10th BMMC Biennial Meeting on Microbial Carbohydrates

10th BMMC

Abstract Book

September 9-12, 2025

Hirszfeld Institute of Immunology & Experimental Therapy,
Polish Academy of Sciences, Wrocław, Poland

10th Biennial Meeting on Microbial Carbohydrates

The conference website: https://bmmc2025.hirszfeld.pl/

Local Organizing Committee

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RHLSERVICE

History of the BMMC

The 10th Biennial Meeting on Microbial Carbohydrates (BMMC) conference continues the long-standing tradition of regular meetings of the international scientific community interested in the microbial glycans in terms of chemical analysis of their structures, synthesis and biosynthesis, carbohydrate biochemistry, intermolecular interactions and biological activity.

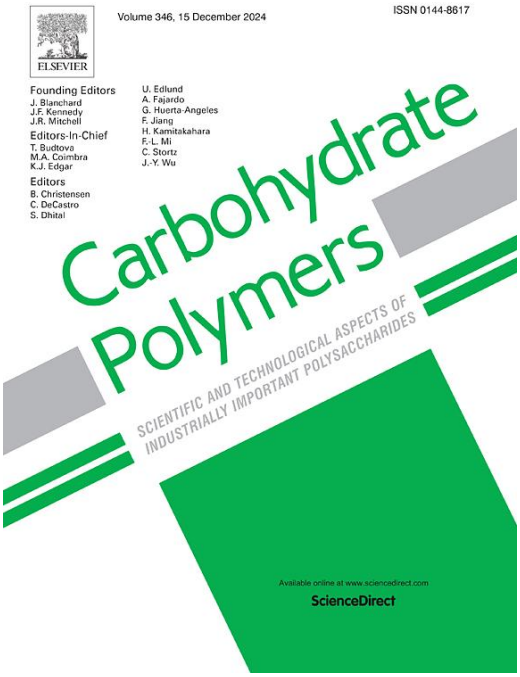
The lectures and communications presented cover a wide range of microorganisms, including bacteria, viruses, parasites, archaeons, and microalgae. Major topics dealt with important healthcare issues such as infectious diseases and vaccination. The overall message is complemented by reports on the latest analytical methods, bioinformatics and databases that provide indispensable support for glycan research. The conference dates back to 1988. It has evolved from the early Baltic Meeting, and its reach has since expanded geographically far beyond the first three countries involved.

The Scientific Committee comprises distinguished researchers in the glycobiology community, the research teams' leaders from Austria, Germany, Finland, Ireland, Italy, and Poland, representing the wide gamut of subjects in the carbohydrate chemistry and glycobiology. The conference aims to integrate research teams and to encourage the participation of PhD students and researchers from all over the world. Frequently, the conference engagement and presentations have resulted in successful collaborations, post-doctoral positions or joint projects and publications. After 20 years, the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław has the privilege to be once again the organizer of the conference.

Year	Location	Chair
2000	Borstel, Germany	Otto Holst
2002	Moscow, Russia	Yuriy Knirel
2004	Wrocław, Poland	Andrzej Gamian
2006	Rostock, Germany	Christian Vogel
2008	Sigtuna, Sweden	Elke Schweda, Stefan Oscarson
2010	Hyytiala, Finland	Mikael Skurnik
2012	Suzdal, Russia	Yuriy Knirel
2014	Gdansk, Poland	Zbigniew Kaczynski
2016	Rostock, Germany	Christian Vogel
2018	Dublin, Ireland	Stefan Oscarson, Paul Murphy
2022	Napoli, Italy	Cristina De Castro
2025	Krzyżowa, Poland	Andrzej Gamian, Jolanta Łukasiewicz

<u>Special Issue of Carbohydrate Polymers</u> will be dedicated to 10th BMMC 2025.
Participants are welcome to contribute to this Special Issue, both Regular and Review articles will be accepted.

https://www.sciencedirect.com/journal/carbohydrate-polymers/about/call-for-papers#10th-bmmc



Guest editors:
Cristina De Castro, Jolanta Łukasiewicz and Immacolata Speciale

Special issue information:

This Special Issue is devoted to collecting the scientific contributions from the authors who participated in the 10th Biennal Meeting on Microbial Carbohydrates.

Manuscript submission information:

Only conference contributors of BMMC can submit their papers at https://www.editorialmanager.com/carbpol/default1.aspx and make sure to select the article type "VSI: 10th BMMC-Research Paper or VSI: 10th BMMC-Review paper" while submitting.

The current submission deadline is 31-March-2026 and the submission portal for new papers will be open from 01-Sep-2025.

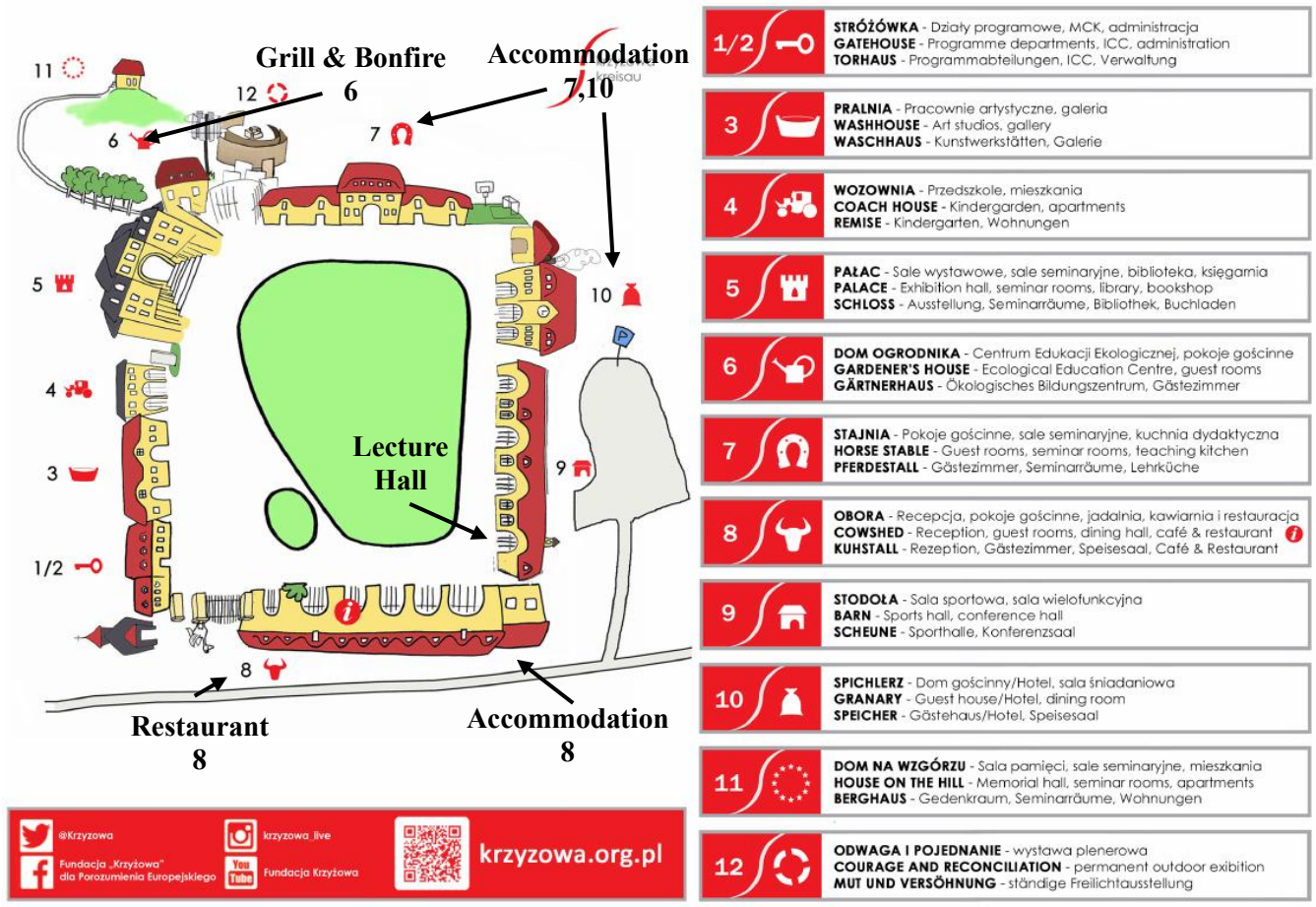
In case of any queries please contact Prof. Cristina De Castro directly at decastro@unina.it

Keywords:
Biochemistry, Genetics and Molecular Biology, Structural Biology, Microbiology, Organic Chemistry, Glycobiology, Microbial Polysaccharides

10th Biennial Meeting on Microbial Carbohydrate

Conference Venue: Krzyżowa Foundation for Mutual Understanding in Europe, Krzyżowa 7

https://www.krzyzowa.pl/en



The Conference and coffebreaks will be held at the Barn (9).

The internet will be available at the login "**Krzyżowa_free_internet**".

There are 3 possible accommodation houses at 7,8,10.

Restaurant is located at Dining Hall (8).

The social event Grill will be held in the Garden (6).

On Wednesday, Sep 10 Guided Tour of the Krzyżowa Center will take place at 5.30 pm. (optional)

„Krzyżowa Foundation for Mutual Understanding in Europe" Guided Tour, app. 1 hour.
During the tour the visitors are introduced to history and work of Krzyżowa Foundation. Our activities cover a variety of formats, starting with youth exchange programs, including conferences and seminars and covering also cultural events, such as music festivals.
The history of the place, though situated in a small village, is rich. 12 November 1989 Tadeusz Mazowiecki and Helmut Kohl, heads of the Polish and the German government respectively, met during a catholic service in Krzyżowa, exchanged the sign of peace and decided to support the project of Krzyżowa as a european educational centre.

The scientific program of the 10th BMMC will cover principal aspects of recent advances in:

- Glycans of Microbes
- Glycan Synthesis
- Structural Chemistry of Carbohydrates
- Glycan Biosynthesis and Recognition
- Glycans in Bacterium/Host Interactions

Keynote speakers:

- Jeroen Codee, Leiden University, The Netherlands
- Cristina De Castro, University of Napoli Federico II, Italy
- Sonsoles Martín-Santamaría, Scientific Researcher at the Department of Cellular and Molecular Biosciences, Center for Biological Research CIB-CSIC, Madrid, Spain
- Miguel A. Valvano, The Wellcome-Wolfson Institute for Experimental Medicine, Queen's University of Belfast, United Kingdom
- Chris Whitfield, University of Guelph, Canada

Scientific Programme

Daily Program of the BMMC 2025

Tuesday, September 9, 2025

TIME	EVENT
13:00 - 14:00	**Transfer of Attendees & Guests from Wrocław to Krzyżowa – the Conference Venue**
14:00 - 19:00	Registration & Accommodation
19:00	**Get-together Dinner**

Wednesday, September 10, 2025

TIME	EVENT
7:30 – 8:45	Breakfast
09:00 - 09:10	**Welcome - Opening of the BMMC2025**
	Session I Chairman: **Zbigniew Kaczyński**
09:10 - 09:55	**Invited Speaker:** Chris Whitfield VANISHING ACT – THE ENIGMATIC *SALMONELLA* T1 ANTIGEN
09:55 - 10:15	**O1** Timm Fiebig NOVEL INSIGHTS INTO PRIMING AND POLYMERIZING GRAM-NEGATIVE GROUP 2 CAPSULAR POLYSACCHARIDES
10:15 - 10:35	**O2** Andrea Iovine STRUCTURAL ELUCIDATION OF THE CAPSULAR POLYSACCHARIDE FROM *LACTIPLANTIBACILLUS PLANTARUM*
10:35 - 10:55	**O3** Raffaele D'Amico FROM ANTARCTIC SEAS TO BIOTECHNOLOGICAL FRONTIERS: INVESTIGATING THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF *PSYCHROBACTER SP.* TAE2020 CAPSULAR POLYSACCHARIDE
10:55 - 11:25	Coffee Break
11:25 - 11:45	**O4** Carolina Fontana STRUCTURE AND BIOSYNTHETIC ASPECTS OF THE O-ANTIGEN POLYSACCHARIDES OF TWO *E. COLI* STRAINS RELATED TO VARIANTS OF O99 AND O20 GENOTYPES
11:45 - 12:05	**O5** Wiesław Kaca INTO THE UNDERSTANDING OF THE MOLECULAR MECHANISMS OF

	POLYMYXIN B RESISTANCE IN *PROTEUS MIRABILIS*
12:05 - 12:25	**O6** Agata Palusiak STRUCTURE OF THE O-ANTIGEN OF THE *KLEBSIELLA OXYTOCA* 0.067 LPS AND ITS CONTRIBUTION TO SEROLOGICAL CROSS REACTIONS
12:25 - 12:40	**Flash Communications I** **F1** Tim A. Mast THE MOLECULAR MECHANISM OF BCS3, THE CAPSULE POLYMERASE FROM *HAEMOPHILUS INFLUENZAE* SEROTYPE B **F2** Małgorzata Marczak MULTILAYER CONTROL OF GLYCOSYLTRANSFERASES BY c-di-GMP AND TYROSINE PHOSPHORYLATION SHAPES EXOPOLYSACCHARIDE OUTPUT IN *RHIZOBIUM LEGUMINOSARUM* BV. *TRIFOLII* **F3** Marco D. Albers TRANSITION TRANSFERASES PRIME GROUP 2 CAPSULAR POLYSACCHARIDE BIOSYNTHESIS IN GRAM-NEGATIVE BACTERIA
13:00 -14:00	Lunch & Coffee
	Session II Chairman: **Miguel A. Valvano**
14:15 - 15:00	**Invited Speaker: Sonsoles Martin-Santamaria** MOLECULAR MECHANISMS OF THE INNATE IMMUNITY RECEPTORS: FROM LPS TO GLYCOLIPIDS AND SMALL MOLECULES
15:00 - 15:20	**O9** Katarzyna Zamłyńska STUDIES ON LIPIDS A FROM *BRUCELLA CITISI* AND *BRUCELLA LUPINI* A NODULE FORMING BACTERIA ISOLATED FROM *CYTISUS SCOPARIUS* AND *LUPINUS ALBUS*, RESPECTIVELY
15:20 - 15:40	**O10** Angela Marseglia STRUCTURAL AND CONFORMATIONAL INSIGHTS INTO BACTERIAL LECTIN RECOGNITION OF HOST RECEPTORS
15:45 - 16:15	Coffee Break
16:15 - 16:35	**O11** Ferran Nieto-Fabregat CATALYTIC INSIGHTS INTO KP34GP57, A PHAGE-DERIVED DEPOLYMERASE TARGETING *KLEBSIELLA* K63 CAPSULAR POLYSACCHARIDES
16:35 - 16:55	**O12** Antonella Aquilone BIOINFORMATIC IDENTIFICATION AND MODELING OF CHLOROVIRUS PBCV-1 MULTIDOMAIN PROTEIN A111/114R
16:55 - 17:10	**Flash Communications II** **F4** Iwona Komaniecka STRUCTURAL CHARACTERIZATION OF A WATER-INSOLUBLE POLYSACCHARIDE FROM *PLEUROTUS CITRINOPILEATUS* AND PREBIOTIC

	POTENTIAL OF ITS ACIDIC HYDROLYSATE **F5** Sabina Koj *PLESIOMONAS SHIGELLOIDES* O68 LIPOPOLYSACCHARIDE. EXPANDING THE ARRAY OF *P. SHIGELLOIDES* STRAINS WITH UNUSUAL HYDROPHOBIC PROPERTIES OF THE O-ANTIGENS **F6** Jakub Smoliński THE *MYCOBACTERIUM BOVIS* BCG SUBSTRAIN PASTEUR CELL CONTAINS A DIVERSE ARRAY OF LIPIDS
17:10 - 19:00	Free time
17:30 - 18:30	**Guided tour of the Krzyżowa Center (optional)**
19:00	**Outdoor Barbecue & Bonfire**

Thursday, September 11, 2025

TIME	EVENT
7:30 – 8:45	Breakfast
	Session III Chairman: **Stefan Oscarson**
09:00 - 09:45	**Invited Speaker: Jeroen D. C. Codée** OF SUGARS AND PHOSPHATES: SYNTHESIS AND APPLICATION OF BACTERIAL GLYCANS AND MIMETICS
09:45 - 10:05	**O13** Gustav Nestor ISOTOPICALLY LABELLED CARBOHYDRATES FOR NMR STUDIES OF PROTEIN-CARBOHYDRATE INTERACTIONS
10:05 - 10:25	**O14** Marie-Claire Jiang SYNTHESIS OF TRISACHARIDE REPEATING UNITS OF *CAMPYLOBACTER JEJUNI* HS:23/36
10:25 - 10:45	**O15** Ornella Serpino STRUCTURAL BIOLOGY APPROACHES FOR THE CHARACTERIZATION OF *SHIGELLA* O-ANTIGEN SPECIFIC MONOCLONAL ANTIBODIES
10:45 - 11:05	**O16** Tomasz Niedziela CHEMOTYPING OF BACTERIAL GLYCANS USING NMR SPECTROSCOPY. HIGH-RESOLUTION MAGIC ANGLE SPINNING NMR IN THE ANALYSIS OF O-ANTIGENS
11:05 - 11:35	Coffee Break
11:35 - 11:55	**O17** Marta Kaszowska STRUCTURAL DIVERSITY AMONG *AEROMONAS SALMONICIDA* O-POLYSACCHARIDES ISOLATED FROM FISH AS AN EPIDEMIOLOGIC TOOL

11:55 - 12:15	**O18** Katarzyna Pacyga-Prus IMPACT OF DEPHOSPHORYLATION ON THE STRUCTURE AND FUNCTION OF B.PAT POLYSACCHARIDE ISOLATED FROM *BIFIDOBACTERIUM ANIMALIS* SSP. ANIMALIS CCDM 218
12:15 - 12:40	**Flash Communications III** **F7** Tapasi Manna SYNTHESIS OF IMMUNOREACTIVE PHOSPHOCHOLINE MODIFIED PARASITIC GLYCAN EPITOPES **F8** Immacolata Speciale CHLOROVIRUS GLYCOSYLTRANSFERASES USED FOR THE CHEMOENZYMATIC SYNTHESIS OF A NEW CLASS OF BIOSURFACTANTS **F9** Antonio Lembo ANALYSIS OF THE KDO BIOSYNTHETIC CLUSTER IN CROV **F10** Anna Turska-Szewczuk STRUCTURE AND GENE CLUSTER ORGANIZATION OF CLOSELY RELATED O-ANTIGENS OF *AEROMONAS SP*. CLASSIFIED TO THE NEW PROVISIONAL SEROGROUP PREVAILING IN POLISH AQUACULTURE **F11** Lourriel Macale NMR STUDIES REVEAL DISTINCT GLYCOSYLATION PATTERNS OF MAJOR CAPSID PROTEINS OF THREE CHLOROVIRUSES, FR483, MT325 AND PBCV-1
13:00 -14:00	Lunch & Coffee
	Session IV **Chairman:** Tomasz Niedziela
14:00 - 14:45	**Invited Speaker: Miguel A. Valvano** LIPID A MODIFICATION BY 4-L-AMINOARABINOSE IN OPPORTUNISTIC BACTERIA: A BALANCING ACT BETWEEN BACTERIAL SURVIVAL AND ANTIBIOTIC HETERORESISTANCE
14:45 - 15:05	**O19** Jacek Rybka HOW *SALMONELLA* ENTERITIDIS EVADES THE INNATE IMMUNITY MECHANISMS USING THE LENGTH OF LIPOPOLYSACCHARIDE MOLECULES
15:05 - 15:25	**O20** Anna Chudzik CHEMICAL CHARACTERISTICS OF GLYCOLIPIDS PRESENT IN *C. ACNES* CELLS AND SECRETED EXTRACELLULAR VESICLES
15:25 - 15:45	**O21** Cedric Battaglino DISSECTING THE ROLE OF BACTERIAL OUTER MEMBRANE VESICLE (OMV)-DEPENDENT COMPLEMENT ACTIVATION IN SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)
15:45 - 16:15	Coffee Break
16:15 - 16:35	**O22** Sabina Górska BUBBLE TROUBLE: HOW BACTERIAL EXTRACELLULAR VESICLES KEEP

	ALLERGIES AT BAY?
16:35 - 16:55	**O23** Paolo Costa ADOPTING A GLYCOENGINEERING STRATEGY TO IMPROVE THE THERAPEUTICAL POTENTIAL OF EXTRACELLULAR VESICLES FROM YEAST
	Flash Communications IV
16:55 - 17:10	**F12** Andrej Sinica EXOPOLYSACCHARIDES PRODUCED BY *LIMOSILACTOBACILLUS FERMENTUM* MM1V: PHYSICAL PROPERTIES AND EFFECTS ON GUT PROBIOTIC STRAINS ADHERENCE **F13** Katarzyna Durlik-Popińska BACTERIAL GLYCANS IN AUTOIMMUNITY. ANTI-LPS *PROTEUS MIRABILIS* S1959 (O3) ANTIBODIES FROM RA PATIENTS TARGET COLLAGEN AND HOST FIBROBLASTS **F14** Agnieszka Kowalczyk REINSTATING THE O10 ANTIGEN TO THE SEROLOGICAL CLASSIFICATION SCHEME FOR *KLEBSIELLA PNEUMONIAE*
17:10 - 18:15	**Poster Session & Coffee**
18:15 - 19:00	Free time
19:00	**Gala Dinner & Dance**

