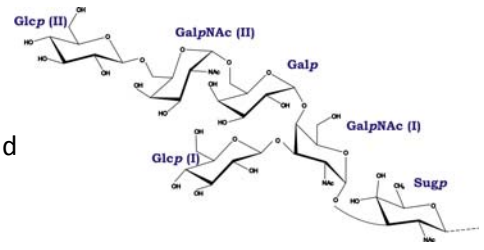


## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



# 4<sup>th</sup> Baltic Meeting on Microbial Carbohydrates – 4<sup>th</sup> BMMC

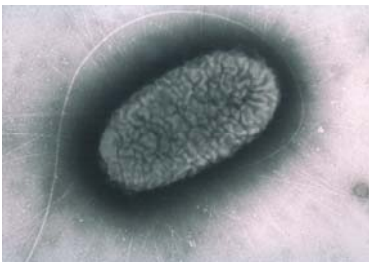
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Hyytiälä Forestry Field Station, September 19-22, 2010

### Contents:

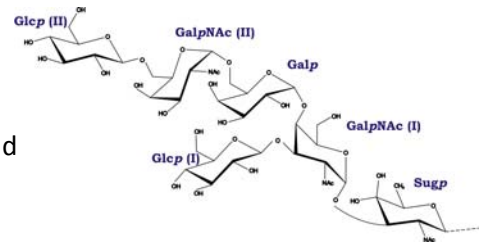
**Organizers**  
**Welcome**  
**General instructions**  
**Accommodation list**  
**Participant list**  
**Programme**  
**Poster sessions**

**Invited speaker abstracts**  
**Oral presentation abstracts**  
**Poster session 1 abstracts**  
**Poster session 2 abstracts**



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### International Organizing Committee

**Andrzej Gamian** (*Wroclaw, Poland*)  
**Jukka Finne** (*Helsinki Finland*)  
**Otto Holst** (*Borstel Germany*)  
**Yuriy Knirel** (*Moscow, Russia*)  
**Timo Korhonen** (*Helsinki Finland*)  
**Stefan Oscarson** (*Dublin Ireland*)  
**Elke Schweda** (*Stockholm, Sweden*)  
**Mikael Skurnik** (*Helsinki Finland*)

### Local Organizing Committee

**Jukka Finne** (*Helsinki*)  
**Timo Korhonen** (*Helsinki*)  
**Mikael Skurnik** (*Helsinki*)

### Poster sessions

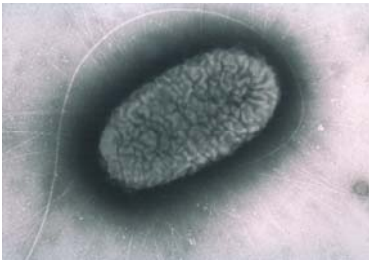
**Mona Svensson** (*Stockholm, Sweden*)  
**Olga Ovchinnikova** (*Moscow, Russia*)  
**Olga Valueva** (*Moscow, Russia*)  
**Salim Islam** (*Guelph, Canada*)  
**Nikolay Kondakov** (*Moscow, Russia*)  
**Carolina Fontana** (*Stockholm, Sweden*)

### Local assistance

**Kaisa Pulliainen** (*Helsinki*)  
**Tuula Ruusunen** (*Forestry Field Station*)  
**Marta Biedzka-Sarek** (*Helsinki*)  
**Kamila Rabsztyn** (*Helsinki*)  
**Elise Pinta** (*Turku*)

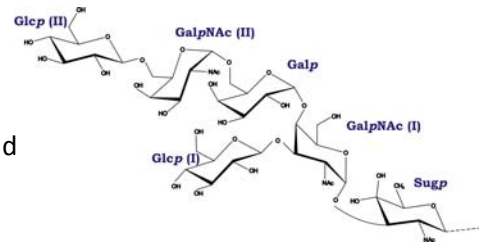
### Dr. Helander & His Power Drops

**Dr. Helander** (*vocals, guitars*)  
**Pepe Ahlqvist** (*vocals, harmonica, guitar*)  
**George Plumber** (*guitars*)  
**Mike Mountain** (*bass*)  
**General Lee** (*drums*)



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



# Welcome

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I welcome all the participants to the 4<sup>th</sup> BMMC meeting and wish that the meeting will be successful both scientifically and socially. The reason of bringing people to a more remote place is that there are less distractions to participants so that they can concentrate fully to both excellent science and networking. High class networking should be possible since we have 91 participants representing 15 different countries.

