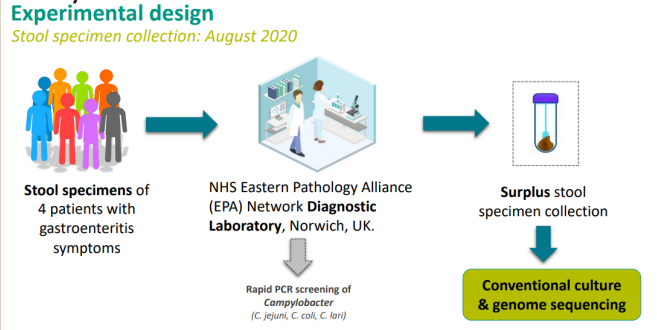
(a) Quadram Institute Bioscience, Norwich, United Kingdom
(b) Eastern Pathology Alliance, Norfolk and Norwich University Hospital, Norwich, United Kingdom
(c) Faculty of Medicine, University of East Anglia, Norwich, United Kingdom

Campylobacter jejuni is the leading cause of gastroenteritis worldwide, usually transmitted through the consumption of poultry products or other contaminated foods. Diagnostic laboratories typically analyse only one colony per specimen or use multiplex PCR assays, resulting in a limitation of genomic information and an inability to characterise multi strain infections.

In this study, the aim was to investigate the diversity of C. jejuni populations within individual stool specimens of four campylobacteriosis patients. Direct plating and pre-culture filtration of one stool specimen per patient was used to culture multiple isolates per stool specimen. Whole genome sequencing and pangenome level analysis were used to investigate genomic diversity of C. jejuni within each patient's stool.

Genetic diversity of C. jejuni population within a single specimen was detected by pangenome analysis. Using sequence types (ST) as a comparison trait, one of the four patients contained two different STs, and those that contained the same ST, SNP analysis revealed diversity with 12-43 core non-recombinant SNP difference. Our results demonstrate the value of comparative genomics in studying C. jejuni population diversity. Diagnostic laboratories' current testing methods do not allow for population level analysis within an infection, potentially leading to misdiagnosis or missed opportunities to identify the source of an outbreak.

By highlighting the limitations of current diagnostic methods, this study provides insight into a dynamic Campylobacter population in campylobacteriosis cases.



INTERACTIONS BETWEEN Campylobacter jejuni ISOLATES RESEMBLE KIN-DISCRIMINATION LIKE BEHAVIOUR

Katarina ŠIMUNOVIĆ (a), Sonja SMOLE MOŽINA (b) Ines MANDIĆ MULEC (a)

(a) Chair of Microbial Ecology and Physiology, Department of Microbiology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia
(b) Chair of Biotechnology, Microbiology and Food Safety, Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

Kin discrimination is a widespread social behaviour that promotes cooperation with highly related individuals and avoidance or exclusion of less related representatives of the same species. It has important implications for bacterial competition, cooperation, horizontal gene transfer and community assembly. Nevertheless, only a few bacterial species have been investigated for consequences of kin discrimination dependent sociality and genetic relatedness between interacting strains is usually not considered.

Campylobacter jejuni is the foodborne pathogen, responsible for ~ 400 million cases of human gastroenteritis each year, the autoimmune disease Guillain-Barre syndrome, and an economic burden of almost 3 billion dollars per year. Yet, almost no knowledge exists on intraspecific interactions of this important foodborne pathogen.

We address this knowledge gap by testing interactions between 24 isolates of C. jejuni with variable phylogenetic relatedness and host/origin in all pairwise combinations (300 combinations altogether) during cooperative swarming, co-cultivation and adhesion to surfaces. Results show that isolates with high (>99.13%) genetic relatedness preferentially merged their swarming colonies, whereas boundary phenotype dominated among swarms of lower (≤99.13%) genetic relatedness.

INTERACTIONS BETWEEN Campylobacter jejuni ISOLATES RESEMBLE KIN-DISCRIMINATION LIKE BEHAVIOUR

During co-cultivation and co-adhesion to a polystyrene surface, we observed the dominance of one of the paired isolates over the other only in pairs with lower genetic relatedness. Finally, we also tested the effect of relatedness on antibiotic resistance transfer among strains during co-cultivation. This study provides the first insight into C. jejuni kin-discrimination behaviour and supports that kinship affects outcomes during intraspecific competition and horizontal gene transfer of this important pathogen.