Friday, September 12, 2025

TIME	EVENT
7:30 – 8:45	Breakfast
	Session V Chairman: **Adam Choma**
08:45 - 09:30	**Invited Speaker: Cristina De Castro** GLYCOSYLATION IN GIANT VIRUSES
09:30 - 09:50	**O24** Stefanie Barbirz A SWEET KEY TO BACTERIOPHAGE INFECTION: PHAGE-GLYCAN INTERACTIONS AT GRAM-NEGATIVE ENVELOPES AND IN BIOFILMS
09:50 - 10:10	**O25** Mikael Skurnik IDENTIFICATION OF RECEPTOR BINDING PROTEINS OF *YERSINIA* PHAGE ΦR1-37 AND ENTEROCOLITICIN THAT USE THE SAME BACTERIAL SURFACE RECEPTOR
10:10 - 10:30	**O26** Łukasz F. Sobala COMPARATIVE GLYCOMICS REVEALS CHANGES IN N-GLYCOME PATTERNS IN

	MULTICELLULARITY AND EARLY ANIMAL EVOLUTION
10:30 - 10:50	**O27** Dandan Yu STRUCTURAL ANALYSIS OF THE GLYCAN PRODUCED BY THE MUTANT IN THE GENE *VNG*1058H OF *HBT. SALINARUM*
10:50 - 11:00	**Closing of the BMMC2025**
11:00 - 12:00	Lunch & Coffee
12:15 - 12:30	**Transfer of attendees & guests from Krzyżowa back to Wrocław**

K1

VANISHING ACT – THE ENIGMATIC *SALMONELLA* T1 ANTIGEN

Chris Whitfield

Department of Molecular & Cellular Biology, University of Guelph, Canada; cwhitfie@uoguelph.ca

The Kauffmann-White serological classification scheme distinguishes isolates of the genus *Salmonella* based on flagellar (H) and lipopolysaccharide (O) antigens. Diversity in the repeat-unit structures of the O-antigen polysaccharides is reflected in 46 O serogroups. In the 1960s, a transient (T1) antigen was identified in an isolate of *Salmonella enterica* Paratyphi B, and it was proposed to be part of an LPS molecule. However, the apparent instability of T1 production limited detailed investigation, so more than 5 decades later, its exact structure, distribution across the genus, and the explanation for its transient property, were still unknown. We used a bioinformatic approach to identify the genes for T1-antigen biosynthesis and reconstituted its stable production in an *Escherichia coli* host background. This enabled determination of the structure of the T1 antigen by combining biophysical, biochemical and genetic methods. The T1 antigen is composed of a linear polysaccharide backbone containing tandemly arranged polymeric domains. The LPS core oligosaccharide proximal region contains a homopolymer of galactofuranose, and its assembly shares similarities with related glycans including mycobacterial (arabino)galactan, and some O antigens from *Klebsiella pneumoniae* and other bacteria. The non-reducing terminal domain is a homopolymer of ribofuranose, resembling an O antigen found in some isolates of *Serratia* sp. The transient property of the T1 antigen was found to result from a phase-variation mechanism acting at the transcriptional level, which has not previously been observed in O-antigen production. Reversible transition between “on” and ‘off’ states is determined by recombinational inversion of the promoter upstream of the T1 genetic locus. The T1 locus is broadly distributed across many *Salmonella* isolates, but is not universal, and the genes are either mutated or absent in most typhoidal serovars and in serovar Enteritidis. The precise role of T1 antigen in *Salmonella* biology remains to be established**.**

Reference

[1] Kelly, S. D., Allas, M. J., Goodridge, L. D., Lowary, T. L. & Whitfield, C. Structure, biosynthesis and regulation of the T1 antigen, a phase-variable surface polysaccharide conserved in many Salmonella serovars. *Nat. Commun.* 15, 6504 (**2024**).

K2

MOLECULAR MECHANISMS OF THE INNATE IMMUNITY RECEPTORS: FROM LPS TO GLYCOLIPIDS AND SMALL MOLECULES

Sonsoles Martín-Santamaría

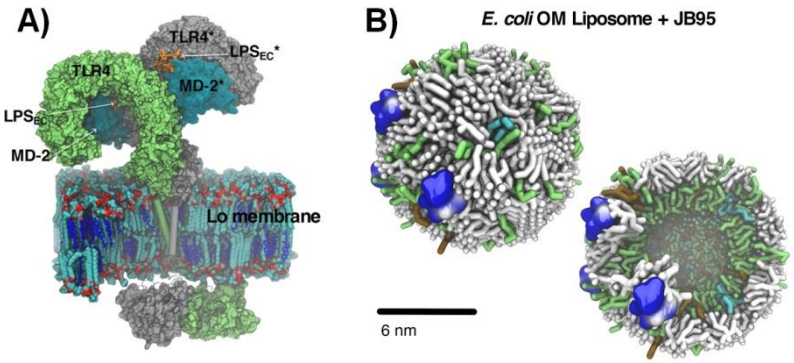
Centro de Investigaciones Biológicas "Margarita Salas", CSIC. C/ Ramiro de Maeztu, 9, 28040-Madrid, Spain.
smsantamaria@cib.csic.es

Toll-like receptors (TLRs) have a primordial role in the activation of the innate immunity through the recognition of pathogen-associated molecular patterns, and have sparked great interest in the therapeutic modulation of the innate immune system. Deep structural understanding of TLRs signaling and mechanism is leading to the discovery of novel molecules with desirable therapeutic properties as antiinflammatory agents or vaccine adjuvants.[1] Our work aims to contribute to increasing this knowledge.

We have addressed the computational characterization of the molecular recognition processes of agonist and antagonist modulators of TLR2, and TLR4, ranging from atypical lipopolysaccharides to glycolipids and small molecules with drug-like structure, and the structural assembly of activated full-length TLR4 and TLR2 models (Figure A) embedded into realistic plasma membranes.[2]

We combine different computational techniques (protein-protein and ligand-protein docking, multiscale molecular dynamics (MD) simulations, accelerated MD simulations, membranes simulation, machine learning (ML)-based techniques) with experimental data (from X-Ray crystallography and NMR), chemical synthesis and biological assays. We here report our last progresses in: i) the atomic details of the novel mechanism of TLR2/TLR4 modulation by low endotoxic atypical LPS from *Ochrobactum intermedium*;[3] ii) differences in both the lipid and carbohydrate moieties of LPS from microbiota *Veillonella parvula*;[4] and iii) the design and structure-activity relationship studies through ML-based techniques of glycolipids as TLR4 modulators; and iv) the finding of small molecules able to mimic LPS properties and lipid A properties in TLR modulation.

Also, studies of pathogen bacterial membranes by liposome models can contribute to understand infection mechanisms.[5] We have captured, by coarse-grained MD simulations, the role of Lipid A on bacterial liposome morphology and physicochemical properties (Figure B), providing a promising tool to predict the activity of novel antimicrobial peptides able to overcome antibacterial resistance.



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[1] Romerio A, Romerio A, Gotri N, Franco AR, Artusa V, Shaik MM, Pasco ST, Atxabal U, Matamoros-Recio A, Minguez-Toral M, Zalamea JD, Franconetti A, Abrescia NGA, Jimenez-Barbero J, Anguita J, Martin-Santamaria S, Peri F. *J. Med. Chem*. **2023**, *66*, 3010-3029.

[2] Matamoros-Recio A, Franco-Gonzalez JF, Perez-Regidor L, Billod JM, Guzman-Caldentey J, Martin-Santamaria S. *Chem. Eur. J*. **2021**, 27(62), 15406-15425.

[3] Matamoros-Recio A, Merino J, Gallego-Jiménez A, Conde-Alvarez R, Fresno M, Martin-Santamaria S. C*arbohydr. Polym.*, **2023**, *318*:121094.

[4] Pither MD, Andretta E, Rocca G, Balzarini F, Matamoros-Recio A, Colicchio R, Salvatore P, van Kooyk Y, Silipo A, Granucci F, Martin-Santamaria S, Chiodo F, Molinaro A, Di Lorenzo F. *Angew. Chem. Int. Ed. Engl.* **2024**, *63*:e202401541.

[5] Franco-Gonzalez JF, Matamoros-Recio A, Torres-Mozas A, Rodrigo-Lacave B, Martin-Santamaria S. *Sci. Rep*. **2022**, *12*, 19474.

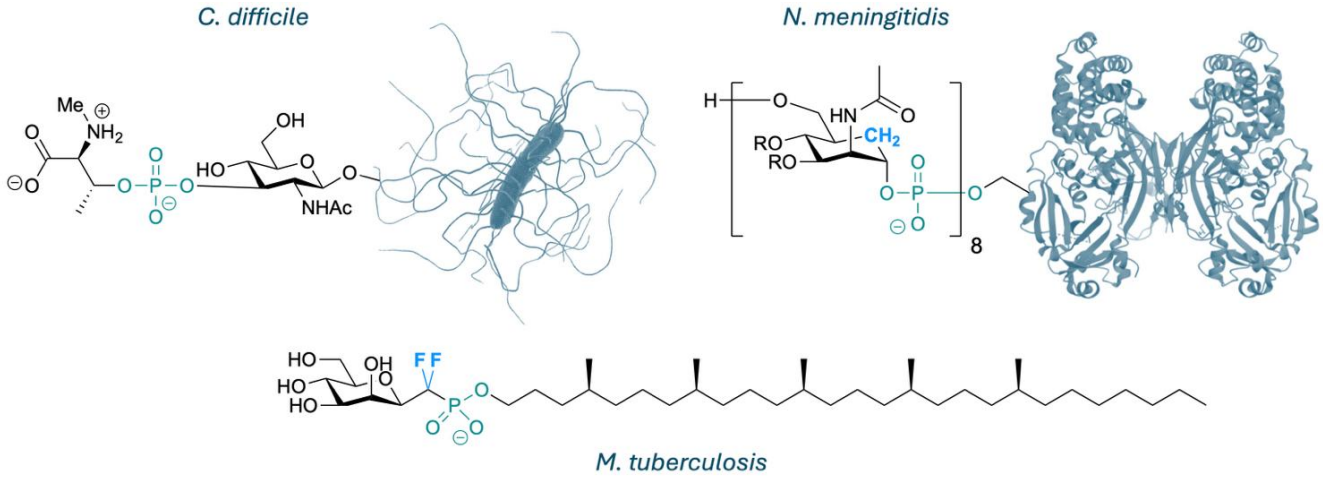
K3

OF SUGARS AND PHOSPHATES: SYNTHESIS AND APPLICATION OF BACTERIAL GLYCANS AND MIMETICS

Jeroen D. C. Codée

Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands

Bacteria decorate the outside of their cell wall with unique carbohydrate-based structures, and these often contain phosphodiester elements. To study how these creative structures are synthesized by bacteria and unravel the role of these glycans in the interaction with the host immune system, well-defined structures are required. Because isolation of these compounds from natural sources in sufficient quantity and purity is exceedingly difficult or impossible, synthetic chemistry has been an important supplier of these valuable molecules. Organic synthesis also allows one to incorporate additional functionalities in the target structures, such as chemoselective functionalization handles, or modify the structures to provide enhanced (metabolic) stability. This presentation shows our recent results in the unravelling of a unique *C. difficile* post-translational modification and the development of stabilized *M. tuberculosis* and *N. meningitidis* phosphoglycans for vaccine development.



K4

LIPID A MODIFICATION BY 4-L-AMINOARABINOSE IN OPPORTUNISTIC BACTERIA: A BALANCING ACT BETWEEN BACTERIAL SURVIVAL AND ANTIBIOTIC HETERORESISTANCE

Miguel A. Valvano

Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, BT9 7BL

I will discuss modalities of Gram-negative opportunistic pathogens we use as models in my laboratory (Burkholderia, Achromobacter, and Enterobacter species) to overcome polymyxins.

These molecules are potent cationic antimicrobial peptides that have become the last resort to treat infections caused by multidrug resistant bacteria. A key component, critical to the action of polymyxins, is the outer membrane lipopolysaccharide (LPS). Bacteria protect themselves from polymyxins by modifying the LPS in several ways that ultimate prevent drug binding. Many of these modifications are intrinsically connected with other bacterial functions including bacterial viability, which result in resistance and heteroresistance to polymyxins.

I will present research demonstrating the intimate relationship of lipid A modifications with bacterial physiology and the participation of efflux pumps in the mechanism of resistance to polymyxins.

K5

GLYCOSYLATION IN GIANT VIRUSES

Cristina De Castro

Department of Chemical Sciences, University of Napoli Federico II, Via Cintia 26, 80126 Napoli, Italy, decastro@unina.it

Giant dsDNA viruses are gaining interest in the field of Glycobiology because, and differently from other viruses, they are able to biosynthesize rare sugars and to glycosylate their own proteins by an apparent host independent process.

Giant dsDNA viruses consist of a large number of species and this lecture will focus on the recent developments that involve members of *Phycodnaviridae* and *Mimiviridae* families.

Regarding *Phycodnaviridae*, to date information is available for *Chloroviruses.* Chloroviruses are large (190 nm in diameter) icosahedral, plaque-forming viruses with an internal lipid membrane; they have genomes of 290 to 370 kb that contain up to 400 protein-encoding genes.[1] The prototype chlorovirus, *Paramecium bursaria chlorella virus* (PBCV-1), infects *Chlorella variabilis*, a symbiont of the protozoan *Paramecium bursaria*. The PBCV-1 major capsid protein Vp54 accounts for about 40% of the viral protein, and it is glycosylated by an unusual and complex oligosaccharide.[2,3] The glycobiology of PBCV-1 will be presented as well as information about its antigenic variants and other related chloroviruses.[4,5,6]

As for *Mimiviridae*, these viruses infect *Acanthamoeba* sp. and were initially identified as bacteria because of their large size along with the heavily glycosylated fibrils of the capsid. Within *Mimiviridae*, information is available for *Mimivirus* and *Megavirus* genera, and this lecture will focus on the recent structural data available on *Mimivirus*.[7]

References

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O1

NOVEL INSIGHTS INTO PRIMING AND POLYMERIZING GRAM-NEGATIVE GROUP 2 CAPSULAR POLYSACCHARIDES

Timm Fiebig[1,2], Christa Litschko[1,2], Valerio Di Domenico[3,4], Julia Schulze[1], Sizhe Li[5], Olga G Ovchinnikova[6], Thijs Voskuilen[5], Andrea Bethe[1], Javier O Cifuente[3,7], Alberto Marina[7], Insa Budde[1], Tim A Mast[1], Małgorzata Sulewska[1], Monika Berger[1], Falk F R Buettner[1,8], Todd L Lowary[9,10,11], Chris Whitfield[6], Jeroen D C Codee[5], Mario Schubert[12,13], Marcelo E Guerin[3,4,7,14]

[1]Institute of Clinical Biochemistry, Hannover Medical School, Hannover, Germany.
[2]German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Hannover, Germany.
[3]Structural Glycobiology Laboratory, Biocruces Bizkaia Health Research Institute, , Barakaldo, Bizkaia, Spain.
[4]Structural Glycobiology Laboratory, Department of Structural and Molecular Biology; Molecular Biology Institute of Barcelona (IBMB), Spanish National Research Council (CSIC), Barcelona, Catalonia, Spain.
[5]Leiden Institute of Chemistry, Leiden University, Leiden, The Netherlands.
[6]Department of Molecular and Cellular Biology, University of Guelph, Guelph, Canada.
[7]Structural Glycobiology Laboratory, Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Derio, Spain.
[8]Proteomics, Institute of Theoretical Medicine, Faculty of Medicine, University of Augsburg, Augsburg, Germany.
[9]Department of Chemistry, University of Alberta, Edmonton, Canada.
[10]Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan.
[11]Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan.
[12]Department of Biosciences and Medical Biology, University of Salzburg, Salzburg, Austria.
[13]Department of Biology, Chemistry and Pharmacy, Free University of Berlin, Berlin, Germany
[14]Ikerbasque, Basque Foundation for Science, Bilbao, Spain

Capsular polysaccharides (CPS) are important virulence factors that protect bacterial pathogens from the host immune system. They are structurally diverse and used as antigens in highly effective glycoconjugate vaccines, in which they are coupled to a carrier protein to induce a T-cell dependent immune response. The manufacturing of glycoconjugate vaccines includes the purification of CPS from pathogen culture. To reduce biohazard and costs, chemical and enzymatic synthesis have been extensively studied as alternatives for CPS production.
In Gram-negative bacteria, capsules are often expressed using ABC transporter-dependent pathways, also referred to as group 2 systems. Group 2 systems are used by human and animal mucosal pathogens that cause urinary tract infections, septicemia and meningitis, including *Haemophilus influenzae* and *Actinobacillus pleuropneumoniae*.
CPS production in group 2 systems can be divided into four steps. In step I, a conserved glycolipid anchor is generated, consisting of phosphatidylglycerol and an oligomer of b-linked Kdo. Step II is uncharacterized and comprises the synthesis of the region that connects glycolipid and CPS. In step III, the serotype-specific CPS is assembled by capsule polymerase(s), which are highly diverse, reflecting the structural variability of their products. Step IV comprises the transport of the polymer from the cytoplasm to the outside of the cell.
Here, we identify the transition transferases that connect the conserved glycolipid anchor with the serotype specific CPS in bacteria expressing a poly(glycosylpolyol phosphate) capsule and demonstrate that these enzymes generate a structurally distinct linker identical to Gram-positive wall teichoic acid. We elucidate how said linker is elongated by the capsule polymerases and show that the transition transferases stimulate the polymerases to produce longer CPS, indicating a concerted process.

In addition, multi-enzyme cascades were established for the generation of the CPS from *Haemophilus influenzae* type b and *Actinobacillus pleuropneumoniae* serotypes 3 and 7, starting from widely available substrates, to pave the way for the biotechnological exploitation of the catalysts. Finally, crystal structures of capsule polymerases from both pathogens were solved to improve our understanding of the catalytic mechanism of these multi-domain enzymes.

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O2

STRUCTURAL ELUCIDATION OF THE CAPSULAR POLYSACCHARIDE FROM *LACTIPLANTIBACILLUS PLANTARUM*

Andrea Iovine [a], Giulia Bisson [b], Immacolata Speciale [a], Anna Notaro [a], Marilena Marino [b], Cristina De Castro [a]

[a] Department of Chemical Sciences, University of Naples Federico II, via Vicinale Cupa Cintia n°26 Napoli – Italy, andrea.iovine@unina.it
[b] Department of Agri-Food, Environmental and Animal Sciences, University of Udine, via Sondrio 2/A, Udine – Italy

In recent times, nutrition has become one of the main health patterns due to disorders related to sedentary lifestyles and therefore consumers consider functional foods as an attractive solution.[1] Among the main ingredients of functional foods are probiotics, food supplements based on live microbes that have beneficial effects on the host organism.[2] Probiotic microorganisms include the lactic acid bacteria (LAB) group, which have several scientifically proven effects on human health, such as antimicrobial activity, immune enhancement and anti-cancer activity. Their probiotic activity derives from the molecules they produce, including polysaccharides, which are used not only as ingredients but also especially as functional food additives. The wide spectrum of applications of microbial polysaccharides is in fact due both to their properties as thickeners or filmogens and, above all, to their immunomodulatory properties, i.e. anti-cancer, anti-inflammatory or antimicrobial.[3] Based on the above, the present work focuses on the study of cell wall polysaccharides produced by *Lactiplantibacillus plantarum*, a gram-positive, mesophilic bacterium belonging to the LAB group, which colonises the human and animal gastrointestinal tract. *L. plantarum* possesses probiotic activity and therefore this bacterium is associated with several beneficial effects due to its ability to: produce antimicrobial and antioxidant molecules, modulate the immune system and strengthen the intestinal microflora.[4] However, the probiotic activity of this bacterium is not fully associated with molecules whose structure and more general chemical nature is known. This is why the present work was based on the extraction, purification and structural characterisation of the capsular polysaccharide produced by this bacterium, with the aim of being able to associate the probiotic function with it.

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O3

FROM ANTARCTIC SEAS TO BIOTECHNOLOGICAL FRONTIERS: INVESTIGATING THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF *PSYCHROBACTER SP.* TAE2020 CAPSULAR POLYSACCHARIDE

Raffaele D'Amico,[a] Diana Olimpo,[a] Ermenegilda Parrilli,[a] Maria Luisa Tutino,[a] Angela Casillo,[a] Maria Michela Corsaro[a]

[a] Department of Chemical Sciences, University of Naples "Federico II ", Complesso Universitario Monte Sant'Angelo, via Cintia 26, Napoli, Italy. e-mail: raffaele.damico@unina.it

Biosurfactants are surface-active biomolecules produced by microorganisms, valued for their specificity, low toxicity, and ease of preparation, making them attractive for various applications.[1] The Antarctic bacterium *Psychrobacter sp*. TAE2020, an aerobic γ-proteobacterium isolated from coastal seawater [2], has shown significant potential in this field. This marine bacterium produces CATASAN, a capsular polysaccharide-protein complex with notable antibiofilm and emulsifying properties. [2,3] The aim of this study is to characterise the structure of the capsular polysaccharide of *Psychrobacter sp*. TAE2020, whose antibiofilm activity against *Staphylococcus epidermidis* has already been demonstrated. This nosocomial pathogen is known for its ability to form biofilms, leading to persistent infections associated with medical devices.[3] The isolated polysaccharide not only reduced biofilm formation but also facilitated the detachment of preformed biofilms. Additionally, the polymer exhibited excellent emulsifying activity, functional across a range of pH levels and temperatures, indicating its potential for various biotechnological applications. [1,2] Preliminary analysis using gas chromatography-mass spectrometry (GC-MS) revealed the presence of galactosamine as main component. In depth structural elucidation will be conducted using 1D and 2D nuclear magnetic resonance (NMR) spectroscopy. This study highlights the multifaceted biotechnological potential of *Psychrobacter sp.* TAE2020 for the development of novel anti-adhesive and emulsifying agents. [2,3]

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O4

STRUCTURE AND BIOSYNTHETIC ASPECTS OF THE O-ANTIGEN POLYSACCHARIDES OF TWO *E. COLI* STRAINS RELATED TO VARIANTS OF O99 AND O20 GENOTYPES

Sylvia Cuchman,[a] S.; María José Benítez-Galeano,[b] Edgardo Gianneechini,[c] Rodolfo Rivero, [c]
Nélida Rodríguez-Osorio,[b] Ilena Corvo, [b] Carolina Fontana [a],*

[a] Departamento de Química del Litoral, CENUR Litoral Norte, UdelaR, Uruguay. *cfontana@fq.edu.uy
[b] División Laboratorios Veterinarios "Miguel C. Rubino", MGAP, Uruguay
[c] Departamento de Ciencias Biológicas, CENUR Litoral Norte, UdelaR, Uruguay

Pathogenic *E. coli* strains can cause diseases such as neonatal calf diarrhea and bovine mastitis, leading to economic losses and reduced livestock productivity. *E. coli* strains SC-UY1, SC-UY4, and SC-UY5 were isolated from mesenteric lymph nodes during calf necropsies in Uruguay, whereas strains SC-UY2 and SC-UY3 were obtained from bovine mastitis cases. NMR spectroscopy analysis of the O-antigen (O-Ag) structures revealed that strains SC-UY2 and SC-UY3 belong to serogroups O1C and O88, respectively, while strain SC-UY5 was identified as serogroup O146 –representing the first report of these serogroups in Uruguay. In contrast, SC-UY1 and SC-UY4 displayed novel O-Ag structures that differ from any previously reported in *E. coli* or other species. These polysaccharides exhibit features of two distinct biosynthetic pathways: a linear heteropolysaccharide typical of the flippase/polymerase-dependent pathway (SC-UY1), and a D-rhamnan-type backbone consistent with the ABC transporter-dependent pathway (SC-UY4); interestingly, the latter O-Ag is decorated with uncommon D-Xyl*f* residues.