We have received support from several sources and I want to thank all of them here:

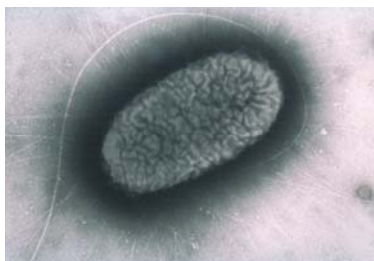
- Finnish Glycoscience Graduate School
- Societas Biochemica, Biophysica et Microbiologica Fenniae (The BioBio Society)
- Helsinki Biomedical Graduate School
- Federation of Finnish Learned Societies
- FEMS Visiting grant
- Dionex (The ICS-5000 Capillary Reagent-Free IC System is on exhibition during the meeting)
- Reactionlab Finland Oy (see the advertisement)

I have tried to keep the meeting expenses at minimum and organized the meeting without a professional congress agency. Therefore we will be on our own during the meeting. We have to organize our own fun during the little free time we have.

I have received a lot of help from the Forestry Field Station personnel and I want to thank them for letting us use the Station for our meeting. In practical matters Kaisa Pulliainen, Marta Biedzka-Sarek, Elise Pinta and Kamila Rabsztyn have been very helpful; thank you very much. I also thank the members of the local and international organizing committees that helped to organize the outstanding scientific program of the meeting. Finally, I want to thank Mona, Olga O., Olga V., Salim, Carolina and Nikolay for organizing the poster sessions.

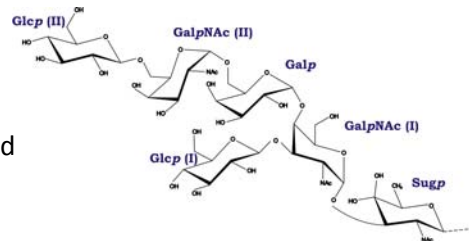
I wish you all enjoyable meeting.

Mikael Skurnik



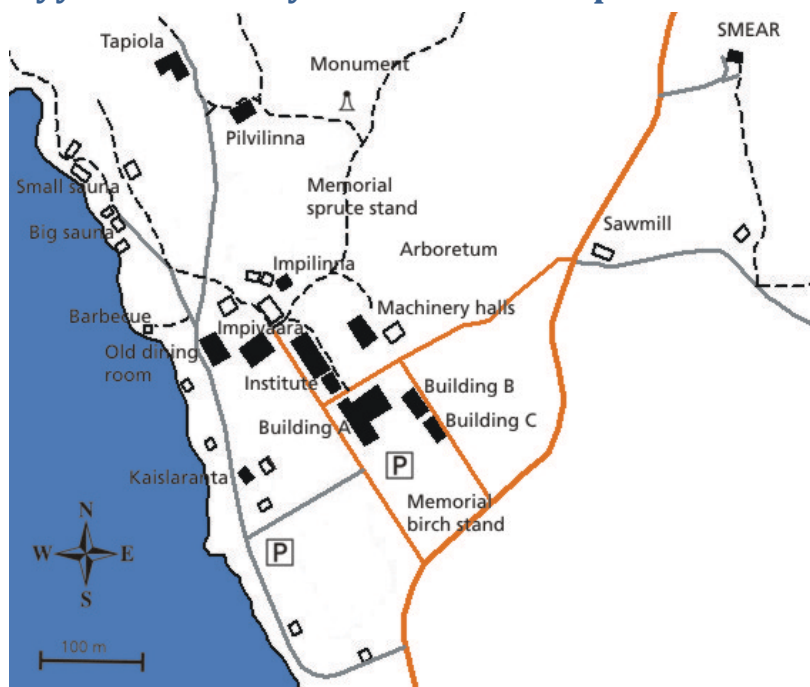
## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



# General instructions

## Hyytiälä Forestry Field Station map



## Registration on 19.9.2010

Building A entrance. Collect your name badge and registration fee receipt. Missing fees can be paid in cash. Late registrations between sessions to Mikael Skurnik or Marta-Biedzka-Sarek .

## Accommodation

- Outside door code to Buildings A and B is 5729
- Room keys are waiting in the room door locks.
- On Wednesday Building A rooms have to be empty after breakfast. Luggage storage is in the gym room.

## Bus transportation

Wednesday September 22, 2010, from Hyytiälä to Tampere departs after lunch at ca. 12.30.

## Lecture hall

Located in the Institute building

## Poster sessions

Located in the gym in Building A ground floor

## Meals and afternoon coffees

Served in Building A dining room.

## WLAN connections and computer rooms

Several guest accounts each for 5 simultaneous users are available. University of Helsinki personnel can use their own accounts.