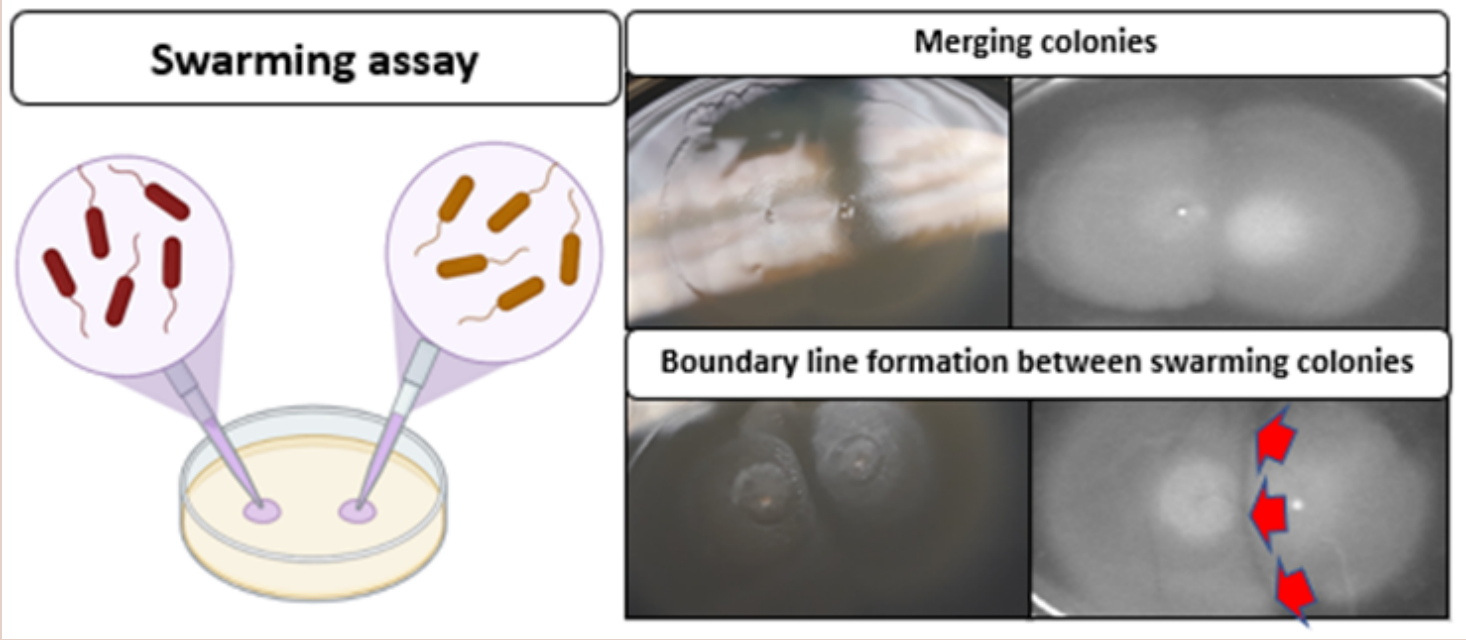


Figure. Swarming assay performance and resulting C. jejuni merging colonies and boundary line formation on soft 0.5% Mueller Hinton agar.