To investigate the genetic basis of these structures, the O-antigen gene clusters located between *galF* and *gnd* were amplified and sequenced using a MinION sequencer (Oxford Nanopore Technologies), yielding sequences of 16,120 bp (SC-UY1) and 20,100 bp (SC-UY4). The online version of SerotypeFinder[1] was used for *in silico* serotyping of the two *E. coli* strains, classifying SC-UY1 as *E. coli* O20 (100% identity for both *wzx* and *wzy*) and SC-UY4 as *E. coli* O99 (99.62% identity for *wzm* and 99.69 % for *wzt*). Notably, these two serogroups had previously been identified in Uruguay using PCR-based genotyping,[2] but the O-Ag structures elucidated herein differ markedly from those described previously for the international reference strains.[3-5]

In particular, the biosynthesis of the *E. coli* O20 O-Ag structure reported in the literature has been attributed to gene clusters located outside the *galF–gnd* locus, suggesting that the later may be non-functional. However, the O-Ag structure of strain SC-UY1 perfectly match the genetic information encoded in the canonical *galF–gnd* gene cluster of the reference strain (GeneBank accession number KJ778793), which shares 99.96% sequence identity with our data. The variability in the expression of the O-Ag may explain the inconsistent results previously observed in the serological reaction performance of *E. coli* O20-specific antisera.[6] Functional annotation of both gene clusters was performed using BLAST analysis and correlated with the structural features of the O-Ags. Remarkably, SC-UY1 features the uncommon presence of D-Xyl*f* residues, branching from the rhamnose backbone –instead of the glucose residues observed in the international reference strain of *E. coli* O99– following a biosynthetic mechanism like that described for *C. youngae* O2.[7]

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O5

INTO THE UNDERSTANDING OF THE MOLECULAR MECHANISMS OF POLYMYXIN B RESISTANCE IN *PROTEUS MIRABILIS*

Wiesław Kaca[1], Wanesa Sasal[1], Aleksandra Omelaniuk[1], Klaudia Musiał[1], Leon Petruńko[1], and Dawid Gmiter[1,2]

[1] Department of Microbiology, Institute of Biology, Jan Kochanowski University in Kielce
[2] Department of Environmental Microbiology and Biotechnology, Institute of Microbiology, University of Warsaw

Proteus mirabilis, an opportunistic human pathogen, is known for its natural resistance to polymyxin B (PB). This resistance is thought to result from the presence of a 4-amino-4-deoxy-L-arabinose (L-Ara4N) moiety in the lipid A component of lipopolysaccharide (LPS). In the genome of the reference strain *P. mirabilis* HI4320, two open reading frames encoding the *arnT* gene—a putative L-Ara4N transferase—have been identified: (1) PMI1047, located within the arn gene operon, and (2) an orphan form, PMI0275.

The PhoPQ and RppAB two-component systems (TCSs) are known to regulate L-Ara4N-dependent polymyxin B resistance in many bacterial species. However, their roles in *P. mirabilis* remain poorly understood.

Our *in silico* analysis revealed a high degree of genetic conservation in the studied genes across *P. mirabilis* strains. Further investigation suggested that PhoP may regulate the expression of the *arn* gene operon as well as *rppA*. Gene expression analysis in the presence of PB showed that both versions of *arnT* were upregulated by PB, with a more pronounced effect observed for PMI0275. Conversely, PB exposure inhibited *phoP* expression while enhancing *rppA* expression.

This study presents, for the first time, a comprehensive analysis of the molecular interactions between TCSs and *arnT* genes in response to PB. Our findings shed new light on the mechanisms underlying polymyxin B resistance in *P. mirabilis*.

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O6

STRUCTURE OF THE O-ANTIGEN OF THE *KLEBSIELLA OXYTOCA* 0.067 LPS AND ITS CONTRIBUTION TO SEROLOGICAL CROSS REACTIONS

Agata Palusiak,[a] Anna Maciejewska,[b] and Jolanta Łukasiewicz[b]

[a] Department of Biology of Bacteria, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland, E-mail: agata.palusiak@biol.uni.lodz.pl
[b] Laboratory of Microbial Immunochemistry and Vaccines, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12,53-114 Wrocław, Poland

K. oxytoca is a member of *Enterobacteriaceae* bacilli, widely distributed in nature and also found in the human gut and oropharnyx microbiota. *K. oxytoca* is emerging as a crucial human pathogen implicated in an antibiotic-associated hemorrhagic colitis, urinary tract infections or bacteremia, including also the hospital-acquired cases [1, 2]. Among many virulence factors, *K. oxytoca* also produces lipopolysaccharide (LPS), an important endotoxin with a crucial role in developing the septic shock, however, in contrast to *K. pneumonia* LPS, its structures and immunogenicity remain poorly understood [2]. Thus, the aim of the present study was serological and structural characterization of the O-polysaccharide of LPS from the *K. oxytoca* 0.067 strain, coming from the urine of a patient of the Łódź area.

LPS was extracted from the *K. oxytoca* 0.067 strain by Westphal method and purified by means of trichloroacetic acid, dialysis and ultracentrifugation. This strain was chosen, since its biomass was recognized by a few *Proteus* antisera in Western blotting in a way characteristic to polysaccharides. Thus, the *K. oxytoca* 0.067 LPS was also tested in Western blotting where it was recognized by the sera specific to some *Proteus* O-serogroups. The observed cross reactions concerned the high-molecular mass fractions of LPS. The high-molecular-mass O polysaccharide obtained after mild acid hydrolysis of the *K. oxytoca* 0.067 LPS was chemically tested by means of mass spectrometry, methylation and sugar analyses, NMR spectroscopy, including 2D experiments COSY, TOCSY, HSQC, NOESY, HMBC, HSQC-TOCSY. The structural analyses revealed that the O-repeating unit of the *K. oxytoca* 0.067 LPS is a homopolymer of galactose composed of four Gal*p* residues and one Gal*f* residue, which form two galactans. The *K. oxytoca* 0.067 LPS showed in Western blotting a banding pattern similar to that of the *K. pneumonia* O1 LPS, which suggests that galactans may be common components of O-antigens not only in *K. pneumonia* LPSs [3], but also in *K. oxytoca* LPSs.

Determining the structure of the O-antigen from *K. oxytoca* 0.067 LPS and detecting its cross-reactions with *Proteus* antisera allow recognizing its role as an immunogenic antigen, which may induce cross-protection. The obtained results will enrich the knowledge of this emerging species of growing clinical importance.

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O7

MECHANISM OF CONTROL OF MEMBRANE FLUIDITY AND THE REGULATION OF THE LPXC MEDIATING THE FIRST COMMITTED STEP OF LIPOPOLYSACCHARIDE BIOSYNTHESIS

Gracjana Klein,[a] Marta Jeschke,[a] Akshay Maniyeri Suresh,[a] Aravind Ayyolath,[a] Satish Raina,[a]

[a] Laboratory of Bacterial Genetics, Faculty of Chemistry, Gdansk University of Technology, Narutowicza 11/12, 80-233 Gdansk, Poland, gracjana.klein@pg.edu.pl

Gram-negative bacteria, such as *Escherichia coli,* maintain a tight balance between lipopolysaccharide (LPS) and phospholipids levels, which are the two essential components of the outer membrane. This is achieved by controlling the flux of the common metabolic precursor *R*-3-hydroxymyristate-ACP into their biosynthetic pathways. This involves the proteolytic control of LpxC by the FtsH-LapB complex. Interestingly, proteolysis of LpxC is adjusted according to the cellular demand for LPS by LapC, since LapC can bind LPS and LapB. However, the mechanism of LPS recognition by LapC and the interaction between LapB and LpxC remain unknown. Here, we identified specific amino acid residues in the LapC N-terminal domain that mediate LPS recognition, which was supported by isothermal titration calorimetry. Furthermore, several mutations in the *lapB* gene were isolated as extragenic suppressors, identifying the involvement of the TPR2 and TPR3 domains of LapB in the interaction with LpxC. LpxC amounts can also be regulated by ppGpp, fatty acid composition and acetyl-CoA levels. However, the mechanism by which they are sensed remains unknown. Our data reveal that increasing the ratio of unsaturated to saturated fatty acids by overexpression of the *gnsA* gene can stabilize LpxC and suppress Ts phenotype *lapC* mutants, while inducing ppGpp synthesis confers lethality that can be overcome by loss-of-function mutations in the *lapB* gene. We further show that LpxC amounts are modulated by WaaH, which mediates the inner core modification by GlcUA on the heptose III. Overall, this work describes the mechanism of LPS recognition by LapC, how LapB interacts with LpxC, and how membrane fluidity contributes to LpxC stability.

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O8

MECHANISM OF CONTROL OF MEMBRANE FLUIDITY AND THE REGULATION OF THE LPXC MEDIATING THE FIRST COMMITTED STEP OF LIPOPOLYSACCHARIDE REQUIRES A NEW PLAYER GNSA

Satish Raina,[a] Gracjana Klein,[a]

[a] Laboratory of Bacterial Genetics, Faculty of Chemistry, Gdansk University of Technology, Narutowicza 11/12, 80-233 Gdansk, Poland, satish.raina@pg.edu.pl

The cell envelope of diderms is comprised of an asymmetric outer membrane containing lipopolysaccharide (LPS) in the outer leaflet and phospholipids (PL) facing inwards, an inner membrane, which are separated by a thin layer of peptidoglycan. Bacteria, such as *Escherichia coli*, have a coordinated mechanism to maintain the homeostasis of these three essential components. The establishment of cross-talk between cell envelope machineries underlines this intricate metabolic network, which is the generation of acetyl-ACP by malonyl-ACP decarboxylase. Acetyl-ACP supports the initiation of fatty acid synthesis (FASII) and phospholipid synthesis by entering FASII. Our recent study showed that *E. coli* utilizes three major hubs to coordinate LPS and PL biosynthesis. One such hub is made by LapB, whose interactome contains at least 30 proteins, involved in LpxC degradation and coordination with PL biosynthesis via interaction with FabZ. LapB also interacts with another protein, LapD. LapD constitutes the second hub, since its partners include committed enzymes of FAS II and the acetyl-CoA carboxylase enzyme. As acyl-ACP is also used in the biosynthesis of PL and LPS, its availability acts as a regulatory checkpoint to maintain their balance. The third major point of regulation involves the hub constituted by the acyl carrier protein AcpP and a new thioesterase, TesD. This hub regulates the activity of PlsB, mediating the first step in phospholipid synthesis to generate lysophosphatidic acid from long-chain ACP and *sn*-glycerol-3 phosphate. Here, we describe the functional analysis of TesD and a new regulatory protein, SsrA, involved in the generation of acetyl-ACP.

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O9

STUDIES ON LIPIDS A FROM *BRUCELLA CITISI* AND *BRUCELLA LUPINI* A NODULE FORMING BACTERIA ISOLATED FROM *CYTISUS SCOPARIUS* AND *LUPINUS ALBUS*, RESPECTIVELY

Katarzyna Zamłyńska[a], Kamil Żebracki[a], Zbigniew Kaczyński[b], Małgorzata Pac-Sosińska[c], Iwona Komaniecka[a], and Adam Choma[a]

[a] Department of Genetics and Microbiology, Institute of Biological Science, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland, E-mail: katarzyna.zamlynska@mail.umcs.pl
[b] Department of Medical Chemistry, Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308, Gdansk, Poland
[c] Department of Virology and Immunology, Institute of Biological Science, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland

Brucella citisi and *B. lupini* (previously classified as *Ochrobactrum citisi* and *O. lupini*) belong to *Brucellaceae* family in the *Rhizobiales* order. This genus comprises about twenty species, including: human/mammalian pathogens - *B anthropic* and *B. abortus;* rhizosphere/endophyte bacteria - *B. oryzae* and *B. rhizosphaerae;* as well as legume endosymbionts - *B. cytisi* and *B. lupini* nodulating *Citisus scoparius* and *Lupinus albus,* respectively.[1] *Brucella cytisi* strain ESC1T and *B. lupini* LUP21T (type strains) were used in the study. The LPSs preparations were isolated from bacterial pellets with the classical hot 45% phenol/water extraction. LPS suspensions were hydrolyzed with 1 % acetic acid. The liberated lipids A were separated by centrifugation from hydrophilic components and next purified by a two-phase Bligh–Dyer system (chloroform/methanol/hydrolysate 2/2/1.8 (v/v/v)). Both lipids A preparation were structurally characterized based on chemical methods as well as MALDI-TOF mass spectrometry and nuclear magnetic resonance spectroscopy. 2,3-diamino-2,3-dideoxy-D-glucose (D-Glc*p*N3N) was identified as the only sugar component of the lipid A backbones. Fatty acid analysis revealed the presence of: 14:0(3-OH), 16:0(3-OH), and traces of 18:0(3-OH). All of them as a primary fatty substituents of a sugar backbone were amide-linked residues. A lactobacillic (19:0cyc) and a very long chain fatty acid - 27-hydroxyoctacosanoic (28:0(27-OH)) acids were found as ester-linked secondary substituents. *B. cytisi* and *B. lupini* lipid A preparations contained several glycolipid species. Fatty acid distribution revealed to be asymmetrical, and acylation pattern was established as (4:2). The very long chain fatty acid was in turn partly esterified by a 3-hydroxybutyroyl residue. Two unsubstituted 3-hydroxyfatty acids were linked directly to the proximal D-Glc*p*N3N residue. Phosphate residues were connected to the distal and proximal D-Glc*p*N3N in the lipid A backbones of about half of lipid A molecules pool. The final structure of the lipids A sugar backbone was confirmed by analyzing the 2D NMR spectra. Having the genome sequences of both investigated strains, a comparative analysis of selected genes encoding enzymes of the lipid A biosynthetic pathway was also performed.

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O10

STRUCTURAL AND CONFORMATIONAL INSIGHTS INTO BACTERIAL LECTIN RECOGNITION OF HOST RECEPTORS

Angela Marseglia[a*], Mario Fares[b,c,d], Alexander Titz[b,c,d], Roberta Marchetti[a]

[a] Department of Chemical Sciences, University of Naples Federico II, Via Cintia 4, 80126 Naples, Italy. E-mail address: angela.marseglia@unina.it
[b] Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Campus E8.1, 66123 Saarbrücken, Germany.
[c] Deutsches Zentrum für Infektionsforschung (DZIF), Standort Hannover – Braunschweig, Germany
[d] Department of Chemistry, PharmaScienceHub, Saarland University, 66123 Saarbrücken, Germany

Lectins play a crucial role in bacterial colonization of host tissues and have emerged as promising therapeutic targets due to their involvement in host-pathogen interactions [1]. The increasing threat of antimicrobial resistance (AMR) is rendering standard antibiotics ineffective, necessitating the development of novel treatment strategies against multidrug-resistant pathogens, particularly Gram#negative ESKAPE pathogens. Targeting bacterial lectins, such as LecA and LecB in *Pseudomonas aeruginosa*, has been shown to effectively interfere with bacterial virulence and biofilm formation, offering a promising alternative to traditional antibiotics [2].

In this context, an orthologue of LecA, named EclA, has been identified in *Enterobacter cloacae*, a member of the human gut microbiota that can also act as an opportunistic pathogen in immunocompromised patients.
The crystal structure of EclA, in complex with methyl α-L-selenofucoside, revealed a unique two-domain architecture, consisting of a dimeric N-terminal LecA-like domain and a novel dimeric C-terminal carbohydrate-binding domain. This domain adopts an unprecedented intertwined β-sheet dimeric structure, suggesting a binding mode distinct from previously characterized bacterial lectins and indicating a potential role for EclA as a cross-linker for specific host glycans. Glycan array analysis demonstrated high specificity of EclA for fucosylated blood group antigens, particularly LewisA and H-type II [3], in contrast to LecA, which binds galactose-containing sugars.

Despite these findings, the molecular mechanisms underlying EclA's interaction with fucosylated ligands remained unresolved. To address this gap, a multidisciplinary approach was employed, using saturation transfer difference (STD) NMR to obtain ligand epitope mapping, trNOESY for the identification of the bioactive conformation, and computational studies to generate a three-dimensional model of the complexes. Additionally, isothermal titration calorimetry (ITC) was used to quantify binding thermodynamics and affinities, providing a comprehensive understanding of EclA-ligand interactions. These findings not only deepen insights into *E. cloacae* adhesion mechanisms but also lay the groundwork for developing anti-adhesion therapies targeting bacterial lectins in drug-resistant infections.

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O11

CATALYTIC INSIGHTS INTO KP34GP57, A PHAGE-DERIVED DEPOLYMERASE TARGETING *KLEBSIELLA* K63 CAPSULAR POLYSACCHARIDES

Ferran Nieto-Fabregat,[a]* Flavia Squeglia,[b] Roberta Marchetti[a]

[a] Department of Chemical Sciences and Task Force for Microbiome Studies, University of Naples Federico II, Via Cinthia 4, 80126, Naples, Italy. E-mail: ferran.nietofabregat@unina.it
[b] Institute of Biostructures and Bioimaging, CNR, Napoli, Italy.

Capsular polysaccharides (CPS) play a critical role in bacterial immune evasion and virulence, especially in hypervirulent strains of *Klebsiella pneumoniae*.[1] Bacteriophage-encoded depolymerases have emerged as attractive candidates for therapeutic or biotechnological applications due to their ability to degrade CPS in a highly specific manner. Among them, KP34gp57 is a tailspike depolymerase with demonstrated activity against the K63 CPS serotype, yet its catalytic mechanism and substrate recognition strategy remain poorly understood.[2] Thus, we combined biophysical and computational approaches to characterise the interaction of KP34gp57 with exopolysaccharidic substrates derived from the K63 serotype of *Klebsiella pneumoniae*. Through Saturation Transfer Difference (STD) NMR, kinetic profiling, and cavity prediction analyses, we provide insights into the enzyme's substrate specificity, binding mode, and a plausible catalytic mechanism that complements and extends the available crystallographic data.

To do so, the exopolysaccharide was extracted, characterised, and subjected to enzymatic digestion, isolating oligosaccharides of different lengths. Among them, the trisaccharide was used for defining the epitope mapping, while the hexasaccharide was used as minimal hydrolysable substrate, allowing us to define the cleavage site and suggesting a retaining mechanism of the hydrolysis.

Additionally, to probe the catalytic mechanism, a 1-μs molecular dynamics simulation of the apo form of the protein was performed. The analysis revealed the putative catalytic dyad involved in the EPS recognition process. This hypothesis was reinforced by cavity prediction and residue scoring using GlycanInsight.thus defining the catalytic architecture of KP34gp57. Altogether, our findings support a retaining Fuc(α1–3)-hydrolase activity in KP34gp57 and allows to propose a mechanistic model in which active site flexibility facilitates effective depolymerisation of high molecular weight CPS. Site-directed mutagenesis and advanced QM/MM simulations are currently underway to clarify the enzymatic mechanism at molecular level.

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O12

BIOINFORMATIC IDENTIFICATION AND MODELING OF CHLOROVIRUS PBCV-1 MULTIDOMAIN PROTEIN A111/114R

Antonella Aquilone,[a] Immacolata Speciale,[a] Anna Notaro,[a] Antonio Molinaro,[a] Cristina De Castro[a]

[a]: Department of Chemical Sciences, University of Naples Federico II, via Cintia 26-80126, Napoli (IT) - a.aquilone@ssmeridionale.it

Paramecium bursaria chlorella virus 1 (PBCV-1) is a large, double-stranded DNA virus that is known for its unusual N-glycosylation process. One of the unique aspects of PBCV-1 is its capability, unlike other viruses, to encode most, if not all, of the components required to glycosylate its major capsid protein called VP54.[1] The structure of the four N-linked glycans from the PBCV-1 VP54 is a nonasaccharide, not found elsewhere in the three domains of life.[2] Interestingly, all chloroviruses known to date share a unique conserved central core composed of four monosaccharides: an N-linked glucose, to which a xylose unit and a hyperbranched fucose are linked, and a galactose bounded to the latter. Additional monosaccharides decorate this core N-glycan, and their combination is unique for the virus, considered so that they can be considered as a molecular signature.[3] The atypical structures of this N-glycan have prompted the investigation of the genome of this virus to understand which enzymes are involved in N-glycosylation, revealing that PBCV-1 encodes at least eight putative glycosyltransferases (GTs).[4] To date, the function of some of them has been demonstrated, and here we focus attention on the protein responsible for the assembly of the oligosaccharide core: A111/114R.[5] Using bioinformatic approaches, such as HHPred, Blast, and structural alignments on the Express server, it was understood that the protein is composed of three transferase domains, each with a specific function, similar to the A064R protein, another PBCV-1 protein. In detail, the N-terminal domain (1–250 aa) is a galactosyltransferase (GalT), the central domain (260–491 aa) is a xylosyltransferase (XylT), and the C-terminal domain (560–860 aa) is a fucosyltransferase (FucT).[5] Investigation via Alphafold was used to create a model of the enzyme, useful to identify the boundaries between the domains. Based on this information, several vectors were designed for expression in bacterial cells to experimentally evaluate the function of each domain. Preliminary biochemical data confirm the predicted activity of the protein, and current studies are now in progress to define the activity of each single domain.