MICROPLASTIC AS A CARRIER OF BACTERIAL COMMUNITIES

Manca KOVAČ VIRŠEK

Institute for Water of the Republic of Slovenia

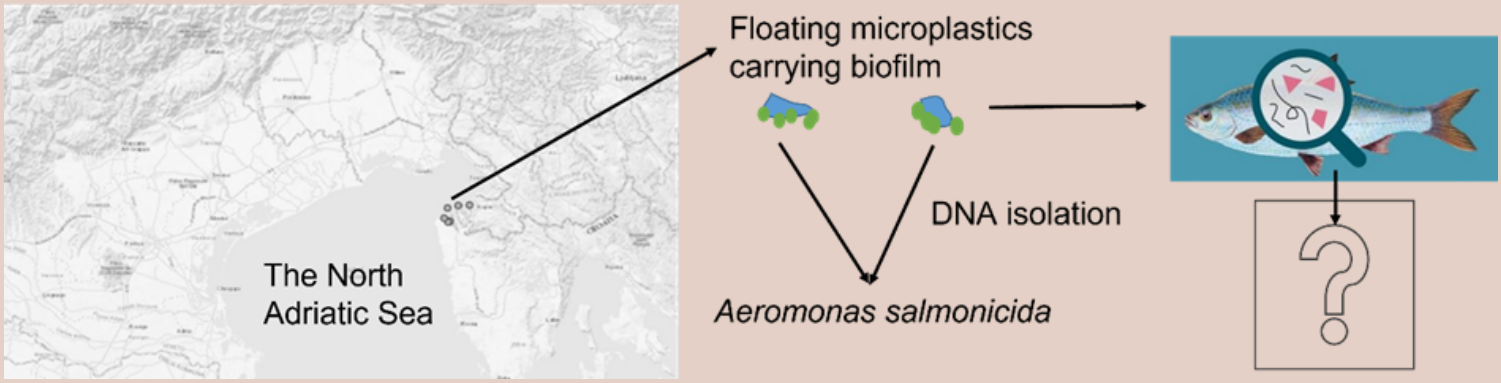
Microplastics are widespread in different environments and they circulate between earth, water, and air ecosystems. Its presence in the environment has numerous negative effects (chemical and biological), including but not limited to providing space for the growth of organisms and serving as a vector for the long-distance transfer of microorganisms. The hydrophobic surface of plastics and their long life span promote microbial colonization and biofilm formation on plastic microparticles. A community composed of heterotrophs, autotrophs, predators, and symbionts that inhibit and live on plastic particles was named a "plastisphere" (Zettler et al., 2013[1]). A plastisphere developed in the marine environment is taxonomically distinct from microbial communities in its surrounding. In recent years, a growing number of pathogenic types of microorganisms have been discovered in plastispheres from different parts of the world. Bacteria of the genus Vibrio spp. were the most frequently reported (Bowley et al., 2021[2]), although they are generally sparse in the open sea.

One of the first studies about bacterial communities' composition on microplastics, which are still uncommon, was published in 2017, in which bacteria from microplastics caught in the Slovenian sea were analysed (Kovač Viršek et al., 2017[3]). DNA from microplastic particles was isolated by three different methods, followed by PCR amplification of 16S rDNA, clone libraries preparation, and phylogenetic analysis. 28 bacterial species were identified on the microplastic particles including Aeromonas spp. and hydrocarbon-degrading bacterial species. Based on the 16S rDNA sequences the pathogenic fish bacteria Aeromonas salmonicida was identified for the first time on microplastics.

MICROPLASTIC AS A CARRIER OF BACTERIAL COMMUNITIES

It is currently known that microplastics are transmitted through the food chain and that they can function as a substrate for pathogenic microorganisms, but to address whether microplastics act to increase disease occurrence additional variables have to be considered:

1) the attachment processes and microbial interactions on the particle surface;
2) the rate and distance of transport of pathogen - colonised particles across oceans and concurrent plastisphere changes;
3) vertical transport processes to the benthos, where ingestion and trophic transfer occurs; and
4) the uptake and retention of particles into organisms and the likelihood of disease transfer occurring as a result (Bowley et al., 20212).



1 Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A. (2013). Life in the "plastisphere": microbial communities on plastic marine debris. Environmental science & technology, 47(13), 7137-7146.
2 Bowley, J., Baker-Austin, C., Porter, A., Hartnell, R., Lewis, C. (2021). Oceanic hitchhikers–assessing pathogen risks from marine microplastic. Trends in microbiology, 29(2), 107-116.
3 Viršek, M. K., Lovšin, M. N., Koren, Š., Kržan, A., Peterlin, M. (2017). Microplastics as a vector for the transport of the bacterial fish pathogen species Aeromonas salmonicida. Marine pollution bulletin, 125(1-2), 301-309.

DNA SEQUENCING OF STRAINS (Campylobacter as model pathogen)