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O13

ISOTOPICALLY LABELLED CARBOHYDRATES FOR NMR STUDIES OF PROTEIN-CARBOHYDRATE INTERACTIONS

Gustav Nestor

Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, gustav.nestor@slu.se

Carbohydrates used in structural studies by NMR are rarely 13C-labelled, preventing exploitation of the 13C spectral dispersion in 3D or higher order NMR experiments, which would alleviate severe resonance overlap in their 1H NMR spectra. This is in part associated with the lack of easily accessible isotope-labelled sugar molecules. In this presentation, I will describe our strategy for isotopic labelling, which is based on uniformly 13C-labelled sugars and, in the case of aminosugars, also 15N-labelling. I will discuss challenges with the production of isotopically labelled sugars, the lack of suitable NMR experiments for such compounds, and what information that can be collected from protein-carbohydrate interactions. With inspiration from protein NMR experiments, we are adapting NMR experiments for tailored analysis of the structure and dynamics of isotopically labelled carbohydrates. Our aim is to provide NMR tools for detailed characterization of glycan moieties in glycoproteins, carbohydrate-binding proteins, and carbohydrate-active enzymes.

Examples of isotopic labelling of glycoproteins, cellulose and hyaluronan[1] will be shown, as well as conformational analysis of the *N*-acetyl group of 13C,15N-labelled *N*-acetylglucosamine by measurement of eight *J*-couplings.[2] Using 13C-labelled carbohydrates and 15N-labelled proteins for NMR studies on protein-carbohydrate interactions, we can map the binding of both the protein and the carbohydrate binding interface.[3-4] In addition, individual sugar hydroxyl protons in hydrogen bonds with the protein can be observed for characterization of the hydrogen-bonding network.[5]

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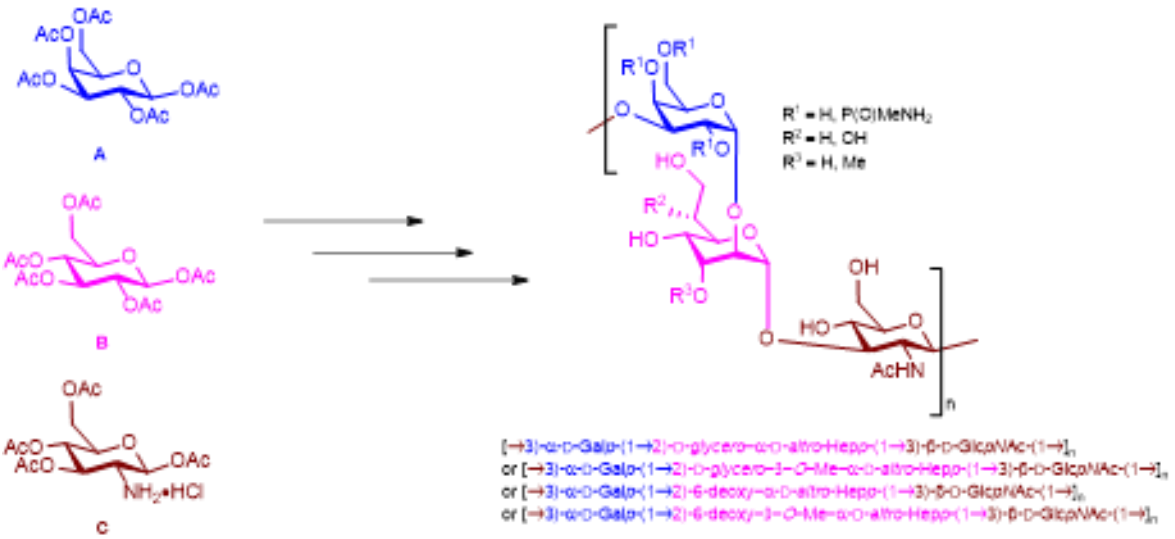
O14

SYNTHESIS OF TRISACCHARIDE REPEATING UNITS OF *CAMPYLOBACTER JEJUNI* HS:23/36

Marie-Claire Jiang[a, b], David A. Clément[a], Davide Dell'Accantera[a], Laurence A. Mulard[a]

[a] Chimie des Biomolécules, Department of Structural Biology and Chemistry, Institut Pasteur, Université Paris Cité, CNRS UMR3523, F-75015 Paris, FRANCE, marie-claire.jiang@pasteur.fr
[b] Université Paris Cité, ED N°563, 4 avenue de l'observatoire, 75006 Paris, FRANCE

Diarrhoeal diseases are the third cause of death in children under the age of 5, accounting for 400 000 deaths globally each year.[1] Bacteria of the genus *Campylobacter* fall within the four most common causes of diarrhoeal diseases.[2] Infections, especially frequent in young children in developping countries, are often attributed to *C. jejuni*.[3] Considering the threat of anti-microbial resistance (AMR), means of prevention against infection are to be developped, including vaccination. Conjugate vaccines based on capsular polysaccharides (CPS) represent an attractive strategy to mitigate the burden of campylobacteriosis.[4] This approach has been successfully applied to the developpement of a semi-synthetic conjugated vaccine against *Shigella flexneri* 2a by our group.[5]



Here, focus is on, *C. jejuni* HS:23/36, one of the most common serotypes, the CPS repeating unit (RU) of which is a trisaccharide composed of a D-galactose (A) bearing a non-stoechimoetric methyl phosphoramidate (MeOPN) moiety, a rare D-*altro*-heptose that can be D-*glycero* or 6-deoxy, and/or 3-*O*-methyl (B) and an *N*-acetyl D-glucosamine (C). Design and large scale synthesis of orthogonally protected A, B and C monosaccharides (up to 50 g) will be presented. Next, the assembly of the most promising building blocks into frame-shifted trisaccharides ready for elongation at both ends will be discussed.

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O15

STRUCTURAL BIOLOGY APPROACHES FOR THE CHARACTERIZATION OF *SHIGELLA* O-ANTIGEN SPECIFIC MONOCLONAL ANTIBODIES

O. Serpino[a, b], G. Vezzani [b], R. Alfini [b], E. Boero [b], R. Di Benedetto [b], G. Batani [c], M. Ridelfi [c], E. Roscioli [c], O. Rossi [b], C. Sala [c], F. Micoli [b], A. Molinaro [a], C. Giannelli [b], A. Silipo [a]

[a] Department of Chemical Sciences, University of Naples Federico II, Via Cintia 4 Naples, Italy
[b] GSK Vaccines Institute for Global Health, Via Fiorentina 1 Siena, Italy
[c] Monoclonal Antibody Discovery Laboratory, Fondazione Toscana Life Sciences, Siena, Italy

Shigella is leading cause of diarrheal infections, especially in developing countries1. Recent estimates report approximately 270 million diarrhea episodes due to *Shigella*, with around 212,000 deaths of which 64,000 are in children under 5 years in underdeveloped countries2. Antibiotic resistance of *Shigella* is increasing and common therapeutic antibiotics against shigellosis have become progressively less efficient. The World Health Organization (WHO) has listed *Shigella* as a priority pathogen for the development of new therapeutic solutions3. Currently there are no licensed vaccines available against *Shigella* but several candidates, based on the O-antigen (O-Ag) moiety of lipopolysaccharides (LPSs) of the outer membrane of these bacteria, are in development4. In fact, the O-Ag is involved in many interactions between pathogen and host and has been recognized as a key protective antigen5. Multi-component vaccines are needed to cover *Shigella* O-Ag diversity and have good coverage. Recently, monoclonal antibodies (mAbs) against *Shigella* O-Ag have been isolated from subjects vaccinated with a *Shigella* 4-component vaccine6 in development or after exposure to *Shigella* challenge. Importantly functional potent mAbs were identified that demonstrated ability to protect mice from challenge in a dose dependent manner. In this study we have developed innovative tools to characterize the O-Ag epitopes responsible for protection and cross-reactivity, avoiding the use of synthetic oligosaccharides. The O-Ag was extracted from Generalized Modules for Membrane Antigens (GMMA), exosomes released from different *Shigella* strains7 engineered to reduce the O-Ag size6, and next subjected to different purification steps. Chemical and structural characterization of the O-Ag was performed via compositional, MS and NMR analysis. Finally, ligand-based NMR approaches were successfully applied to the characterization of O-Ag specific human mAbs, to map recognition and binding process and define epitope mapping ligands bioactive conformation. The methodology developed opens the possibility to generate many O-Ag oligomers with various structural features to better characterize anti-O-Ag specific mAbs, providing useful information on epitope structural characteristics essential for protection, which can be relevant for *Shigella* vaccine design or refinement. This work also supports the use of mAbs as alternative intervention to fight *Shigella* disease.

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O16

CHEMOTYPING OF BACTERIAL GLYCANS USING NMR SPECTROSCOPY. HIGH-RESOLUTION MAGIC ANGLE SPINNING NMR IN THE ANALYSIS OF O-ANTIGENS

Tomasz Niedziela

Department of Immunochemistry, Hirszfeld Institute of Immunology & Experimental Therapy, Polish Academy Of Sciences, Wrocław, PL, tomasz.niedziela@hirszfeld.pl

Bacteria expose on the surface complex glycans that are essential for structural integrity and interactions with hosts. Gram-negative bacteria produce lipopolysaccharides (LPS), which are the major component of the outer cell membrane - essential for physical organization and function. They constitute the most exposed antigens in non-encapsulated bacteria and targets for specific antibodies.

High-resolution magic angle spinning NMR spectroscopy (HR-MAS NMR) is a unique technique, that allows for the investigation and validation of glycans directly on the surface of intact bacteria[1]. The structural identities of the O-antigens on the surface of bacteria, but also in the isolated LPS and O-specific polysaccharides[2] can be assessed. When combined with the structure reporter group concept[3] the technique is suitable for detection of chemical differences between strains, without a prior knowledge of the glycan structures, as the changes in the HR-MAS NMR spectra provide immediate distinction between differing structures. This approach allows to chemotype and classify bacteria according to the NMR detected structural features of the surface glycans. The possible applications of the HR-MAS NMR technique in chemotyping of bacterial surface carbohydrates, including the benefits and downsides as well as prospects will be presented and discussed.

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O17

STRUCTURAL DIVERSITY AMONG *AEROMONAS SALMONICIDA* O-POLYSACCHARIDES ISOLATED FROM FISH AS AN EPIDEMIOLOGIC TOOL

Karolina Ucieklak [a], Sabina Koj [a], Olga Rojewska [a], Laëtitia Le Devendec [b], Sandrine Baron [b], Marta Kaszowska [a]

[a] Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, R. Weigla 12, Wroclaw, Poland
[b] Mycoplasmology-Bacteriology and Antimicrobial Resistance unit, Ploufragan-Plouzané-Niort Laboratory, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Ploufragan, France

Aeromonas salmonicida is an important pathogen of fish, producing the systemic disease furunculosis. Since the annual worldwide losses of farmed fish due to diseases involve millions of dollars, this pathogen has been subjected to considerable investigation [1]. One of the principal virulence factors of this pathogen is an A-layer that consists principally of a 2-dimensional crystalline tetragonal protein (A-protein) array, which is tethered to the cell by lipopolysaccharide (LPS). The A-layer appears to cover most of the surface of virulent *A. salmonicida*, although some LPS may also be exposed. This complex is responsible for adherence to fish macrophages, resistance to makrophage cytotoxicity and increasing the bacterial surface hydrofobicity. The LPS is one of the major structural and immunodominant molecules of the outer membrane. It consists of three domains: lipid A, core oligosaccharide, and O-specific polysaccharide (O-antigen). The chemical structure of LPS molecule together with the A-layer can strongly determine the bacterial resistance against antibiotics, what has been known as a problem for many decades. Before our studies, only one structure of *A. salmonicida* lipid A, one core oligosaccharide and three O-polysaccharide have been identified (named A, B, C) and published [2-7].

The 51 of *A. salmonicida* strains from different fish farms (geographical distribution) and from ill and healthy fish have been isolated. The O-polysaccharides were compared by using ^{1}H,^{13}C high-resolution magic angle spinning (HR-MAS) NMR spectroscopy. The structure A has been identified in 42 strains, the structure B in 7. The structure C has not been identified. Additionally, the O-polysaccharide structure in strains 11/A/658 and 10/A/646 (both isolated from ill fish) has been identified as a new one [8]. This new structure was investigated by ^{1}H and ^{13}C NMR spectroscopy and matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The following structure of the new linear hexasaccharide repeatig unit of A. salmonicida O-antigen has been established:

→3)-b-L-Rhap-(1→3)-α-D-ManpNAc-(1→2)-β-D-Glcp-(1→3)-α-L-Rhap2OAc4OAc-(1→3)-β-D-ManpNAc-(1→3)-α-D-Glcp-(1→

Identification of *A. salmonicida* O-polysaccharides (different O-serotypes) presented in ill fish could be very useful for veterinarian either to confirming the etiologic agent of disease or in improving fish farms biosecurity, by providing a quick tool to detect the presence of pathogenic *A. salmonicida* O-polysaccharides before the level of the pathogen reaches density and causes disease. The structural identification of *A. salmonicida* O-polysaccharides could contribute to reducing and improving the usage of antibiotics in fish farms, which considered a potential hotspot for antimicrobial resistance dissemination.

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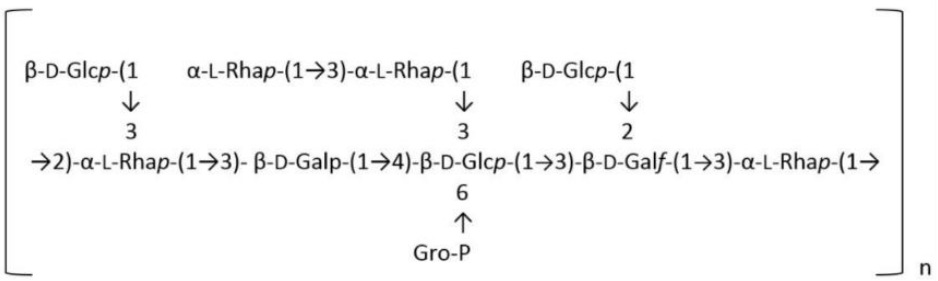
O18

IMPACT OF DEPHOSPHORYLATION ON THE STRUCTURE AND FUNCTION OF B.PAT POLYSACCHARIDE ISOLATED FROM *BIFIDOBACTERIUM ANIMALIS* SSP. ANIMALIS CCDM 218

Katarzyna Pacyga-Prus[a], Corine Sandström[b], Dagmar Šrůtková[c], Martin Schwarzer[c], Sabina Górska[a]

[a] Department of Microbiology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114, Wroclaw, Poland
[b] Department of Molecular Sciences, Swedish University of Agricultural Sciences, Box 7015, SE-750 07, Uppsala, Sweden
[c] Laboratory of Gnotobiology, Institute of Microbiology, Czech Academy of Sciences, 549 22, Novy Hradek, Czech Republic

A growing number of publications are focusing on the impact of different bacteria strains on the prevention or treatment of various diseases. However, some of them shifted their focus from live and proliferating microorganisms to their smaller, easy-to-define components, that exhibit similar or enhanced properties compared to their bacteria of origin. These molecules associated with beneficial effects on the host include among others polysaccharides (PS), lipoteichoic acids, proteins or peptidoglycan, and were named by ISAPP (International Association of Probiotics and Prebiotics) as "postbiotics"[1]. B.PAT PS is a surface antigen isolated from the surface of *Bifidobacterium animalis* ssp. animalis CCDM 218. In our work, we aimed to describe its structure and properties and understand the relation between those two factors. Thorough investigation showed that B.PAT is a nonasaccharide of molecular mass approximately 1.96 x 104 consisting of glucose, galactose, and rhamnose residues substituted by glycerol phosphate and creating the following repeating unit:



To explore the role of B.PAT, we decided to investigate the impact of its glycerol phosphate substitution on exhibited properties. For this reason, we prepared a dephosphorylated counterpart (called B.- MAT) and tested their immunomodulatory properties on bone marrow-derived dendritic cells (BMDCs). Investigation of obtained results indicated that the loss of glycerol phosphate in the structure of B.PAT resulted in increased production of IL-6, IL-10, IL-12, and TNFα. Moreover, both PSs were able to enhance the effect of known immunomodulatory bacteria - Lacticaseibacillus rhamnosus GG, however, this effect remained stronger for B.MAT. Further analysis performed on Caco-2 and HT-29 cells showed the enhanced ability of a dephosphorylated compound to suppress IL-1βinduced inflammatory response. Finally, the prediction of 3D models of both PSs indicated that the loss of Gro-P strongly affected the spatial structure of B.PAT. Thus, we suggest that the dephosphorylation of polysaccharides may lead not only to structural and spatial changes but also impact the properties exhibited by those molecules. These findings indicate, that the introduction of modifications to bacterial compounds may induce better functionalities of bacterial polysaccharides, which can be exploited in the treatment of various diseases and may constitute an alternative to probiotics.

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O19

HOW *SALMONELLA* ENTERITIDIS EVADES THE INNATE IMMUNITY MECHANISMS USING THE LENGTH OF LIPOPOLYSACCHARIDE MOLECULES

Eva Krzyżewska-Dudek[1], Vinaya Dulipati[2], Katarzyna Kapczyńska[1], Mateusz Noszka[3], Carmen Chen[2], Juha Kotimaa[2,4], Marta Książczyk[5], Bartłomiej Dudek[6], Gabriela Bugla-Płoskońska[5], Krzysztof Pawlik[3], Seppo Meri[2,7], Jacek Rybka[1#]

[1] Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
[2] Department of Bacteriology and Immunology, Translational Immunology Research Program, University of Helsinki, Helsinki, Finland
[3] Department of Microbiology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
[4] VTT Technical Research Centre of Finland Ltd, Espoo, Finland
[5] Department of Microbiology, Faculty of Biological Sciences, University of Wrocław, Wrocław, Poland
[6] Platform for Unique Models Application (P.U.M.A), Department of Pharmaceutical Microbiology and Parasitology, Faculty of Pharmacy, Wroclaw Medical University, Wroclaw, Poland
[7] HUSLAB Diagnostic Center, Helsinki University Central Hospital, Helsinki, Finland

Bacterial resistance to bactericidal activity of the serum is a crucial virulence factor for the development of systemic infections. The most important component of innate immunity in serum is the complement system, which consists of a cascade of proteins activated upon the recognition of foreign antigens. The recognition and activation of complement cascade leads to inflammatory response, attraction of phagocytic cells and elimination of microorganisms.

Non-typhoidal *Salmonella* (NTS) infections are a serious public health problem. *Salmonella* has the ability to escape bactericidal action of the complement systemusing various mechanisms. Surprisingly, one of the mechanisms is the adaptation of the length of the lipopolysaccharide (LPS) molecule. LPS is a main component of the outer membrane of Gram-negative bacteria, consisting of three structural domains: lipid A, core oligosaccharide and O-specific chain (O-antigen). O-antigen is built of varying number repeating oligosaccharide units (from one to over one hundred repeating units). In some bacteria, including *Salmonella*, the distribution of LPS molecules length creates a distinct modal pattern. We investigated the effect of the O-antigen modal length composition of LPS molecules on the surface of *S.* Enteritidis cells on its ability to evade host complement responses. Using specific deletion mutants we analysed the role of different O-antigen fractions in complement evasion, additionally, we assessed the aspect of LPS O-antigen chain length distribution in *S.* Enteritidis virulence *in vivo* in the *Galleria mellonella* infection model. Data obtained in the complement activation assays and *in vivo* study clearly demonstrated that *S.* Enteritidis bacteria require LPS with long O-antigen to resist the complement system and to facilitate bacterial survival *in vivo* in the *Galleria mellonella* model.

O20

CHEMICAL CHARACTERISTICS OF GLYCOLIPIDS PRESENT IN *C. ACNES* CELLS AND SECRETED EXTRACELLULAR VESICLES

Anna Chudzik [a], Marzena Ciesielska [a], Marta Kaszowska [b], Wojciech Jachymek [b], Katarzyna Kapczyńska [a], Katarzyna Pacyga-Prus [c], Andrzej Gamian [a], Mariola Paściak [a]

[a] Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław
[b] Department of Immunochemistry, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław
[c] Laboratory of Microbiome Immunobiology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland

The genus *Cutibacterium* comprises facultative anaerobic microorganisms that are part of the human skin microbiota and also opportunistic pathogens. In the membrane lipid profile of *C. acnes* strains, we were able to find two or four glycolipids. Due to the content of sugar components, these glycolipids may present antigenic properties.

Extracellular vesicles are spherical nanostructures secreted by prokaryotic cells that transport a variety of substances, including proteins, nucleic acids, cellular metabolites, bacterial virulence factors, and other molecules. Due to these characteristics, they can selectively stimulate the host immune system, rendering them suitable for use as vaccine components. In our studies, we isolated cellular glycolipids present in 4 *Cutibacterium acnes* phylotypes. Glycolipids were identified not only in whole-cell extracts but also in EVs secreted by these bacteria. Depending on the TLC retention factors, glycolipids were designated GL1, GL2, GL3 and GL4. Glycolipids were analysed by TLC, GC-MS, MALDI-TOF MS and NMR.

Of the whole-cell extracts, only one phylotype of *C. acnes* (DSM 16379) contained all 4 glycolipids. The other phylotypes (DSM 1897, PCM 2334, and NCTC 13655) contained glycolipids GL1 and GL3. In lipid extracts obtained from EVs, the presence of glycolipid GL1 (all phylotypes) and glycolipid GL3 (DSM 1897 and NCTC 13655) were also found. Based on the GC-MS and MALDI-TOF MS analysis data, GL1 contains 2 fatty acid chains and 2 glucose residues attached to a glycerol backbone. GL3 contains the same sugar-glycerol backbone and three fatty acyl residues.

In-vitro tests were performed using PWR-1E human prostate cells and MSU1.1 fibroblasts to determine GL1 and GL3 toxicity and pro-inflammatory potential.

The chemical and biological characteristics of *C. acnes* glycolipids are the starting point for further research on the antigenic properties of these compounds in the context of searching for new vaccine components and diagnostic tools. Studies on the function of glycolipids in EVs are also very promising research goals.

O21

DISSECTING THE ROLE OF BACTERIAL OUTER MEMBRANE VESICLE (OMV)- DEPENDENT COMPLEMENT ACTIVATION IN SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)

Cedric Battaglino[a*], Irina Bondaruk[b*], Katarzyna Kasperkiewicz[b#], Paula Czyszczoń[c*], Beata Filip-Psurska[c], Kamil Malik[b], Mikael Skurnik[d], Maciej Cedzyński[a], Mariusz Gadzinowski[e], Jolanta Łukasiewicz[c#] Anna Swierzko[a#]
([*]-equal contribution and first authorship; [#]-equal contribution and senior authorship)

[a] Laboratory of Immunobiology of Infections, Institute of Medical Biology, PAS, cbattaglino@cbm.pan.pl
[b] Faculty of Natural Sciences, University of Silesia
[c] Department of Immunochemistry, L. Hirszfeld Insitute of Immunology and Experimental Therapy, PAS
[d] Department of Bacteriology and Immunology, University of Helsinki
[e] Department of Engineering of Polymer Materials, Centre of Molecular and Macromolecular Studies, PAS

Originating from the outer membrane, outer membrane vesicles (OMVs) contain cell surface structures, including lipopolysaccharide (LPS, endotoxin). Even after eliminating living pathogen cells, those small (50-300 nm) vesicles are still capable of reaching distant organs and triggering immune response (including complement activation). They have been proposed to be involved in, inter alia, horizontal gene transfer, biofilm formation, killing of competing microbial cells, resistance to antibiotics, adherence to host cells, immunomodulation, and SIRS development. They were shown to consume serum bactericidal factors thus contributing to the survival of parental (and co-infecting) bacteria. We investigated the contribution of OMV#induced complement activation to systemic inflammatory response syndrome (SIRS).

Our model bacterium was Yersinia enterocolitica O:3 (YeO3) able to multiply in a broad range of temperature, characterised by temperature-regulated expression of virulence factors and lipopolysaccharide with exceptional structure organization.We investigated OMVs secreted by serum-sensitive (virulence plasmid-cured) YeO3 wild type (YeS) bacteria grown at 4°C, 22°C and 37°C. Furthermore, OMVs from three variants expressing LPS of Ra (YeR1), Rd1 (M196) and Re (M205) chemotypes, cultivated at 37°C, were tested. OMVs were isolated from sterile culture supernatants by ultracentrifugation(with/without collection on 100 kDa membrane) and their concentrations and size wereanalysed by NTA (nanoparticle tracking analysis) and TEM. The OMVs-induced complement activation was tested by ELISA and Western Blot. To induce SIRS, C57BL/6 mice were treated i.p. with YeS_37°C OMVs. The decomplementation was triggered by i.p. injection of cobra venom factor (CFV). The WBC and serum de Ritis ratio as well as liver IL1β1-, IL6-, TNFα#and complement C3- gene expression was analysed.

The highest secretion of OMVs was found for M196 bacteria. According to NTA analysis, the amount of OMVs produced by M196_37°C (33,0x1010/ml) was 3 times higher than by YeS_37°C bacteria (9,3x1010/ml) and 6 times higher than YeR1_37°C and M205_37°C bacteria (5,9x1010 and 5,0x1010/ml, respectively). The amount of OMVs secreted by YeS cultivated at 22°C and 4°C was 1,5-2 times lower than at 37°C (6,5 x1010 and 5,0x1010/ml).
The trend towards smaller size of LPS-mutant-derived OMVs in comparison with those of YeS strain was observed. The difference in temperature- (but not LPS chemotype)-dependentOMVs size distribution profile was noticed. Regardless of the growth temperature and LPS#chemotype, all preparations of OMVs were capable of inducing complement C3 activation in normal human serum. Calcium and magnesium chelators markedly inhibited C3 activation, but supplementation of EGTA with Mg, recovered it only partially. Furthermore, complement lectin pathway-dependent C4 activation and terminal complement complex (TCC) formation were observed. Treatment of mice with a sublethal dose of YeS_37°C OMVs caused some symptoms of SIRS. These symptoms were significantly reduced in CVF-treated mice, indicating complement involvement in OMV-induced SIRS.

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O22

BUBBLE TROUBLE: HOW BACTERIAL EXTRACELLULAR VESICLES KEEP ALLERGIES AT BAY?

Gorska Sabina[a] , Kozakiewicz Dominika[a] , Sysak Angelika[a] , Razim Agnieszka[a] , Pacyga-Prus Katarzyna[a] , Jakubczyk Dominika[a]

[a] Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
sabina.gorska@hirszfeld.pl

Allergies are a major problem. They are becoming increasingly prevalent around the world. It is therefore necessary to find an easy and safe way to treat and prevent allergies. Scientific evidence suggests that bacteriotherapy, particularly probiotic strains, could be advantageous in combatting allergies. One relatively new area of research is the use of extracellular vesicles (EVs) produced by probiotic bacteria. These EVs may offer a safer alternative to using live bacteria. In my presentation, I will summarise the current evidence on the potential of bacteria-derived EVs for allergy treatment, drawing on findings from our recent investigations. I will present the physicochemical and immunomodulatory properties of EVs, and discuss their potential role in allergy prevention. Furthermore, I will demonstrate how EVs interact with host cells and which signalling pathways may be involved in their activity. Our findings highlight the strong potential of bacterial EVs as postbiotic therapeutic agents. However, further research is needed to fully explore their clinical applications, particularly in mucosal vaccination and targeted immunotherapy.

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O23

ADOPTING A GLYCOENGINEERING STRATEGY TO IMPROVE THE THERAPEUTICAL POTENTIAL OF EXTRACELLULAR VESICLES FROM YEAST

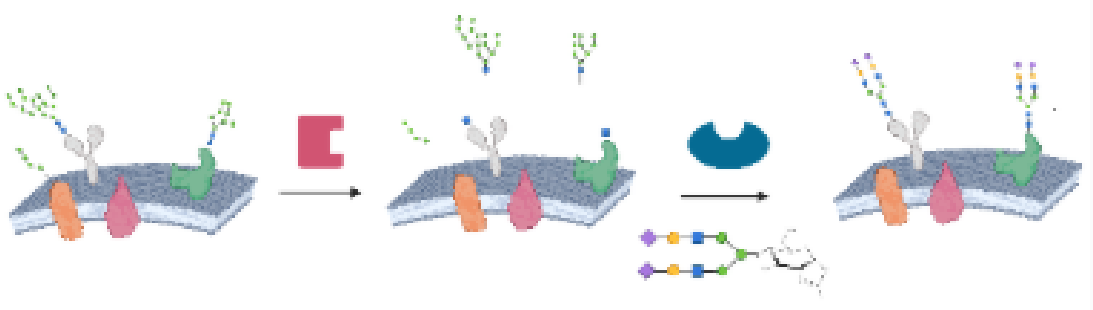
Paolo Costa [a], David Slilaty [a], Joseph Trani [a], Curtis Logan [a], Maxence Le Palud [b], Francois Le Mauff [c], Samy Cecioni [b], Christopher Brett [a], David Kwan [a]

[a] Biology Department, Concordia University, 7141 Sherbrooke St W, Montreal, Quebec H4B 1R6, paolo.costa@concordia.ca
[b] Chemistry Department, Universite de Montreal, 1375 Avenue Thérèse-Lavoie-Roux, Montréal, QC H2V 0B3
[c] Department of. Medicine, Microbiology, and Immunology, McGill University, 1001 Decarie Blvd, Montreal, Quebec H4A 3J1

Extracellular vesicles (EV) mediate several physiological responses in all domains through the delivery of their content to surrounding cells [1]. In recent years, the interest in EV has increased exponentially, given the potential application as drug delivery carriers. Importantly, the specific glycan profiles of EV membrane proteins strongly shape EV functionalities. Using a glycoengineering approach, glycans presented on the EV surface can be edited, introducing new properties, potentially attractive for the development of novel EV-mediated therapies.

In this project, EV from *Saccharomyces cerevisiae* are studied. Interestingly, budding yeast could be exploited to produce large amounts of therapeutical EV in a short time. However, while human EV have been extensively characterized, research on EV from *S. cerevisae* and yeasts in general is still scarce [2]. Indeed, to better understand the potential application of *S. cerevisae* EV, the role of the glycans decorating the membrane proteins must be addressed as well. If *S. cerevisae* EV shall be conceived as drug delivery carriers, first the surface glycans need to be characterized. Secondly, it must be understood whether these glycans might induce unexpected immune responses in the body. To test this, a glycoengineering approach was followed: first wild-type yeast-glycans are trimmed from EV and then substituted with human-like glycans. Eventually, EV different functionalities and immunogenic potential are studied, via cell-based assays.

Thermal heat-shock, Nanoparticle Tracking Analysis (NTA) and Size Exclusion Chromatography (SEC) are adopted to purify EV. GC/MS analysis Lectin Western Blot are used to elucidate glyco-profiles. To modify glycans, glycosyltransferase enzymes have been screened and selected. First, mannose-rich glycans have been identified on EV surface. Secondly, these glycans have been stripped off by two selected enzymes. Additionally, human-like glycans have been successfully purified from egg yolk powder and tagged to EV by transglycosilation reactions. In the next months, natural and modified EV are going to be tested with THP-1 cells differentiated into M1-like macrophages to detect the pro-inflammatory cytokine TNF-alpha.



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O24

A SWEET KEY TO BACTERIOPHAGE INFECTION: PHAGE-GLYCAN INTERACTIONS AT GRAM-NEGATIVE ENVELOPES AND IN BIOFILMS

Nina K. Bröker[a], Mareike Stephan[b], Hannah-Maria Monks[c], Antonia Nowicki[c], Salvatore Chiantia[d], Stefanie Barbirz[c]

[a] Department of Medicine, Science Faculty, HMU Health and Medical University, Potsdam, Germany.
[b] Department of Colloid Chemistry, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.
[c] Department of Medicine, Science Faculty, MSB Medical School Berlin, Germany.
[d] Physical Biochemistry, University of Potsdam, Potsdam, Germany.

The encounter with a cellular boundary is a central step in a viral life cycle to start transfer of genetic material into a host cell. Bacteriophages meet a plethora of bacterial surface structures, most of them highly diverse envelope glycans, but also glycan-based biofilms. Phage-host co-existence in these glycan matrices is tightly balanced, as they play dual roles both as protective shields and as phage receptors. We study how bacteriophages interact with polysaccharides as part of the bacterial envelope or of biofilms to understand how bacterial glycans control bacteriophage function. We have analyzed bacteriophage mobility in polysaccharide-based biofilms by defining microviscosity parameters of the glycan matrix. Tailed bacteriophages infecting Gram-negative bacteria can usethe outer O-antigen polysaccharide layer for infection. In intact cells, the O-antigen serves as infection control point, whereas in outer membrane vesicles, the O-antigen mediates extracellular phage concentra#tions. In addition, isolated lipopolysaccharide (LPS) fragments form membrane-like assemblies expos#ing O-antigen to inactivate phages. Using a set of Salmonella model phages of different tail architec#tures and fluorescence techniques we show that phage particles work as molecular machines that can be triggered to release their DNA by O-antigen containing membranes. In particular, we employ glycan-containing model membranes that mimic the Gram-negative envelope. With this we investigate how the interplay of bacteriophage enzymatic activity, OM properties and phage tail architecture leads to opening of the phage particle, illustrating the high regulatory power of the Gram-negative OM glycan environment on bacteriophage infection.

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O25

IDENTIFICATION OF RECEPTOR BINDING PROTEINS OF *YERSINIA* PHAGE ΦR1-37 AND ENTEROCOLITICIN THAT USE THE SAME BACTERIAL SURFACE RECEPTOR

Mikael Skurnik[1], Rahime Tetik[2], Muhammad Suleman Qasim[1,3], Jana Sachsenröder[2], Ralf Dieckmann[2,4], Carlos G. Leon-Velarde[5], Arnab Bhattacharjee[1], Göran Widmalm[6] and Eckhard Strauch[2]

[1] Department of Bacteriology and Immunology, Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki FI
[2] Department Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany
[3] RNAcious Laboratory, Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki FI
[4] Department 1 – Infectious Diseases, Robert Koch Institute, Berlin, Germany
[5] Laboratory Services Division, University of Guelph, Guelph, Ontario, Canada
[6] Department of Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91, Stockholm, Sweden

Bacteriophage φR1-37 and enterocoliticin, a tailocin, both use lipopolysaccharide (LPS) outer core (OC) hexasaccharide of *Yersinia enterocolitica* serotype O:3 as receptor. We determined the sequence of the gene cluster directing the biosynthesis of enterocoliticin and demonstrated that it is sufficient for the enterocoliticin production. The gene cluster comprises altogether 42 predicted genes, and the predicted function of 37 gene products could be annotated based on homology to known gene products. The genes *g298* and *g297* of phage φR1-37 were identified to encode for the tail fiber protein and its trimerization chaperone, respectively. These proteins could not be separated when purified from an expression construct; however, the function of purified Gp298-Gp297 complex as the receptor binding protein (RBP) of φR1-37 was confirmed. Based on local similarity to Gp298, the *orf39* gene product of enterocoliticin gene cluster was predicted to encode for the enterocoliticin RBP. The comparison of the predicted RBPs identified a long homologous region of 80-85 amino acid residues between the RBPs being the only obvious explanation for the receptor binding. AlphaFold was used to generate structural models of the binding sites and docking experiments with tetra- and pentasaccharide ligands generated by CarbBuilder were carried out to confirm the interactions with the OC. Interestingly, a database search with the predicted binding site motifs revealed its wide presence in diverse phage tail proteins indicating that the motif might be a specialized carbohydrate-binding structure.

O26

COMPARATIVE GLYCOMICS REVEALS CHANGES IN N-GLYCOME PATTERNS IN MULTICELLULARITY AND EARLY ANIMAL EVOLUTION

Łukasz F. Sobala,[a] Aleksandra Kuniec,[a] Gonzalo Bercedo Saborido,[b] Koryu Kin[b] Alexander Ereskovsky,[c]

[a] Laboratory of Glycobiology, Hirszfeld Institute of Immunology and Experimental Therapy, PAS, Weigla 12, 53-114 Wrocław, Poland, lukasz.sobala@hirszfeld.pl
[b] Multicellgenome Laboratory, Institut de Biologia Evolutiva (CSIC–Universitat Pompeu Fabra), Pssg. de la Barceloneta 37-49, 08003 Barcelona, Spain
[c] IMBE, CNRS, IRD, Aix Marseille University, Station Marine d'Endoume, Rue de la Batterie des Lions,13007 Marseille, France

Self vs. non-self cell recognition and division of labor between groups of cells are key requirements of animal multicellularity. In mammals, N-glycosylation is tissue-specific and cancer cells are known to have altered glycosylation patterns. However, there is little N-glycomics data available from early branching animals and protistan, unicellular microorganisms closely related to animals, leaving the evolutionary patterns in the dark. To overcome this gap, we conducted a systematic, comparative N-glycomics study that includes representatives of early branching animal groups (ctenophores, sponges, placozoans and cnidarians), as well as their closest protist relatives from the group Holozoa (filastereans, choanoflagellates and ichthyosporeans) and of additional eukaryotic outgroups. We also created a proteomic database to make Holozoan unicellular-to-multicellular transition studies more accessible[1].

Here, we report a huge variety of N-glycan structures, including novel compositions. The data suggest that N-glycan complexity is positively correlated with organismal complexity and linked to lifestyle. Ichthyosporeans, which have a complex life cycle and often are animal parasites, synthesize a wide variety of N-glycan structures, similar to animals. In contrast, facultatively multicellular protists (*C. owczarzaki, S. rosetta*) synthesize simpler oligomannose N-glycans, despite possessing the genes encoding for glycan branching. Our results indicate that the N-glycan biosynthetic pathway became more important for obligate multicellularity, both as a mechanism of protein quality control and a way to synthesize recognition tags. This study provides a foundation for future work on non-canonical species by establishing several reference stage-specific glycomes. Further, single species-focused studies are needed to unravel the significance of the observed structures for each organism.

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O27

STRUCTURAL ANALYSIS OF THE GLYCAN PRODUCED BY THE MUTANT IN THE GENE *VNG*1058H OF *HBT. SALINARUM*

Dandan Yu,[a] Antonio Molinaro,[a] Anna Notaro, [a] Zlata Vershinin, [b] Marianna Zaretsky, [b] Cristina De Castro, [a] * and Jerry Eichler[b]*

[a] Department of Chemical Sciences, University of Naples Federico II, Via Cinthia 4, 80126, Napoli, Italy.
dandan.yu@unina.it
[b] Department of Life Sciences, Ben-Gurion University of the Negev, Beersheva, Israel

N-glycosylation is a conserved post-translational modification present across all domains of life, including Eukarya, Bacteria, and Archaea. The halophilic archaeon *Halobacterium salinarum*, the first known non-eukaryotic organism to carry out N-glycosylation, decorates its glycoproteins with sulfated oligosaccharides that are assembled on dolichol phosphate or dolichol pyrophosphate lipid carriers [1-2]. Although some enzymes involved in these glycosylation pathways have been characterized, the specific enzymes responsible for adding certain sugars—such as L-iduronic acid (L-IdoA)—have yet to be identified.

L-IdoA, which is the C-5 epimer of D-glucuronic acid (D-GlcA), is a well-known component of glycosaminoglycans (GAGs) in animals and is also found in certain bacterial polysaccharides.*Halobacterium salinarum* is unique in incorporating L-IdoA into an N-linked glycan, specifically within a tetrasaccharide structure. However, the biosynthetic pathway for producing IdoA in archaea remains unclear [3,4]. In this study, we generated a deletion mutant lacking a predicted D-glucuronyl C5-epimerase *VNG*1058H, which is hypothesized to catalyze the formation of L-IdoA at the third position of the tetrasaccharide. We then analyzed the N-glycan profile of the mutant to determine the gene's function. Our results represent the first identification of this type of enzyme in archaea and shed light on the complexity and evolutionary variation of archaeal N-glycosylation pathways.

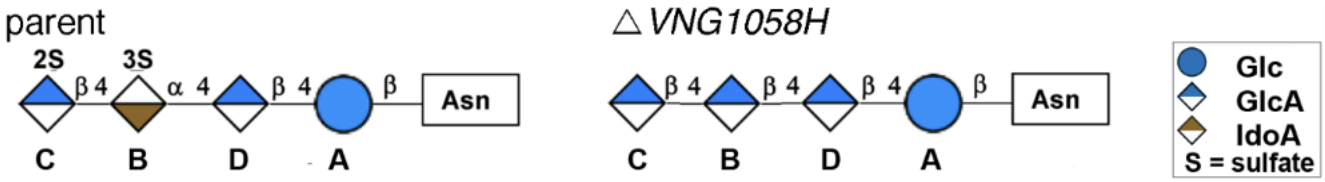

Fig.1 The structure of the oligosaccharides produced by the wild type strain and the mutant.

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F1

THE MOLECULAR MECHANISM OF BCS3, THE CAPSULE POLYMERASE FROM *HAEMOPHILUS INFLUENZAE* SEROTYPE B

Tim A. Mast[1], Valerio Di Domenico[2], Julia Schulze[1], Christa Litschko[1,3], Andrea Bethe[1], Adriá Alcaide i Jimenez[2], Juan Carlos Villegas Ruíz[2], Javier O. Cifuente[4], Mario Schubert[5], Timm Fiebig[1,3] and Marcelo E. Guerin[2]

[1]Institute of Clinical Biochemistry, Hannover Medical School, Hannover, Germany.
[2]Structural Glycobiology Laboratory, Department of Structural and Molecular Biology; Molecular Biology Institute of Barcelona (IBMB), Spanish National Research Council (CSIC), Barcelona Science Park, c/Baldiri Reixac 4-8, Tower R, 08028 Barcelona, Catalonia, Spain.
[3]German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Hannover, Germany.
[4]Instituto Biofisika (UPV/EHU, CSIC), University of the Basque Country, E-48940, Leioa, Spain.
[5]Department of Biology, Chemistry and Pharmacy, FU Berlin, 14195 Berlin, Germany.

Bacterial capsules represent one of the main virulence factors in pathogen-host interactions and have fundamental importance in the outcome of many human infectious diseases. Here we focus in Bcs3, the multi-enzymatic machinery responsible for the biosynthesis of the heteropolymeric capsule of *Haemophilus influenzae* serotype b (Hib), the most clinically relevant serotype that causes severe infections in infants and children.