METAGENOMES (WGS) Sample processing to genomic interpretation

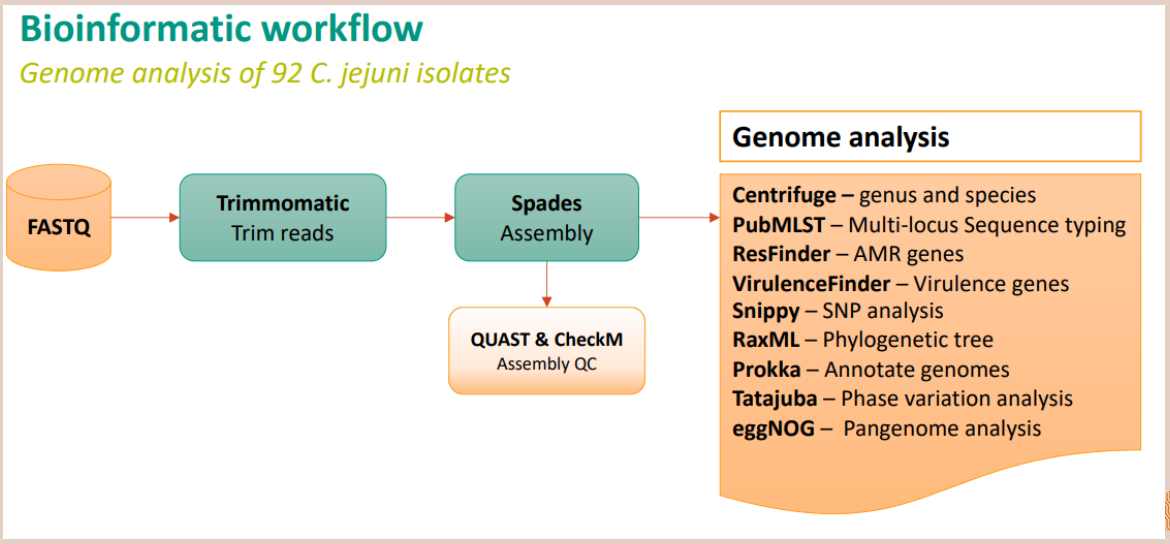
Bilal DJEGHOUT

Quadram Institute Bioscience, Norwich, United Kingdom

The session will address DNA sequencing of strains (Campylobacter as the model pathogen) on short and long read sequencing technologies (Illumina and Nanopore).
Steps in the analysis of sequencing data utilising the Galaxy platform and other in silico methods to characterise Key Campylobacter attributes. The analysis will involve species definition, MLST, AMR genes, virulence genes, core genome phylogenetic RaXML trees and data visualisation.

Metagenomic sequencing's challenges and sample preparation: what considerations are required, how to extract (using stool and chicken liver as model samples). Aspects of sequencing including quality, concentration, sequencing depth, and coverage.

A hands-on MinION sequencing demonstration to illustrate what and how it is done, including quality control interpretation.



PROTEIN PROFILE OF Campylobacter jejuni IN DIFFERENT BIOFILM COMMUNITIES

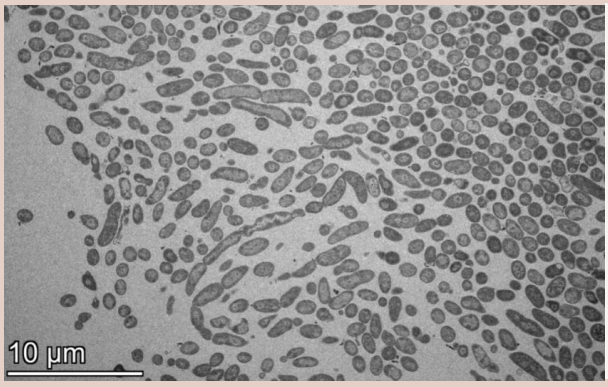
Blaž JUG, Anja KLANČNIK

Chair of Biotechnology, Microbiology and Food Safety, Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Campylobacter jejuni bacteria are the most common cause of bacterial intestinal infections in humans. The main source of infection is consumption of contaminated food, especially poultry meat (1). C. jejuni bacteria form biofilms on surfaces (e.g., production equipment, carcasses, food), which facilitates their survival. Control of biofilms at all stages of production and safe food supply is therefore key to preventing persistent contamination and resistant strains as agents of campylobacteriosis in humans (2).

The aim of this study is to investigate whether the biofilm of Pseudomonas fragi bacteria, the causative agents of food spoilage, modulate the protein profile of C. jejuni. Protein analysis revealed increased synthesis of: (i) TatA, TatB, and TatC proteins, which are the major components of the Tat transport protein system across the inner membrane into the periplasmic space of bacteria, and (ii) CheA and CheW proteins, which are involved in chemotaxis recognition (3). Protein analysis also revealed decreased synthesis of proteins involved in the stress response to oxidative (AhpC, DpS) and heat stress (GrpE, GroEL, HupB).

Knowledge of the interactions of C. jejuni bacteria with other bacteria in multispecies biofilms will enable to highlight key cellular mechanisms as potential targets for developing successful control strategies for these foodborne pathogenic bacteria.



1. European food safety authority in European centre for disease prevention and control. 2022. The European Union one health 2021 zoonoses report. EFSA J, 20:7666
2. Klančnik A., Šimunović K., Sterniša M. Ramić D., Smole Možina S., Bucar F. 2021. Anti-adhesion activity of phytochemicals to prevent Campylobacter jejuni biofilm formation on abiotic surfaces. Phytochem Rev, 20, 55-84
3. Jug B., Jamnik P., Smole Možina S., Sterniša M., Klančnik A. Mikrobiolog.si, 3 (2021) 51-55.

TEMPORAL TRANSCRIPTOMIC ANALYSIS OF Campylobacter jejuni BIOFILMS

Manca VOLK, Anja KLANČNIK

Chair of Biotechnology, Microbiology and Food Safety, Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Campylobacter jejuni are commensal bacteria in the intestines of poultry and other farm animals, from where they can be transmitted to humans through contact or contaminated food (Elmi et al., 2021). In 2021 alone, infections with bacteria of the Campylobacter genus accounted for 62% of all reported infections with bacteria causing zoonoses (EFSA, 2022). C. jejuni require very specific conditions for their growth, but are neverthless widespread in the environment. Their survival under unfavourable environmental conditions are enabled by numerous adaptive mechanisms and transition to resistant forms, such as biofilm (Soro et al., 2020). The structural changes that occur during biofilm formation are the result of the expression of a group of genes that differ from those expressed in planktonic culture (Stoodley et al., 2002), but the molecular mechanisms involved in the regulation of biofilm formation in C. jejuni are still unknown (Klančnik et al., 2021).

The aim of our research is to use the RNA sequencing method to gain insight into the genes that are expressed differently in biofilm than in planktonic culture and their changes at different stages of biofilm development. Bacteria were cultured at different time points (16 h, 24 h, 48 h, and 72 h), and then RNA was isolated and sequenced. Genes with an absolute log2 $\geq$ 1 and an adjusted p-value $\leq$ 0.05 were selected as differentially expressed. At 16 h biofilm, the total number of differentially expressed genes was 225 (69 with increased expression level, 156 with decreased expression level), at 24 h biofilm 443 genes (207 with increased expression level, 236 with decreased expression level), at 48 h biofilm 383 genes (182 with increased expression level, 201 with decreased expression level), and at 72 h biofilm 372 genes (191 with increased expression level, 181 with decreased expression level).

TEMPORAL TRANSCRIPTOMIC ANALYSIS OF Campylobacter jejuni BIOFILMS

In the following work, we will perform a functional analysis of the genes, which will provide us with data on which groups of biological processes and functions the differentially expressed genes can be classified into. Studying the dynamics of biofilm development is important to reveal key mechanisms that may be targets for new alternative strategies to control and prevent the formation of biofilms of the pathogenic bacterium C. jejuni in the process of safe food production.

Keywords: Campylobacter jejuni, biofilm formation, RNA sequencing, gene expression