Bcs3 possesses a dimeric architecture with each subunit comprising five regions, including the three enzymes CriT, CrpP and CroT. Based on structural data, we provide insight at molecular level how processive chain elongation is achieved in bacterial capsule polymerases and demonstrate that several separate binding sites are required to coordinate this complex processes. These binding sites address two fundamentally different concepts required to assemble the chain: one type of binding site achieves processivity through tethering of the nascent chain to the capsule polymerase, allowing the enzyme complex to finish said chain with priority. The other type of binding site acts as a threading help by specifically binding the non-reducing end of the nascent chain and pointing it towards the active center required at a specific time-point of the catalytic circle. Our findings demonstrate that the assembly of complex polymers by multi-enzyme machines is a highly coordinated process and that intricate mechanistic knowledge is required to exploit these catalysts for biotechnological applications.

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F2

MULTILAYER CONTROL OF GLYCOSYLTRANSFERASES BY c-di-GMP AND TYROSINE PHOSPHORYLATION SHAPES EXOPOLYSACCHARIDE OUTPUT IN *RHIZOBIUM LEGUMINOSARUM* BV. *TRIFOLII*

Aleksandra Horbowicz-Teresińska,[a] Kamila Rusek,[a] Martyna Moryl,[a] and Małgorzata Marczak[a]*

[a] Department of Genetics and Microbiology, Faculty of Biology and Biotechnology, Maria CurieSkłodowska University, 20033 Lublin, Poland; email: malgorzata.marczak@mail.umcs.pl

Biosynthesis of the branched octasaccharide repeat unit of the *Rhizobium leguminosarum* bv. *trifolii* exopolysaccharide (EPS) relies on ten glycosyltransferases (GTs). Transcriptional mapping of the *pss* gene cluster revealed three additional weak promoters immediately upstream of *pssI*, *pssH* and *pssG*, indicating potential conditional modulation of their expression. We focused on the poorly characterised sidechain enzymes PssG, PssH and PssI by combining systematic mutagenesis, a bacterial twohybrid (BTH) screen, pulldown assays and *in vitro* enzymology. The interactome placed PssI among the most connected nodes, forming robust heterotypic contacts with PssG, PssF and the polymerisation complex, whereas PssH interacted mainly with itself. Pulldown experiments confirmed a stable PssG–PssI heterocomplex that probably channels branched subunits to the polymerase [1]. *In vitro*, cdiGMP specifically inhibited PssI activity towards UDPGlc, leaving PssG unaffected. Consistent with this messenger sensitivity, PssI carries canonical PilZdomain RxxxR and DxSxxG motifs in its Cterminal region, reinforcing its role as a cdiGMPresponsive regulator. Massspectrometry of immunoprecipitated PssI-derived peptides revealed phosphorylation on a conserved tyrosine residue (Tyr241), indicating addiational posttranslational control layer. Deletion of *pssG*, *pssH* or *pssI* individually reduced EPS to 40–70 % of the wildtype level while preserving the HMW:LMW profile. The double mutant Δ*pssHΔpssI* synthesised only ~15 % EPS with a broadened size distribution; Δ*pssGΔpssI* abolished production, whereas Δ*pssGΔpssH* overproduced polymer (~150 %). These data highlight complementary yet nonredundant roles: PssH could act as an elongator, PssI—as a cdiGMPresponsive, and tyrosinephosphorylated—regulator, and PssG would buffer the system within a heterocomplex with PssI. A multilayer network of protein–protein interactions, phosphorylation and secondmessenger signalling thus finetunes EPS yield and architecture, offering new levers for engineering polysaccharides with customised properties.

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F3

TRANSITION TRANSFERASES PRIME GROUP 2 CAPSULAR POLYSACCHARIDE BIOSYNTHESIS IN GRAM-NEGATIVE BACTERIA

Marco D Albers[a,b], Christa Litschko[a,b], Valerio Di Domenico[c,d], Julia Schulze[a], Sizhe Li[e], Olga G Ovchinnikova[f], Thijs Voskuilen[e], Andrea Bethe[a], Javier O Cifuente[c,g], Alberto Marina[g], Insa Budde[a], Tim A Mast[a], Małgorzata Sulewska[a], Monika Berger[a], Falk F R Buettner[a,h], Todd L Lowary[i,j,k], Chris Whitfield[f], Jeroen D C Codee[e], Mario Schubert[l,m], Marcelo E Guerin[c,d,g,n], Timm Fiebig[a,b]*

[a] Institute of Clinical Biochemistry, Hannover Medical School, Hannover, Germany, albers.marco@mh-hannover.de.
[b] German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Hannover, Germany.
[c] Structural Glycobiology Laboratory, Biocruces Bizkaia Health Research Institute, Cruces University Hospital, 48903 Barakaldo, Bizkaia, Spain.
[d] Structural Glycobiology Laboratory, Department of Structural and Molecular Biology; Molecular Biology Institute of Barcelona (IBMB), Spanish National Research Council (CSIC), Barcelona Science Park, c/Baldiri Reixac 4-8, Tower R, 08028 Barcelona, Catalonia, Spain.
[e] Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC, Leiden, The Netherlands.
[f] Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON N1G 2W1, Canada.
[g] Structural Glycobiology Laboratory, Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, Building 801A, 48160, Derio, Spain.
[h] Proteomics, Institute of Theoretical Medicine, Faculty of Medicine, University of Augsburg, Universitätsstrasse 2, 86159 Augsburg, Germany.
[i] Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada.
[j] Institute of Biological Chemistry, Academia Sinica, Taipei, Nangang 11529, Taiwan.
[k] Institute of Biochemical Sciences, National Taiwan University, Taipei 10617, Taiwan.
[l] Department of Biosciences and Medical Biology, University of Salzburg, Salzburg, Austria.
[m] Department of Biology, Chemistry and Pharmacy, Free University of Berlin, Berlin, Germany
[n] Ikerbasque, Basque Foundation for Science, 48009 Bilbao, Spain.

Capsules are long-chain carbohydrate polymers that envelop the surfaces of many bacteria, protecting them from host immune responses. Capsule biosynthesis enzymes are potential drug targets and valuable biotechnological tools for generating vaccine antigens. Despite their importance, it remains unknown how structurally variable capsule polymers of Gram-negative pathogens are linked to the conserved glycolipid anchoring these virulence factors to the bacterial membrane. Using Actinobacillus pleuropneumoniae (App) as example, we demonstrate that CpsA and CpsC generate a poly(glycerol-3-phosphate)-linker to connect the glycolipid with capsules containing poly(galactosylglycerol-phosphate) backbones. We reconstruct the entire capsule biosynthesis pathway in App serotypes 3 and 7, identify a tetratricopeptide repeat domain in the capsule polymerase CpsD as essential for elongating poly(glycerol-3-phosphate), and show that CpsA/C stimulate CpsD to produce longer polymers. We identify the CpsA/C product as wall teichoic acid (WTA) homolog, demonstrating similarity between the biosynthesis of Gram-positive WTA and Gram-negative capsules.[1]

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F4

STRUCTURAL CHARACTERIZATION OF A WATER-INSOLUBLE POLYSACCHARIDE FROM *PLEUROTUS CITRINOPILEATUS* AND PREBIOTIC POTENTIAL OF ITS ACIDIC HYDROLYSATE

Iwona Komaniecka[a],*, Adam Choma[a], Adam Waśko[b], Marek Stankevič[c], Marek Siwulski[d], Paulina Adamczyk[e],Kamila Wlizło[e], Adam Junka[f] and Adrian Wiater[e]

[a] Department of Genetics and Microbiology, Institute of Biological Science, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland, E-mail: iwona.komaniecka@mail.umcs.pl
[b] Department of Biotechnology, Microbiology and Human Nutrition, University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland
[c] Department of Organic Chemistry and Crystallochemistry, Faculty of Chemistry, Institute of Chemistry Sciences, Maria Curie-Skłodowska University, Gliniana 33, 20-614 Lublin, Poland
[d] Department of Vegetable Crops, Poznan University of Life Sciences, Dąbrowskiego 159, 60-594 Poznań, Poland
[e] Department of Industrial and Environmental Microbiology, Institute of Microbiology and Biotechnology, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland
[f] Platform for Unique Model Application, Department of Pharmaceutical Microbiology and Parasitology, Pharmaceutical Department, Wroclaw Medical University, Borowska 211, 50-534 Wrocław, Poland

Oligosaccharide prebiotics play an important role in regulating the diversity of human gut microbiota. The hydrolysis of microbial-derived polysaccharides is one of the method for the production of potential prebiotic oligosaccharides.[1] In the present work, the structural features of water-insoluble polysaccharide isolated from fruiting bodies of *Pleurotus citrinopileatus* (the golden oyster mushroom) was investigated. The aim of this study was to formulate preparation of oligosaccharides as well, and to perform an initial evaluation of their prebiotic potential. To characterize an isolated material chemical and spectroscopic techniques were introduced, including: composition and linkages analyses, 2D NMR spectroscopy studies, FT-IR and Raman spectroscopy, specific rotation and viscosity analyses. It was found that the water-insoluble polysaccharide extracted from fruiting bodies of *P. citrinopileatus* was composed almost exclusively of D-glucose (89.2%). The linkage analysis showed that (1→3)-linked Glc*p* was the main component of the backbone chain (82.1%), and (1→4)-linked hexose was the minor one (6%). The glucan also contained small amounts of two types of doubly substituted hexose residues, i.e. →3,4)- and →3,6)-linked residues. The FT-IR and Raman spectra showed the presence of α-(1→3) linkages. 2D NMR studies of the water-insoluble polysaccharide indicated that investigated polymer possess a structure of (1→3)-α-D-glucose-based polysaccharide. In order to detect (1→3)-α-D-glucan in the cell wall of *P. citrinopileatus* the specific fluorophore-labelled antibodies (Alexa Fluor 488 goat anti-mouse IgM) were used and it was observed its accumulation over the entire length of the hyphae, which confirmed a high amounts and the importance of this polymer in the cell wall structure. The mixture of glucooligosaccharides (GOS) used in further studies was obtained *via* acid hydrolysis of this (1→3)-α-D-glucan preparation, as previously described by Wiater and co-workers [2]. The GOS contained glucose (26%) and a mixture of (1→3)-α-D-glucooligosaccharides with a degree of polymerization (DP) from 2 to 10 (74%), as indicated based on the HPLC analysis. The *in vitro* fermentability of (1→3)-α-D-glucan hydrolysate by potential probiotics (*Bifidobacterium infantis*, *B. bifidum*, *B. adolescentis*, *Lactobacillus acidophilus*, *L. casei, L. plantarum, L. rhamnosus* and *L. fermentum*) was investigated and compared to the reference prebiotics, i.e., fructooligosaccharides and inulin. The GOS was utilized for growth by all of the *Lactobacillus* strains tested and showed a strong bifidogenic effect.

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F5

PLESIOMONAS SHIGELLOIDES O68 LIPOPOLYSACCHARIDE. EXPANDING THE ARRAY OF *P. SHIGELLOIDES* STRAINS WITH UNUSUAL HYDROPHOBIC PROPERTIES OF THE O-ANTIGENS

Sabina Koj,[a] Karolina Ucieklak,[a] Monika Dzieciątkowska,[b] Jolanta Łukasiewicz,[a] Czesław Ługowski[a] and Tomasz Niedziela[a],*

[a] Department of Immunochemistry, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland, E-mail: sabina.koj@hirszfeld.pl
[b] Anschutz Medical Campus, University of Colorado, Denver, USA

Plesiomonas shigelloides is a potential human and animal pathogen that has been implicated in outbreaks of food poisoning with acute gastroenteritis. With the gradual increase in antibiotic-resistant strains of *P. shigelloides* development of vaccine therapy is of urgent interest.

We have investigated the complete structure of lipopolysaccharide (LPS) from *P. shigelloides* O68 (strain CNCTC 138/92). The studies by ^{1}H and ^{13}C NMR spectroscopy, complementary mass spectrometry and chemical methods confirmed that the *P. shigelloides* O68 O-specific polysaccharide (O-PS) is composed of a trisaccharide repeating unit with the structure:

→4)-α-D-Glc*p*6OAc-(1→4)-β-D-Glc*p*NAcyl3NAc-(1→3)-β-Fuc*p*NAc4N-(1→

in which β-D-Glc*p*NAcyl3NAc is a 3-acetamido-2,3-dideoxy-glucosamine acylated with D-3-hydroxybutyric acid. The O-PS is attached to a non-phosphorylated core undecasacharide through→3)-β-D-Glc*p*NAc-(1→ residue in the outer core region of the O68 LPS. The inner core region comprises the common structural element of *P. shigelloides* core oligosaccharides that resembles these of O17 and O36 serotypes. Additionally, the presence of glycine linked to the amine group of the→6)-α-D-Glc*p*N-(1→ residue as well as a distal α-Kdo-(1→ residue substituting the →5)-α-Kdo-(1→ residue at position 4 were determined. The innermost segment of *P. shigelloides* O68 LPS, that is lipid A is composed of β-D-Glc*p*N4P-(1→6)-α-D-Glc*p*N1P disaccharide substituted with 14:0(3-OH), 12:0(3-OH), 14:0(3-*O*-14:0) and 12:0(3-*O*-12:0) acyl groups at C-2, C-3, C-2' and C-3', respectively.
To summarize, *P. shigelloides* O68 lipopolysaccharide comprises the typical architectural elements of lipid A, whereas its core oligosaccharide and O-specific polysaccharide represent new structures among bacterial O-antigens. The presence of O-, N-acetyls and D-3-hydroxybutyryl group make the *P. shigelloides* O68 O-PS more hydrophobic than typical enterobacterial O-polysaccharide. This finding expands the array of *P. shigelloides* strains with the phenol-phase soluble S-type LPS. Among *P. shigelloides* O-PS structures identified so far[1], 6 serotypes have typical hydrophilic O-PS (O12, O17, O22, O24, O36, O51, O74) while serotypes: O1, O33, O37, O51, O74 represent the unusual structures. The presence of deoxy and amino sugars, scarce hydroxyl groups, high degree of acetylation, non-sugar elements, N-acyls and rare substituents such as α-D-Lenose (O37), pneumosamin (O1), 3-hydroxybutanoyl (O1) and 3-hydroxy-2,3-dimethyl-5-oxoproline (O74)[2] within their O-PS all contribute to the exceptional hydrophobicity of LPS. These uncommon structures that possibly affect the biological properties of the *P. shigelloides* LPS are likely to play a role for pathogen survival and bacterial growth in unfavourable environments. Therefore, further studies of the distinctive structural variations and biochemical diversity of *P. shigeloides* LPS could facilitate our understanding of the pathogenicity of these bacteria and allow to design the potential antimicrobial agents.

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F6

THE *MYCOBACTERIUM BOVIS* BCG SUBSTRAIN PASTEUR CELL CONTAINS A DIVERSE ARRAY OF LIPIDS

Smoliński Jakub,[a] Paściak Mariola,[a]

[a] Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wroclaw, Poland

Mycobacterium bovis BCG (Bacillus Calmette-Guérin) is a widely used vaccine strain that contains live attenuated mycobacteria and is used for both the prevention and prophylaxis of tuberculosis (TB) and the immunotherapy of bladder cancer. However, despite its benefits, clinical studies and literature have shown that administration of BCG can lead to adverse effects, including systemic infections or sepsis, and many patients do not complete therapy with satisfactory results. Among the various substrains, BCG Pasteur is extensively studied due to its stable genetic structure and broad application in resarch [1,2].

Mycobacteria are known for their high lipid content, which constitutes 40% of their dry mass, and their cell walls can contain up to 60% lipids. The outer layer of the mycomembrane consists of various lipid molecules, such as trehalose-containing glycolipids, trehalose mono- and dimycolate (TMM and TDM), and species-specific lipids like phthiocerol dimycocerosate (PDIM), phenolic glycolipids (PGL), sulfo-glycolipids (SGL), glycopeptidolipids (GLP), and mycolic acids. Lipids play a key role in pathogenicity, modulation of the immune response, and the overall biology of mycobacteria [3-5].

BCG encompasses a range of substrains that exhibit genetic and biochemical differences. It is unknown whether and how these differences affect the efficacy of BCG. Compared to other BCG strains, early strains do not synthesize phthiocerol dimycocerosates (PDIM) and phenolic glycolipids (PGL), two lipid virulence factors. Various studies indicate that the loss of PDIMs/PGLs reduces the virulence and protective efficacy of BCG [5].

Understanding the lipid composition of the late BCG Pasteur strain may provide insights into its mechanisms of action and potential differences in immune response compared to other substrains. The aim of this study is to characterize some of the lipids of the *Mycobacterium bovis* BCG Pasteur substrain using advanced analytical techniques such as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry and thin-layer chromatography (TLC).

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F7

SYNTHESIS OF IMMUNOREACTIVE PHOSPHOCHOLINE MODIFIED PARASITIC GLYCAN EPITOPES

Tapasi Manna[a], Lena Nuschy[b], Iain Wilson[b*], Alla Zamyatina[a*]

[a] Institute of Organic Chemistry, Department of Natural Sciences and Sustainable Resources, BOKU University, Muthgasse 18, A-1190-Vienna, alla.zamyatina@boku.ac.at
[b] Institute of Biochemistry, Department of Natural Sciences and Sustainable Resources, BOKU University, Muthgasse 18, A-1190-Vienna

Phosphorylcholine (PC) is a zwitterionic modification found on bacterial proteins and polysaccharides, as well as on glycans in fungi, helminths, and certain insects[1-2] and is often considered a danger signal recognised by mammalian cells to activate the complement system in the bloodstream.[3] Since phosphocholine (PC) is also a structural component of platelet#activating factor (PAF), the dynamic regulation of PC expression in some bacteria within the host respiratory tract is influenced by the ability of PC-modified epitopes to bind to host epithelial cells via the PAF receptor.[4] During systemic spread, PC expression is subsequently downregulated to evade complement-mediated immune response. PC-modified glycans on eukaryotic pathogens such as helminths are known to be recognized by antibodies present in the sera of parasite-infected animals. In nematodes, PC modifications on glycans have been shown to exert immunomodulatory effects on host immune cells, contributing to a reduction in allergic and inflammatory responses through a shift toward a Th2-type immune response. While several genes have been associated with the biosynthesis of PC-containing glycans, primarily based on knockout studies that demonstrate modifications in lipopolysaccharide structures, direct biochemical evidence for enzymatic PC transfer to a sugar substrate has been reported in only one case.[5-6]

To facilitate effective immune profiling and the identification of novel phosphocholine (PC) transferases, we have chemically synthesized several PC-modified bacterial and parasitic glycan epitopes, along with their unmodified counterparts, equipped with amino group#terminated linkers suitable for glycan array studies. Binding data from glycan arrays, which reveal interactions between synthetic PC-containing glycans and their potential protein counterparts in the host immune system, provide a molecular basis for a deeper understanding of their immunomodulatory activity.

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F8

CHLOROVIRUS GLYCOSYLTRANSFERASES USED FOR THE CHEMOENZYMATIC SYNTHESIS OF A NEW CLASS OF BIOSURFACTANTS

Immacolata Speciale,[a] Rosanna Culurciello,[b] Elio Pizzo,[b] Irene Russo Krauss, [a,c] Antonio Molinaro, [a] Cristina De Castro[a]

[a] Department of Chemical Sciences, University of Naples Federico II, via Cintia 4, Fuorigrotta, Italy, immacolata.speciale@unina.it
[b] Department of Biology, University of Naples Federico II, via Cintia 4, Fuorigrotta, Italy
[c] CSGI – Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase, Firenze, Italy.

Chloroviruses are of particular interest because of their ability to encode most, if not all, of the enzymes required to glycosylate their major capsid protein, making them not so strictly dependent on the host [1], in contrast to the accepted doctrine for all other viruses. Notably, scientific evidence suggests that the glycosylation process occurs in cytoplasm rather than in the host endoplasmic reticulum and Golgi apparatus [2]. Consequently, most recombinant virus-encoded glycosyltransferases (GTs) are soluble and easier to work with. Here, we present the first potential application of a chlorovirus-derived enzyme.

In detail, the prototype of chloroviruses *Paramecium bursaria* chlorella virus (PBCV-1) encodes at least eight GTs (A064R, A071R, A075L, A111/114R, A219/222/226R, A301R, A473L, A546L)[1] that are predicted to be involved in the glycosylation process of its major capsid protein. To date, the function of A064R [3] and A075L [4] enzymes has been experimentally validated. Interestingly, A064R is a protein composed of three domains, two rhamnosyltransferases and one methyltransferase, each capable of working independently. We propose to use the first two rhamnosyltransferases in a novel chemoenzymatic pathway to produce a new class of biosurfactants, rhamnoxylolipids (RXLs) (Fig.1). The idea arose from

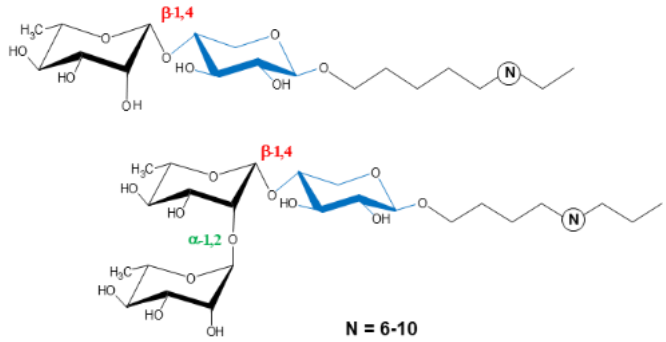

Fig. 1. Examples of mono- and di-rhamnoxylolipids.

the fact that the most widely used biosurfactants to date are rhamnolipids (RLs), although their production encounters several problems. Indeed, they are essentially produced by fermentation of opportunistic bacteria, namely strains of *Burkholderia, Acinetobacter* or, mainly, of *Pseudomonas* [5]. Moreover, any microbial fermentation produces a mixture of rhamnolipid congeners with variations in the chain length and in the degree of unsaturation of the fatty acid chains, as well as differences in the number of rhamnose molecules, with the overall consequence that different production batches do not have the same composition. Our strategy aims to covercome these limitations by enabling the safe, controlled and reproducible synthesis of structurally defined RXLs, creating a library of compounds that could replace rhamnolipids in the dermatology industry.