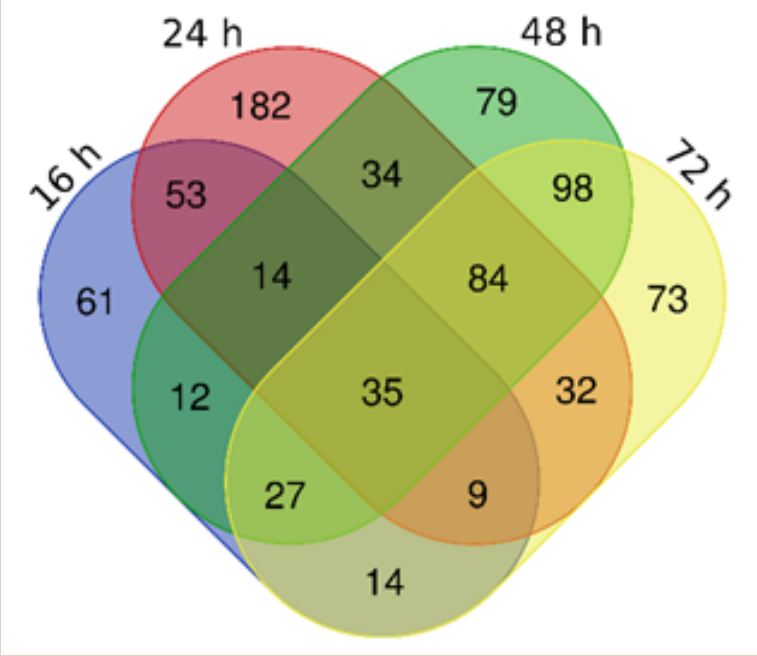


Figure: Venn diagram of differentially expressed genes at different time points of biofilm development at 16 h, 24 h, 48 h and 72 h. Results are normalized to genes expressed in planktonic culture (16 h).

Literature:

Authority, E. F. S. European Centre for Disease Prevention and Control. 2022. The European Union One health 2021 Zoonoses Report. EFSA Journal, 20(12), e07666.

Elmi, A., Nasher, F., Dorrell, N., Wren, B., Gundogdu, O. 2021. Revisiting Campylobacter jejuni virulence and fitness factors: role in sensing, adapting, and competing. Frontiers in Cellular and Infection Microbiology, 10, 607704.

Klančnik, A., Šimunović, K., Sterniša, M., Ramić, D., Smole Možina, S., Bucar, F. 2021. Anti-adhesion activity of phytochemicals to prevent Campylobacter jejuni biofilm formation on abiotic surfaces. Phytochemistry Reviews, 20(1), 55–84.

Soro, A. B., Whyte, P., Bolton, D. J., Tiwari, B. K. 2020. Strategies and novel technologies to control Campylobacter in the poultry chain: A review. Comprehensive Reviews in Food Science and Food Safety, 19(4), 1353–1377.

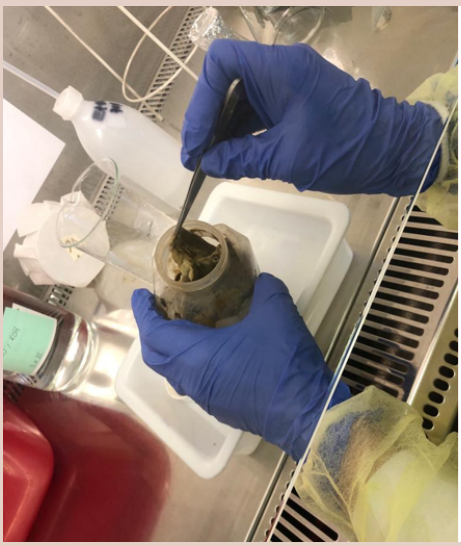
Stoodley, P., Sauer, K., Davies, D. G., Costerton, J. W. 2002. Biofilms as complex differentiated communities. Annual Review of Microbiology, 56, 187–209.

ISOLATION OF MICROPLASTICS FROM POULTRY FECES

Živa KOLENC (a), Manca KOVAČ VIRŠEK (b), Majda GOLOB (c), Anja KLANČNIK (a)

(a) Chair of Biotechnology, Microbiology and Food Safety, Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
(b) Institute for Water of the Republic of Slovenia
(c) University of Ljubljana, Veterinary Faculty, National Veterinary Institute, Institute of Microbiology and Parasitology, Department of Bacteriology and Mycology

We have started developing a method for isolation of microplastic (MP) from poultry feces. For quality assurance, most plastic tools were excluded and replaced by glassware, cleaned with MiliQ water, work was performed in the laminar flow box, white cotton clothes were worn and nitrile gloves were used. Poultry caeca were previously frozen at -20 °C and thawed in the fridge overnight for MP isolation. From each sample of caeca, fat tissue was removed using scissors, tweezers, and a scalpel. Each sample was transferred into an Erlenmeyer flask and weighed, followed by digestion in 10 % KOH with a ratio of 5:1 (V/w). The flasks were covered with gauze, parafilm and aluminium foil, leaving a small hole to allow gas exchange, and incubated for 48 hours (37 °C and 80 RPM). After 48 hours, the samples were filtered using a vacuum filter system and 60 µm nylon net filter. The filter cake was washed with 70 % ethanol and stored in a glass petri dish at -20 °C awaiting microscopic analysis.



LIST OF INVITED SPEAKERS AND ORGANIZERS

Sonja SMOLE MOŽINA

Biotechnical Faculty, Dep. Of Food Science and Technology, Chair of Biotechnology, Microbiology and Food Safety; University of Ljubljana, Ljubljana, Slovenia

University lecturer and head of the Chair of Biotechnology, Microbiology and Food Safety
Scientific interest: She started Campylobacter group in 2000 and the work on Antimicrobial activity of bioactive agents for the control of food-borne pathogen and spoilage bacteria in vitro and in vivo in real food system. Main research areas are microbial ecology of food and food processes and characterization of microbial response to stressful environmental conditions and their resistance in food production environment, and utilisation of bioactive compounds of plant origin and food industry by-products to improve food safety, quality, sustainability and functionality.

https://cris.cobiss.net/ecris/si/sl/researcher/5981
https://www.researchgate.net/profile/Sonja-Smole-Mozina

Nicol JANECKO

Quadram Institute Bioscience, Norwich, UK

Career Track Group Leader, Microbes in the Food Chain, Quadram Institute Bioscience, Norwich with a Campylobacter and metagenomics research group focusing on the transmission of Campylobacter in the food chain.
Scientific interest: Research interest are rooted in One Health principles to study disease dynamics using innovative laboratory metagenomic and bioinformatic tools to transform scientific applications into bring into public health impacts. Understanding the survival, transmission, antimicrobial resistance, and population genomics of foodborne pathogens has been my long-term research interest. We utilise culture-based and culture independent genomic and metagenomic approaches to characterize bacterial populations in different ecological niches.

https://quadram.ac.uk/people/nicol-janecko/
https://www.linkedin.com/in/nicol-janecko-9b854252/

Ozan GUNDOGDU

Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

Scientific interest: The head of the foodborne enteric pathogen group at the LSHTM where we study the physiology and pathogenesis of Campylobacter (most common bacterial cause of human gastroenteritis in the world) and other related enteric microorganisms (e.g. Listeria spp. and Vibrio spp.). Importantly, we translate fundamental microbiological knowledge to real life settings (i.e. by implementing intervention strategies). We use classical molecular microbiology techniques, and link bioinformatic skills applying cutting-edge omics based approaches e.g. genomics, transcriptomics and metagenomics to answer relevant research questions.

https://www.lshtm.ac.uk/aboutus/people/gundogdu.ozan
https://orcid.org/0000-0002-3550-0545
https://www.linkedin.com/in/ozan-gundogdu-16768a102/
https://www.researchgate.net/profile/Ozan-Gundogdu

Blaž STRES

National Institute of Chemistry, Ljubljana, Slovenia

Research Scientist and Full Professor at Kemijski inštitut - National Institute of Chemistry
Scientific interest: the focus is on understanding how our view of the system is shaped by methodological bias and the interconnection of multiscale layers of information. The focus of current work is on anaerobic systems that range from full-scale biogas reactors, optimization experiments, over the transcription and production of effective enzymes within high-altitude wild ruminant ecosystems, towards human intestinal tract microbiome, using top-down multi 'omics approaches and HPCC. His efforts are focused on developing and using novel bioinformatic tools for high-throughput analyses of amplicon and metagenomic and metatranscriptomic datasets and metabolomics.

https://www.linkedin.com/in/blaz-stres-39b9273/
https://orcid.org/0000-0003-2972-2907
https://cris.cobiss.net/ecris/si/sl/researcher/11176

Mojca MILAVEC

National Institute of Biology, Ljubljana, Slovenia

Research Scientist at National Institute of Biology, Ljubljana, Slovenia
Deputy Head of Metrology at the Bacteriology and Metrology Unit of the Department of Biotechnology and Systems Biology and Deputy Head of the quality system at the Department Challenges associated with established and novel technologies for nucleic acid measurements as well as standardisation and quality control of these technologies. Health and food safety.

https://orcid.org/0000-0002-5794-2109
https://cris.cobiss.net/ecris/si/sl/researcher/11191

Majda GOLOB

Institute of Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia

Skills and expertise: Antimicrobials, Antibiotic Resistance, Bacterial Antibiotic Resistance, Bacterial Pathogenesis, Microbial Isolation, Nosocomial Infection, Diagnostic Microbiology, MIC, Clinical Microbiology, Antimicrobial Susceptibility Testing, Antimicrobial Resistance

https://www.researchgate.net/profile/Majda-Golob?ev=brs_overview
https://orcid.org/0000-0001-9526-0152
https://cris.cobiss.net/ecris/si/sl/researcher/33033