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F9

ANALYSIS OF THE KDO BIOSYNTHETIC CLUSTER IN CROV

Antonio Lembo[a], Immacolata Speciale[a], Anna Notaro[a], Antonio Molinaro[a], Matthias Fischer[b], Hiroyuki Ogata[c] and Cristina De Castro[a]

[a] Department of Chemical Sciences, University of Naples Federico II, Via Vicinale Cupa Cintia 26, Naples, Italy, antonio.lembo@unina.it
[b] Max Plank Institute for Marine Microbiology, Max Plank Institute
[c] Bioinformatics Center, Institute for Chemical Research of Kyoto University

Cafeteria roenbergensis virus (CroV) is a double-stranded DNA giant virus (692 kb) that infects the marine unicellular heterotrophic nanoflagellate *C. burkhardae* and it is the first giant virus reported to infect zooplankton[1]. Viruses should be seen as integral components of any ecosystem where they contribute to the maintenance of the balance between species and resources. Indeed, bacterivorous nanoflagellates such as *C. burkhardae*, make up a significant portion of the ocean's protozoan communities. Unlike mammalian viruses (*e.g.* HIV-1, SARS-CoV-2), which exploit host-encoded enzymes to build glycans that echo those of the host, most giant viruses encode their own enzymes with the result that their polysaccharides differ from those produced by their hosts[2]. Gene annotation suggests that CroV encodes a partial Kdo biosynthetic pathway (fig. 1); each of the three viral proteins, crov265, crov266, crov267, presents a dual activity that in some cases (C-termini of crov266 and crov267) seem not to be related to CMP-Kdo production (fig. 1). This study aims to provide new insights into the CroV Kdo biosynthetic pathway by analysing the features of the encoded proteins by bioinformatic analysis.

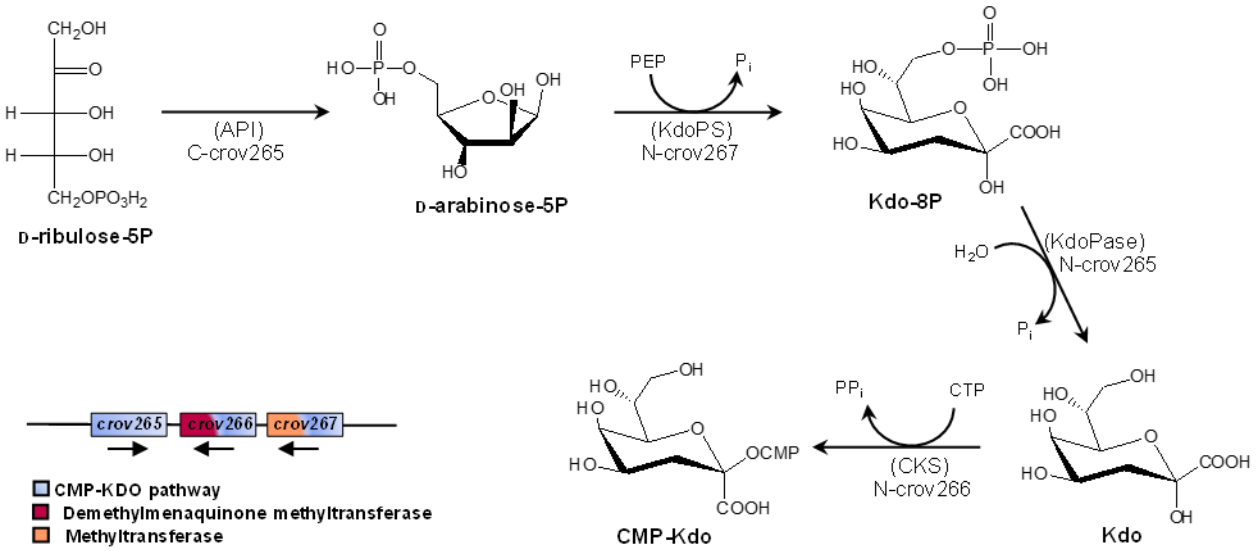

Figure 1. Putative Kdo biosynthetic pathway in CroV.

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F10

STRUCTURE AND GENE CLUSTER ORGANIZATION OF CLOSELY RELATED O-ANTIGENS OF *AEROMONAS* SP. CLASSIFIED TO THE NEW PROVISIONAL SEROGROUP PREVAILING IN POLISH AQUACULTURE

Anna Turska-Szewczuk,[a]* Maria Kurzylewska-Kubaczyńska,[a,b] Arkadiusz Bomba,[c] Katarzyna Dworaczek,[a] and Dominika Drzewiecka[d]

[a] Department of Genetics and Microbiology, Institute of Biological Sciences, M. Curie-Sklodowska University, Akademicka 19, 20-033 Lublin, Poland, anna.turska-szewczuk@mail.umcs.pl
[b] Department of Medical Microbiology, Medical University of Lublin, Chodzki 1, 20-093 Lublin, Poland
[c] Department of Omics Analyses, National Veterinary Research Institute, Partyzantow 57, 24-100 Pulawy, Poland
[d] Laboratory of General Microbiology, Department of Biology of Bacteria, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Aeromonas bacteria, whose natural habitat is the aquatic environment, are mainly known as opportunistic pathogens of farmed fish (carp, trout, tilapia, and salmon) [1]. These Gram-negative bacilli are characterized by high heterogeneity of surface antigenic structures, and based on the O antigen, they are classified into 44 recognized O-serogroups (America and Asia) [2], and an unknown number of provisional O-serogroups, with additional immune specific variants (e.g. Europe, including Poland) [3,4]. The diversity of the O antigen structure is related to the genetic variability of the O antigen gene cluster (OGC), which is key to its biosynthesis. The switching within the O serotype results from alterations in the sets of the OGC genes as an adaptation and consequence of pressure from the host's immune system. Molecular serotyping methods based on data from *in silico* analysis of OGC gene clusters, combined with studies of O antigen structure, is currently a method used in serotyping enteric bacteria [5]. The organization of O-antigen synthesis clusters has been well-studied in *E. coli*, *Salmonella*, and *Shigella* but is only partially known in *Aeromonas* [6,7]. In the latter, the OGC is usually mapped between housekeeping genes *acrB* and *oprM*, which encode the A subunit of the multidrug pump and the outer membrane protein, respectively, in comparison to enterobacterial OGCs. Structural and immunochemical studies of *Aeromonas* O-antigens specific for serogroups dominant in Poland, combined with bioinformatics analysis of OGC clusters, will allow the use of molecular serotyping to characterize *Aeromonas* immunotypes.

Here we report on the immunochemical studies of the *Aeromonas* sp. O-antigens and the genetic basis of their synthesis. In addition, a comparison of the structure of the related O-antigens, and the genetic organization of their O-antigen synthesis regions was performed. Western blotting and ELISA experiments facilitated identification of common structural elements within the related O-antigens and recognition of epitopes that define the specificity of the immunotypes. The bioinformatics analyses of OGC showed the presence of key genes for the synthesis of O-units (activated sugars, glycosyltransferases, and initial glycosyltransferases) and their translocation and polymerization, as well as additional genes differentiating the regions. It was found that the composition of the studied OGC regions is consistent with the O-antigen structure. These are the first O-serotypes that have been completely recognized both genetically and structurally. The analyses undertaken in this study also facilitated the identification of genes as potential markers for molecular serotyping of the representatives of the dominant *Aeromonas* serogroup in Polish aquaculture of carp and trout.

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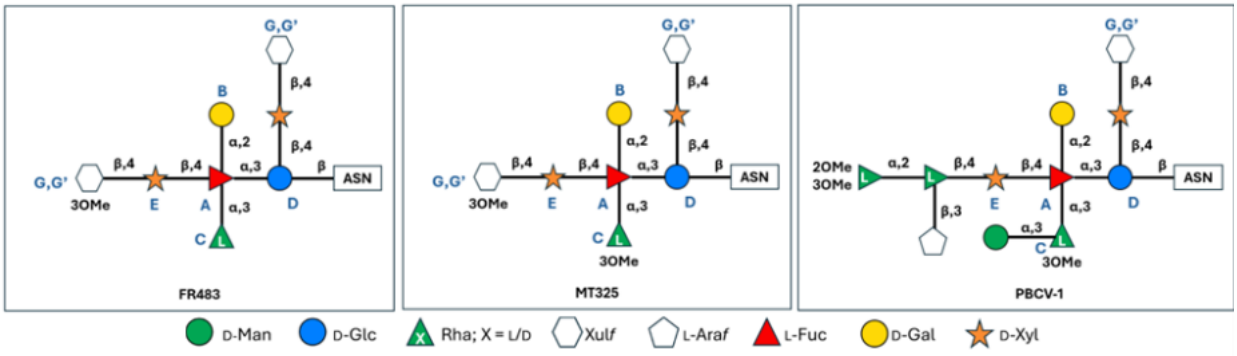
F11

NMR STUDIES REVEAL DISTINCT GLYCOSYLATION PATTERNS OF MAJOR CAPSID PROTEINS OF THREE CHLOROVIRUSES, FR483, MT325 AND PBCV-1

Lourriel Macale[a], Immacolata Speciale[a], Anna Notaro[a], Irina Agarkova[b], Antonio Molinaro[a], James L. Van Etten[b], and Cristina De Castro[a]

[a] Department of Chemical Sciences, University of Naples Federico II, lourriel.macale@unina.it
[b] Department of Plant Pathology, University of Nebraska-Lincoln

Chloroviruses (family *Phycodnaviridae*) are a group of viruses that exhibit unique glycosylation patterns not commonly found in other forms of life. Viruses under this family, however, show heterogeneity through the different non-stoichiometric substituents linked to their N-glycan core structures. Here, we compared the N-glycans decorating the major capsid proteins of three different chloroviruses, FR483, MT325, and PBCV-1 through NMR studies. Analyses showed that these three viruses share a common core N-glycan structure containing an N-linked β-glucose, a hyperbranched L-fucose, a proximal D-xylose and a terminal D-galactose. However, a differential modification of the substituents was revealed, particularly, the modification of the rhamnose and D-xylose residues linked to the L-fucose core glycan. The observed diversity of glycoforms is attributable to the different virus-encoded glycosyltransferases.



N-glycosylation of major capsid proteins of chloroviruses, FR483, MT325, and PBCV-1

Reference

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Acknowledgement: We would like to acknowledge the Glyco-N Doctoral Network under Horizon Europe (HORIZON) and Marie Skłodowska-Curie Actions Doctoral Networks (MSCA-DN) funded by the European Union under Grant Agreement 101119499.

F12

EXOPOLYSACCHARIDES PRODUCED BY *LIMOSILACTOBACILLUS FERMENTUM* MM1V: PHYSICAL PROPERTIES AND EFFECTS ON GUT PROBIOTIC STRAINS ADHERENCE

Andrej Sinica,[a],* Roman Bleha,[a] Mariana Canelas Pereira,[a,b] Šárka Horáčková,[b] Blanka Vrchotová,[b] Gabriela Krausová,[c] Iva Mrviková, [c] Ivan Saloň [d] and Jiří Štětina [b]

[a] Department of Carbohydrates and Cereals, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, Czech Republic, sinicaa@vscht.cz
[b] Department of Dairy, Fat and Cosmetics, University of Chemistry and Technology Prague, Technická 5, 16628 Prague, Czech Republic
[c] Dairy Research Institute, Ltd., Ke Dvoru 791/12a, 160 00, Prague, Czech Republic
[d] Department of Chemical Engineering, University of Chemistry and Technology Prague, Technická 5, 16628 Prague, Czech Republic

Lactic acid bacteria produce exopolysaccharides (EPS) that promote human digestion [1,2]. *Limosilactobacillus fermentum* is known as probiotic species [3,4]. Strain *L. fermentum* MM1V isolated from human milk (LF-MM1V) exhibit antibiotic properties against pathogenic bacteria. This strain also produce EPS isolated after 36 h cultivation in MRS broth: skimmed milk (1:1) at 37 °C in 5 % v/v CO_2 atmosphere. The crude EPS was purified with proteases (pepsin, pronase) and then fractionated by preparative chromatography yielding four polysaccharide fractions F1-4 (Fig. 1a). The EPS contained mainly mannose, glucose and galactose units in the molar ratio of 2:2:1; the presence of N-acetyl-galactosamine was detected by FTIR and NMR. GPC analysis indicated two peaks corresponding to molecular masses of 3.10×10^5 g mol^{-1} and 6.05×10^4 g mol^{-1}. The 2% w/w aq. solution of the EPS exhibits shear-thinning behavior at the shear rate region of 0.01 – 100 s^{-1} (Fig. 1b). This solution also showed viscoelastic properties with a crossover frequency ω^* ($G' = G''$) of ~13.8 s^{-1} (Fig. 1c). The EPS was tested on the adherence of four commercially used probiotic strains *in vitro* [5]. For the *Bifidobacterium animalis* BB-12® and *Escherichia coli* strains, adherence increased more than twofold. In contrast, for the lactobacilli strains LA5 and Shirota, EPS exhibited an anti-adhesive effect. Observed findings highlight species- and strain-specific differences in response to this EPS.

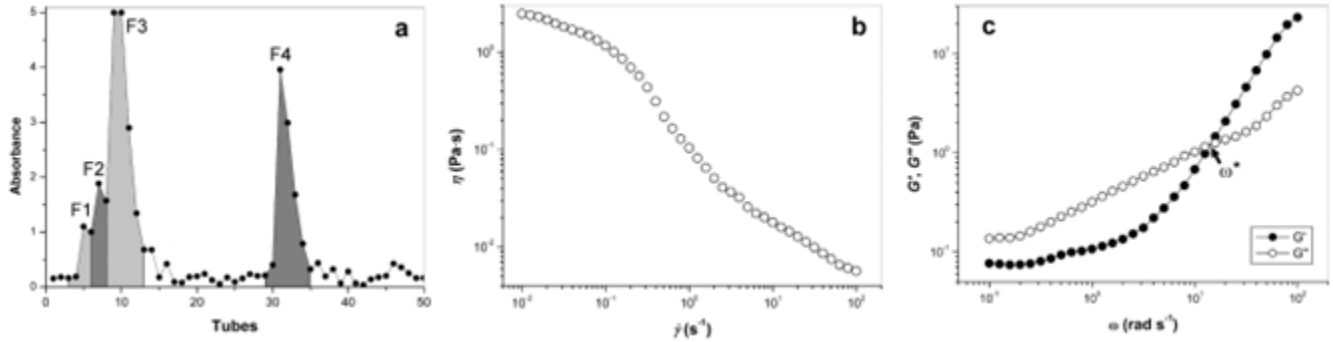

Figure 1. Fractionation (a), steady state behavior (b) and viscoelastic properties (c) of EPS (2 % w/w) produced by LF-MM1V.

This work was supported by the Ministry of Agriculture of the Czech Republic (project QK22010186).

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F13

BACTERIAL GLYCANS IN AUTOIMMUNITY. ANTI-LPS *PROTEUS MIRABILIS* S1959 (O3) ANTIBODIES FROM RA PATIENTS TARGET COLLAGEN AND HOST FIBROBLASTS

Katarzyna Durlik-Popińska,[a] * Dominika Drzazga,[a] Sylwia Nawrot, [a] Aneta Węgierek-Ciuk, [a] Paulina Żarnowiec[a], Katarzyna Gałczyńska, [a] Przemysław Bornicki, [b] Wiesław Kaca[a]

[a] Department of Microbiology, Institute of Biology, Jan Kochanowski University in Kielce, 25-369 Kielce, katarzyna.durlik-popinska@ujk.edu.pl, dominika.drzazga@ujk.edu.pl, sylwia.nawrot@ujk.edu.pl, aneta.wegierek-ciuk@ujk.edu.pl, paulina.zarnowiec@ujk.edu.pl, katarzyna.galczynska@ujk.edu.pl, wieslaw.kaca@ujk.edu.pl
[b] Świętokrzyskie Rheumatology Centre, St. Luke's Hospital, 26-200, Końskie, Poland; przemek_bornicki@wp.pl
*- corresponding author – katarzyna.durlik-popinska@ujk.edu.pl

Rheumatoid arthritis (RA) is among the most prevalent autoimmune disorders globally, characterized by chronic inflammation and the presence of disease-specific autoantibodies such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). Microbial factors, including components of Gram-negative bacteria such as lipopolysaccharides (LPS), can be implicated in the induction of autoantibody responses [1]. In this study, we investigated the reactivity of IgG-class antibodies affinity purified from the sera of 12 RA patients and 12 healthy donors against *Proteus mirabilis* S1959 (O3 serogroup) LPS. Using flow cytometry and ELISA methods, we observed that anti-LPS antibodies from RA patients exhibited enhanced binding to collagen-producing human VH10 fibroblasts compared to controls ($p < 0,05$). Subclass analysis revealed a predominance of IgG1 and IgG3 isotypes and with elevated IgG4 level in the RA group ($p < 0,05$). Statistical analysis demonstrated a positive correlation between fibroblast-binding IgG3 and IgG4 anti-LPS antibodies and serum concentrations of RF and ACPA ($r > 0,5$, $p < 0,05$). To explore potential epitope mimicry, we assessed antibody binding to type I collagen and a panel of 22 synthetic haptens representative of LPS structures from various *Proteus* serogroups. Results revealed reduced epitope specificity, as antibodies displayed variable cross-reactivity regardless of hapten structure or incorporated amino acid. Moreover, complement activation assays indicated higher C1q binding to VH10 fibroblasts opsonized with anti-LPS O3 antibodies, suggesting their potential to initiate inflammatory cascades. These findings support the hypothesis that RA patient sera contain elevated levels of cross-reactive, LPS-specific IgG antibodies capable of targeting host fibroblasts and activating the classical complement pathway. This mechanism may contribute to the chronic inflammation observed in RA and highlights the role of bacterial antigens in the modulation of autoimmune responses.

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F14

REINSTATING THE O10 ANTIGEN TO THE SEROLOGICAL CLASSIFICATION SCHEME FOR *KLEBSIELLA PNEUMONIAE*

Agnieszka M. Kowalczyk,[a,b] Steven D. Kelly,[a] Shawna Zhu,[a] Bradley R. Clarke,[a] and Chris Whitfield[a],*

[a] Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON N1G 2W1, Canada
[b] Department of Biomedical Chemistry, University of Gdańsk, Gdańsk, Poland

Klebsiella pneumoniae is an important human pathogen, responsible for a range of serious community and hospital-acquired infections. Resistance to current antibiotics has revitalized interest in immunotherapeutic strategies to counter this pathogen. Capsular K antigens and lipopolysaccharide (LPS) O antigens are both being pursued as potential candidates for immunotherapies. We have investigated the structure and biosynthesis of many of the currently recognized O antigens, but population genomics studies have identified additional genetic loci that are not correlated with any of the known *K. pneumoniae* polysaccharide structures. One of these is designated OL103. Comparison of sequences and structural models of the OL103 gene products with known enzymes facilitated a proposed repeat-unit structure composed of ribofuranose (Rib*f*) and L-rhamnose (L-Rha), as well as a non-reducing terminal methyl group. A similar structure was once reported as the *Klebsiella* O10 antigen [1] but O10 was subsequently removed from the serotyping scheme because the reference strain was found to belong to the *Enterobacter aerogenes* species. To pursue the relationship of OL103 to the original O10 reference strain, we sequenced the genome of the *E. aerogenes* reference strain and discovered an O-antigen locus sharing high levels of sequence similarity with the *K. pneumoniae* OL103 locus. The original O10 structure was derived from methylation data, so we confirmed the structure by NMR. LPS was isolated from dried bacteria using a hot phenol-water extraction and purified by 50% TCA and dialysis. After hydrolysis with 2% acetic acid, the released polysaccharide was separated by size-exclusion chromatography. The structure was determined using one- and two-dimensional NMR spectroscopy, by recording the following spectra: ^{1}H NMR, COSY, TOCSY, NOESY, HSQC, and HMBC. This data revealed a polysaccharide with a linear pentasaccharide composed of 2 Rib*f* and 3 L-Rha monosaccharide residues, with a sequence of linkages identical to the previously reported structure.

E A D B C

→3)-α-L-Rha*p*-(1→3)-β-D-Rib*f*-(1→4)-α-L-Rha*p*-(1→3)-β-D-Rib*f*-(1→4)-α-L-Rha*p*-(1→

In addition, the published structure predicts a terminal 3-*O*-methyl-L-Rha. While signals for the methyl group were observed in the spectra, its precise linkage was not established from the NMR data although its position is currently being pursued. Collectively, these data highlight the emerging power of sequence data to offer a preliminary prediction of polysaccharide components. Furthermore, the results call for the return of O10 to the *K. pneumoniae* serological classification scheme.

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P1

CHARACTERIZATION OF THE CLINICAL ISOLATE AND EXOPOLYSACCHARIDE OF THE *FLAVONIFRACTOR PLAUTII* PCM 3108

Daria Artyszuk [a]*, Wojciech Jachymek [a], Marcelina Pyclik [b], Paweł Migdał [c], and Jolanta Łukasiewicz [a]

[a] Laboratory of Microbial Immunochemistry and Vaccines, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wroclaw, Poland, daria.artyszuk@hirszfeld.pl
[b] Laboratory of Laboratory of Microbiome Immunobiology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences
[c] Laboratory of Instrumental Analysis and Preparation, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences

The human digestive tract is one of the most complex microbial ecosystems. Microbiota has a great influence on the maintenance of homeostasis or the development of several diseases, such as inflammatory bowel diseases (IBD). IBD include Crohn's disease (CD) and ulcerative colitis (UC) which pathology is characterized by chronic inflammation of digestive tract. It is known that patients with IBD have a greater amount of certain specific species of bacteria compared to healthy individuals[1].