Bilal DJEGHOUT

Quadram Institute Bioscience, Norwich, UK

Research Scientist at Quadram Institute Bioscience
Scientific interest: The microbiologist with an interest in both biology and molecular biology of zoonotic pathogens within different ecological spheres. I joined the Quadram Institute Bioscience as a Post-doctoral research scientist in June 2019 to work on a Gates funded project studying Campylobacter in the food chain , as well as optimising a targeted metagenomic culture-independent method for studying Campylobacter populations in different ecological spheres.

https://quadram.ac.uk/people/bilal-djeghout/
https://www.linkedin.com/in/drbilaldjeghout/
https://orcid.org/0000-0002-2680-6219
https://www.researchgate.net/profile/Bilal-Djeghout

Katarina ŠIMUNOVIĆ

Biotechnical Faculty, Department of Microbiology, Cair of Microbial Ecology and Physiology, University of Ljubljana, Ljubljana, Slovenia

Scientific interest: In the field of microbiology, Campylobacter, microbial interaction, genetic tools, the application of a patent and the launch of a start-up company for the development and marketing of innovations (probiotics for chickens) and thus significantly contributed to the reputation of the University of Ljubljana. She is a co-founder of Elogium company.

https://cris.cobiss.net/ecris/si/sl/researcher/44992
https://orcid.org/0000-0001-6526-4208
https://www.researchgate.net/profile/Katarina-Simunovic
https://www.linkedin.com/in/katarina-%C5%A1imunovi%C4%87-366723a5/

Manca KOVAČ VIRŠEK

Institute for Water of the Republic of Slovenia, Ljubljana, Slovenia

Scientific interest: in the field of environmental pollution, with long-term experience in the field of microplastic research in the aquatic environment, including experience in microplastic sampling and analysis. Leads the development of coordinated methodologies for monitoring microplastics in the marine environment in Slovenia in accordance with the implementation of the marine strategy directive; the MSFD Marine Litter Technical Group and the UNEP-Marine Action Plan, Member States for Marine Microplastics Monitoring and Microplastics Intercalibration Studies.

http://www.izvrs.si/dt_team/kovac-virsek-manca/?lang=en
https://cris.cobiss.net/ecris/si/sl/researcher/33777
https://www.linkedin.com/in/manca-kovac-virsek-5a8a0361/
https://www.researchgate.net/profile/Manca-Virsek

Anja KLANČNIK

Biotechnical Faculty, Dep. Of Food Science and Technology, Chair of Biotechnology, Microbiology and Food Safety; University of Ljubljana, Ljubljana, Slovenia

Scientific interest: focused on food-related bacteria, pathogenic Campylobacter, Listeria transmitted via food, including microplastic as vector for pathogen transmission thorough food chain. Campylobacter stress response and resistance in food against physical / chemical stresses, examined on several cell levels: genetic, proteomic, physiology response. Antimicrobial / resistance-modulatory activity of bioactive agents for the control of food-borne pathogen and spoilage bacteria in vitro and in vivo in real food system. Biofilm-specific resistant phenotype and persisted cells and influence on bacterial survival, adhesion properties, virulence properties, signalling and bacterial communication.

https://www.linkedin.com/in/anja-klancnik-b7a7b442/
https://orcid.org/0000-0003-1632-5785
https://cris.cobiss.net/ecris/si/sl/researcher/15503

Biotechnical Faculty University of Ljubljana

https://www.bf.uni-lj.si/en/

The main goal of the educational program is to educate - based on the Faculty's own research and other achievements - highly skilled professionals in the management of natural resources and the thereby related production technologies. The programme includes undergraduate and postgraduate studies as well as a variety of forms of informal education, enabling the acquisition of basic knowledge for work as well as for research activities and the constant updating and broadening of such knowledge.

The Faculty's scientific and research work combines basic, applied and developmental research work, enabling the rapid transfer of research results into practice. A part thereof consists of technical and consultative work enabling the Faculty teaching staff to be up-to-date with and able to solve the everyday needs and problems of practice, resulting at the same time in new ideas for their research and educational activities. The circle: basic research - applied research - education - specialized work and development must be complete and unbroken. Such approach calls for a close connection with the production needs of individual professions and implementers of societal development. The Faculty wishes to be a part of the arena of life.