Flavonifractor plautii is a strictly anaerobic, Gram-variable bacterium belonging to the Clostridiales[2,3]. It is a component of the human gut microbiome and is well known for its ability to metabolize a wide range of flavonoids, however, that is the only one clearly defined property of this species. *F. plautii* is characterized by slow and often minimal growth, thus its phenotypic identification is a challenge for microbiologists. Until now, six cases of infections due to *F. plautii* have been described[4-5]. Moreover, the enrichment of the *F. plautii* has been indicated in patients with colorectal carcinoma, IBD, irritable bowel syndrome and other gastrointestinal tract disorders[6].

The purpose of this study was to further analyze the biological properties of clinical isolate *F. plautii* PCM 3108. The complete structure of the exopolysaccharide (EPS) produced by *F. plautii* was determined using chemical analyses and nuclear magnetic resonance (NMR) spectroscopy as the repeating unit of →2)-α-Rha*f*-(1→4)-β-Rha*f*-(1→. Moreover, the impact of EPS on cytokine production and TLR receptors recognition were investigated. Bacterial cultures were also analyzed by transmission electron microscopy (TEM) showing the morphology of bacterial cells and cross-sections presenting the structure of the bacterial cell wall being responsible for the Gram-variation during staining. Furthermore, the ability to produce membrane vesicles by *F. plautii* was also demonstrated.

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P2

THE COMPLETE STRUCTURE OF LIPID A FROM *RHIZOBIUM* SP. CAS 24 THE BACTERIAL ENDOPHYTE OF *CHEMAECYTISUS ALBUS* (HACQ.) ROTHM.

Adam Choma[a]*, Anita Swatek[a], Iwona Komaniecka[a], Zbigniew Kaczyński[b], Sylwia Wdowiak-Wróbel[a] and Monika Marek-Kozaczuk[a]

[a] Department of Genetics and Microbiology, Institute of Biological Science, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland, e-mail: adam.choma@mail.umcs.pl
[b] Department of Medical Chemistry, Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308, Gdansk, Poland

The structure of the lipid A isolated from the lipopolysaccharide of *Rhizobium* sp. CAS 24 is presented. According to 16S rRNA analysis, this endophyte of *Chemaecytisus albus*, named CAS 24, is a soil bacterium belonging to *Rhizobiaceae* family. The structural study, carried out by means of chemical analysis, mass spectrometry, and nuclear magnetic resonance spectroscopy, revealed that lipid A preparation consisted of a mixture of species bearing tri-, tetra- and penta-acyl residues. All of them sharing the same bis-phosphorylated glucosaminyl(1→6)glucosamine backbone. The penta-acyl species of lipid A were substituted by 14:0(3-OH) fatty acids with free 3-hydroxyl groups at positions C-3 and C-3'. Two amide linked acyl residues: 18:0(3-OH) and 18:1(3-OH) occupied positions C-2 and C-2', respectively. The unsaturated acyl residue (18:1(3-OH)) was esterified by a 29-hydroxytricontanoic [30:0(29-OH)] residue at its hydroxyl group. The second species, found in smaller amounts, was a tetra-acylated lipid A, lacking the 14:0 (3-OH) residue on proximal Glc*p*N. Tri-acylated lipid A species was deprived of both ester linked residues. Other species observed on the mass spectra deriving from described above by loss a one or two phosphate groups or by replacement of primary acyl substituents by their chain homologues. It is very probable, that the lack of a phosphate residue at the C-1 position in the some lipid A species is a side effect of the process of releasing lipid A from LPS through mild acid hydrolysis. The presented structure of lipid A of *Rhizobium* sp. CAS 24 is very similar to that previously described for *Sinorhizobium fredii* HH103 by Di Lorenzo and co-workers.[1]

Reference

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P3

CHARACTERIZATION OF LIPOPOLYSACCHARIDES IN ZOONOTIC *S. LIQUEFACIENS* AND *S. MARCESCENS* ISOLATES

Gabriela Cieniuch,[a] Agnieszka Korzeniowska-Kowal,[a] Gabriela Bugla-Płoskońska,[b] and Andrzej Gamian[a]

[a] Polish Collection of Microorganisms, Laboratory of Medical Microbiology, Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, Wroclaw, Poland, gabriela.cieniuch@hirszfeld.pl
[b] Department of Microbiology, University of Wroclaw, Stanislawa Przybyszewskiego 63, Wroclaw, Poland

The characterization of the length of O-specific chain of LPS, as one of the virulence factors in Gram-negative bacteria, for *S. marcescens* and *S. liquefaciens* isolates is very poorly described. Works published in 1988 and 1990 [1] [2] shows that *S. marcescens* isolates, presented in these studies, had a long O-specific LPS chain. No literature data was found about presenting the analysis of the O-specific chain of LPS for *S. liquefaciens* isolated from various sources. Characterization of the length of O-specific chain of LPS was performed for 33 of *S. liquefaciens* and *S. marcescens* isolates. Among 33 isolates tested, 25 belonged to the species *S. liquefaciens* and 8 belonged to the species *S. marcescens*. To isolate LPS from the tested *S. liquefaciens* and *S. marcescens* strains the proteinase K method was used. Whereas the separation of LPS was performed using electrophoresis under denaturing conditions SDS-PAGE.

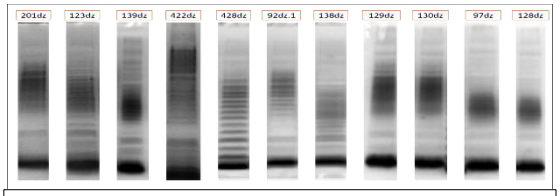

Fig. 1. Image of SDS-PAGE electrophoresis of the O-specific chain of LPS of *S. liquefaciens* isolates from wild boars

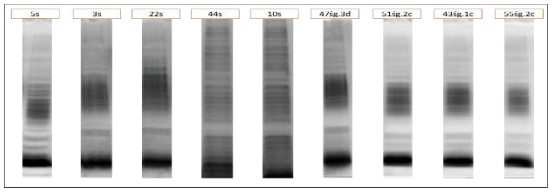

Fig. 2. Image of SDS-PAGE electrophoresis of the O-specific chain of LPS of *S. liquefaciens* isolates from roe deer and pigs

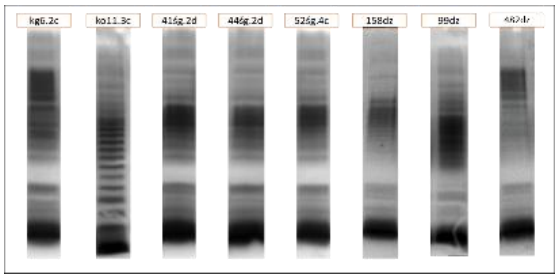

Fig. 3. Image of SDS-PAGE electrophoresis image of the O-specific chain of LPS of *S. marcescens* isolates

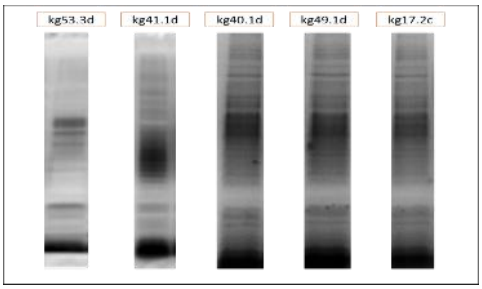

Fig. 4. Image of SDS-PAGE electrophoresis of the O-specific chain of LPS of *S. liquefaciens* isolates from cattle

A modal distribution containing two fractions: L-OAg and LMW-OAg was observed in all tested isolates of *S. liquefaciens* and *S. marcescens* genus. In general, most of *S. marcescens* strains showed some differences between O-antigen, specifically in its subunit's length. The strains isolated from pigs were the only exception where aforementioned subunits of LPS were similar to each other (Fig.3). In the case of *S. liquefaciens* acquired from wild boars, roe deer, cattle and pigs (Fig.1, Fig.2, Fig.4), such similarities were observed in all those strains. The long O-specific LPS chain correlates with bacterial resistance to the complement system.

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P4

IMMUNOHISTOCHEMICAL REACTIVITY OF HUMAN TISSUES WITH MONOCLONAL ANTIBODY AGAINST *ESCHERICHIA COLI* O104 O-SPECIFIC POLYSACCHARIDE CONTAINING SIALIC ACID

Agnieszka Korzeniowska-Kowal[1], Magdalena Geneja-Maciejewska[1], Tomasz Lipiński[2], Piotr Ziółkowski[3], Sławomir Letkiewicz[4], Andrzej Gamian[1]

[1] Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland
[2] Łukasiewicz Research Network-PORT Polish Center for Technology Development, Stablowicka 147, 54-066 Wrocław, Poland
[3] Department of General Pathology, Wrocław Medical University, Marcinkowskiego 1, Wrocław, Poland
[4] Collegium Medicum, Jan Długosz University in Czestochowa, Armii Krajowej Av 13/15, 42-200 Czestochowa, Poland

The serotype O104 of *E. coli* is characterized by the presence of sialic acid in its lipopolysaccharide. Here we report the reactivity of anti-O104 polysaccharide monoclonal antibody with human tissues. The study was conducted under an assumption that the sialic acid based molecular identity of bacterial and tissue structures is not only an understanding the mimicry based bacterial pathogenicity. Cross-reacting antibodies could be used to recognize specific human tissues depending on their histogenesis and differentiation which might be useful for diagnostic purposes. The results indicate that variety of human tissues is recognized by anti-O104 antibodies. Positive reaction has been found in the metastatic colon adenocarcinoma cells in liver, ganglioneuroma cells, ovarian endometrioid carcinoma cells, mammary invasive ductal carcinoma cells and prostate carcinoma cells. The negative reaction with monoclonal antibody anti-O104 has been observed in trophoblast, cartilage, normal squamous epithelium, hematopoietic cells and certain tumors, such as squamous cell carcinoma, myxoid liposarcoma and fibrosarcoma. In general, O104 antibody performed staining of the various tissues with distinct prevalence of glandular epithelium.

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P5

INFLUENCE OF CULTURE MEDIA ON THE GROWTH OF *M. BOVIS* BCG SUBSTRAIN MOREAU IN STATIONARY AND SUBMERGED CULTURES

Katarzyna Machnik,[a] Mariola Paściak[a]

[a] Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, katarzyna.machnik@hirszfeld.pl

In the production of the BCG vaccine, it is of critical importance to optimise the culture process and to minimise losses at each stage. The final product must contain a consistent amount of live BCG bacilli capable of inducing an immune response. Currently, BCG is cultured using the pellicle growth method in stationary flasks. The pellicle growth method involves the use of flasks containing an appropriate liquid medium, where growth occurs exclusively on the surface layer of the medium. The cultivation process is divided into stages, each involving an increase in the number of flasks to expand the biomass area. The entire cycle lasts 4-6 weeks. Finally, the biomass is separated, and a suspension is prepared and homogenised. However, this method of production leads to the formation of aggregates, and poses quality control challenges. Disrupting the pellicle damages the cell wall, resulting in the death of 40-60% of cells. The static culture method for BCG necessitates a substantial amount of working space and involves manual stages that require the input of experienced staff.

The objective of this project is to enhance the growth of the BCG vaccine strain in submerged culture, thereby facilitating the development of novel technology for the large-scale production of M. bovis BCG Moreau biomass in bioreactors.

As part of the pilot experiments for BCG Moreau cultivation, efforts were made to establish a repeatable cultivation cycle. Subsequently, a series of tests were conducted on different culture media. The following culture media were tested: Roisin, Middlebrook 7H9, Dubos, Sauton, and Sauton modifications (addition of glucose, different ZnSO4 concentration, different amino acids). In all culture experiments, the initial step was to establish a stationary culture in a Calmette flask with a 400 ml capacity. Following 22-26 days, the bacteria were transferred for the first time into a stationary culture and a submerged culture, which was shaken with the addition of Tween 80. The stationary cultures were incubated in parallel at 37°C. Following a period of 10-14 days, a second passage was conducted to establish new stationary and shaking flasks. The cultures were incubated under the same conditions as previously described. After 10-12 days, a final biomass harvesting was conducted. The bacteria were inactivated at 100 °C for one hour. The biomass was harvested by centrifugation and freeze-dried. Subsequently, different media were validated, and the biomass obtained from stationary and shaken cultures was compared after two passages. All experiments were conducted by standardised protocols to ensure reproducibility.

A crucial aspect of the pilot study was the verification of the optimal concentration of surfactant, which was determined to be 0.05%. The subsequent stage of the process involved optimising the shaking speed and incubation temperature. The results demonstrated that the greatest biomass was attained in stationary culture on the Sauton medium. Among all the tested shaken cultures, the most optimal growth was observed on the modified Sauton medium, although further verification is required. The results are promising for further growth improvement. The positive outcomes are of considerable significance in advancing the technology and influencing the field of epidemiology, particularly in the context of tuberculosis control.

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P6

STRUCTURE OF THE *ACTINOMYCES ODONTOLYTICUS* POLYSACCHARIDE AND THE SPECIFICITIES OF MONOCLONAL ANTIBODIES DIRECTED AGAINST IT

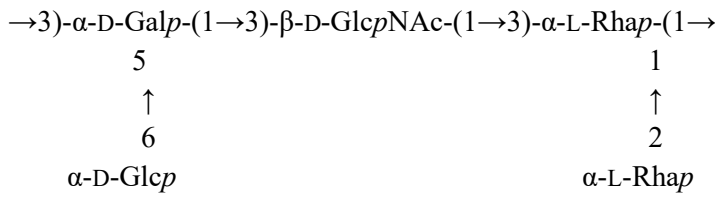
Mariola Paściak,[a] Marta Kaszowska,[b] Adnan Saeed,[a] and Andrzej Gamian[a]

[a] Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wroclaw, Poland
[b] Department of Immunochemistry, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wroclaw, Poland

Actinomycosis is a soft tissue infection that can result in the formation of sinus tracts in the skin. This infection can affect any individual, irrespective of their immune status. In the majority of cases, the clinical symptoms are similar to those of tuberculosis and malignancy. *Actinomyces* species constitute part of the normal microbiota of the oral cavity, gastrointestinal and urogenital tracts. While these microorganisms are normally harmless, they can become pathogenic if they breach the mucosal barrier. Polysaccharides on the surface of bacterial cells play many roles in the ecology of microorganisms. In the case of pathogenic strains, these polysaccharides are responsible for interactions with the host. They are additionally regarded as valuable targets for diagnostic strategies and vaccine development.

This study aimed to determine the structure of the polysaccharide antigen from *Actinomyces odontolyticus* PCM 2399 and generate antibodies against this polysaccharide, which will be useful for further study of bacterial infection of host tissue.

Bacteria *A. odontolyticus* PCM 2399 were cultivated in a liquid thioglycolate medium for 120 hours at 37°C in an anaerobic atmosphere. The polysaccharide was extracted from the cell mass using trichloroacetic acid and a combination of hydrolytic enzymes, including DNase, RNase, and protease. The extract was subsequently purified using ion-exchange chromatography and gel filtration. The composition of the polysaccharide was determined by gas chromatography-mass spectrometry (GC/MS) and subsequently confirmed by nuclear magnetic resonance (NMR) spectroscopy. The chemical analysis revealed that the polysaccharide of *A. odontolyticus* comprises rhamnose, galactose, glucose and glucosamine residues. The following structure of the pentasaccharide repeating unit of *A. odontolyticus* polysaccharide has been established:



Monoclonal antibodies (mAbs) were produced using the hybridoma technique[1, 2]. The specificity of mAbs was evaluated using the ELISA method. Two hybridomas were selected, designated mAbs 12 and mAbs 39, producing mAbs against a polysaccharide antigen. Both mAbs were of the IgM class. The capacity of these monoclonal antibodies to cross-react with different *Actinomyces* spp. antigens, potentially due to the presence of a shared epitope on the cell wall has been demonstrated. Additionally, the mAbs obtained against polysaccharides express reactivity with homologous antigens and cross-reactivity between *A. odontolyticus* and *A. naeslundii*. These results have significant diagnostic implications, as it has been demonstrated that polysaccharides can serve as valuable markers for the diagnosis of actinomycoses [2].

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P7

O-ACETYL GROUPS IN *PROTEUS* O ANTIGENS MAY HAVE A GREAT IMPACT ON THE SEROLOGICAL SPECIFICITY OF THESE BACTERIA

Agata Palusiak[a], Anna Turska-Szewczuk[b], Dominika Drzewiecka[a]

[a] Department of Biology of Bacteria, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland, E-mail: agata.palusiak@biol.uni.lodz.pl
[b] Department of Genetics and Microbiology, Institute of Biological Sciences, Maria Curie-Sklodowska University, Akademicka 19, 20-033 Lublin, Poland

Bacteria within the genus *Proteus* are serologically differentiated and have so far been classified to 85 O serogroups [1]. Some of them are additionally divided into subgroups due to slight structural and serological differences between O antigens within a serogroup [2]. *P. mirabilis* Sr81 and Bm133 come from wound and urea, respectively, of two patients from the Lodz area. Neither the biomass nor the extracted lipopolysaccharide (LPS) of these strains were recognized in ELISA by any of the antisera specific to each known *Proteus* O serotypes. Thus, rabbit polyclonal antisera were obtained for the heat-inactivated cells of *P. mirabilis* Sr81 and Bm133 strains and their reactivities were tested in ELISA with the LPSs representing *Proteus* O serotypes described so far. The obtained results confirmed the previous ones suggesting the uniqueness of *P. mirabilis* Sr81 and Bm133 O antigens, and, at the same time, indicated their mutual similarity. The chemical and structural analyses of the *P. mirabilis* Sr81 O antigen, including 1H, 13C and 31P NMR spectroscopy, revealed that the O repeating unit has a linear structure and is built of four sugar components interlinked by a glycosidic phosphate. This type of linkage is rare among *Proteus* O antigens. The Sr81 O polysaccharide (OPS) is related in its structure to O48 OPS [2]. However, the side O-acetyl group present in O48 OPS and absent from the Sr81 O antigen makes a crucial difference in the serological specificity of these antigens. Only slight cross reactions were observed in ELISA and Western blotting between the O48 LPS and Sr81/Bm133 antisera, while the O48 antiserum did not recognize the Sr81 or Bm133 LPS epitopes, which emphasises the role of side O-acetyl groups in the OPSs specificity. This is also the case of *Salmonella* and *Escherichia coli*, as well as *Aeromonas* O serotypes [3, 4].

Thus, we propose to include the *P. mirabilis* Sr81 and Bm133 strains in the newly created O48a subgroup, differing from the O48 a,b subserotype (the previous O48 serogroup) by the lack of the O-acetyl side group, which substantially influences the serological specificity of the two O48 subgroups.

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P8

ESCHERICHIA COLI P2B POLYSACCHARIDE AS A LIGAND FOR COMPLEMENT LECTIN PATHWAY ACTIVATING FICOLIN-2 and 3

Anna Maciejewska,[a]* Wojciech Jachymek,[a] Paula Czyszczoń,[a] Gabriela Gajek,[b] Katarzyna Kasperkiewicz,[c] Maciej Cedzyński,[b] Jolanta Lukasiewicz, [a] and Anna Świerzko,[b]

[a] Laboratory of Microbial Immunochemistry and Vaccines, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wroclaw, Poland, anna.maciejewska@hirszfeld.pl
[b] Laboratory of Immunobiology of Infections, Institute of Medical Biology, Polish Academy of Sciences, Lodowa 106, 93-232 Łódź, Poland.
[c] Institute of Biology, Biotechnology, and Environmental Protection, Faculty of Natural Sciences, The University of Silesia in Katowice, Jagiellońska 28, 40-032 Katowice, Poland.

Ficolin-2 and ficolin-3 are important factors of innate immunity. They are able to participate in elimination of pathogens through opsonisation of their cells (contributing to phagocytosis) or complement activation *via* the lectin pathway (thanks to co-operation with serine proteases of the MASP family), leading to the direct cell lysis. They may form heterocomplexes, called ficolin-23. While ficolin-3 specificity is restricted to very few microorganisms, ficolin-2 recognises numerous bacteria, fungi, parasites, and viruses. Among its ligands, capsular polysaccharides, lipoteichoic acids, mycobacterial lipoarabinomannan and antigen 85 complex, *Streptococcus pneumoniae* pneumolysin, 1,3-β-glucans, and hemagglutinin of influenza A virus are mentioned. Moreover, artificial ligands like acetylated albumin (Ac-BSA) or N-acetylglucosamine pentamer (GN5-DPPE) were previously used to detect active ficolin-2 molecules (although no ficolin-2-dependent complement activation by Ac-BSA was observed) [1,2].

Here we report interaction of ficolin-2 and ficolin-3 with *Escherichia coli* P2b (serotype O15) bacteria, isolated from blood of preterm neonate, born with signs of intrauterine infection. Both ficolin-2 and ficolin-3 (as well as murine ficolin A, but not human ficolin-1 or mannose-binding lectin) were found to recognise high molecular mass fraction of supernatant obtained after ultracentrifugation of *E. coli* P2b lipopolysaccharide (LPS). Upon recognition of that fraction by ficolin-2/ficolin-3, MASP-1 enzyme became activated leading to complement lectin pathway activation.

Structural analyses of composition of the supernatant indicated the presence of O15 LPS, enterobacterial common antigen (ECA_{PG} and ECA_{CYCLIC}), and capsular polysaccharide (CPS, antigen K) built up of the [→3)-α-D-Glc*p*NAc-(1→P]$_n$. Immunoblotting and ELISA analyses indicated CPS as a functional ligand for ficolin-2 and 3, and excluded O15 LPS and ECA. Identified *E. coli* P2b CPS represents previously described K51 antigen reported as [→3)-α-D-Glc*p*NAc-(1→P]$_n$ with *O*-acetyl groups at 4 and 6 position [3], however contrary to the published structure, identified CPS was devoid of *O*-acetyl groups.

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