WLAN Guest accounts						Computer room
Username	6k5546	6k5547	6k5548	...	6k5555	imap
Password	logger10	logger11	logger12	...	logger	freeway

## **Participant list – 4th BMMC**

### **Participant per country**

Australia	2	Norway	1
Canada	6	Poland	20
Finland	11	Russia	17
France	1	Sweden	6
Germany	11	Switzerland	2
Ireland	5	United Kingdom	3
Italy	2	Unites States	3
Netherlands	1		

#### **MICHAL ARABSKI**

Jan Kochanowski University, Department of Microbiology, Kielce, Poland  
*arabski@ujk.edu.pl*

#### **AGNIESZKA BECZALA**

University of Silesia, Microbiology, Katowice, Poland  
*abeczala@us.edu.pl*

#### **SUSAN BENNETT** (*Accompanying person*)

Massachusetts General Hospital, Medicine, Brookline, MA, USA

#### **MARTA BIEDZKA-SAREK**

National Institute for Health and Welfare, Department of Chronic Disease Prevention, Helsinki, Finland  
*marta.biedzka-sarek@thl.fi*

#### **ALAN BLACK**

University of Dundee, Biological Chemistry and Drug Discovery, Dundee, Scotland  
*a.j.z.black@dundee.ac.uk*

#### **FILIPPO BONACCORSI**

University College Dublin, School Of Chemistry, Centre For Synthesis And Chemical Biology, Dublin, Ireland  
*filippo.bonaccorsi@gmail.com*

#### **BENTE BØRUD**

University of Oslo, Department of Molecular Biosciences, Oslo, Norway  
*bente.borud@imbv.uio.no*

#### **ADAM CHOMA**

Maria Curie-Sklodowska University, Department of Genetics and Microbiology, Lublin, Poland  
*adam.choma@poczta.umcs.lublin.pl*

#### **PAOLO COSTANTINO**

Novartis Vaccines & Diagnostics, Research, Siena, Italy  
*paolo.costantino@novartis.com*

#### **ANNA CZABANSKA**

Research Center Borstel, Structural Biochemistry, Borstel, Germany  
*aczabanska@fz-borstel.de*

#### **MALGORZATA CZERWICKA**

University of GdaDsk, Faculty of Chemistry, GdaDsk, Poland  
*margo@chem.univ.gda.pl*

#### **KATALIN DARAGICS**

University College Dublin, Centre for Synthesis and Chemical Biology, Dublin, Ireland  
*katalin.daragics@ucd.ie*

#### **BARBARA DMOCHOWSKA**

University of Gdansk, Chemistry, Gdansk, Poland  
*bdmochow@chem.univ.gda.pl*

#### **KATARZYNA DUDA**

Research Center Borstel, Structural Biochemistry, Borstel, Germany  
*kduda@fz-borstel.de*

## **Participant list – 4th BMMC**

### **HALINA DZIADZIUSZKO**

Medical University of GdaDsk, Molecular  
Microbiology and Serology, GdaDsk, Poland  
*halim@gumed.edu.pl*

### **CHRIS EDGAR**

University of Dundee, Biological Chemistry And Drug  
Discovery, Dundee, Scotland  
*c.w.edgar@dundee.ac.uk*

### **JÖRG EHRT**

Hannover Medical School, Institute for Cellular  
Chemistry, Hannover, Germany  
*j.ehrit@yahoo.de*

### **YULIA FEDONENKO**

Institute of Biochemistry and Physiology of Plants and  
Microorganisms, RAS, Laboratory of Biochemistry,  
Saratov, Russia  
*fedonenko@yandex.ru*

### **MARIO FELDMAN**

University of Alberta, Biological Sciences, Edmonton,  
Canada  
*mfeldman@ualberta.ca*

### **VALENTINA FEODOROVA**

Scientific and Research Institute for Medical and  
Veterinary Biotechnology, Zoo- and Zooanthroponotic  
Diseases, Saratov, Russia  
*feodorovav@mail.ru*

### **TIMM FIEBIG**

Hannover Medical School, Institute for cellular  
chemistry, Hannover, Germany  
*timmfiebig@yahoo.de*

### **JUKKA FINNE**

University of Helsinki, Department of Biosciences,  
Helsinki, Finland  
*jukka.finne@helsinki.fi*

### **CAROLINA FONTANA**

Stockholm University, Organic Chemitrsy, Stockholm,  
Sweden  
*fontana@organ.su.se*

### **ANDRZEJ GAMIAN**

Institute of Immunology and Experimental Therapy,  
Polish Academy of Sciences, Department of  
Immunology of Infectious Diseases, Wroclaw, Poland  
*gamian@iitd.pan.wroc.pl*

### **MARINA GENING**

N.D. Zelinsky Institute of Organic Chemistry,  
Laboratory of Glycoconjugate Chemistry, Moscow,  
Russia  
*marina@mcl.ru*

### **RITA GERARDY-SCHAHN**

Medical School Hannover, Cellular Chemistry,  
Hannover, Germany  
*Gerardy-Schahn.Rita@mh-hannover.de*

### **OTTO HOLST**

Research Center Borstel, Molecular Infectiology,  
Borstel, Germany  
*oholst@fz-borstel.de*

### **HEATHER HORAN**

University College Dublin, School of Chemistry and  
Chemical Biology, Dublin, I  
*heather.horan@ucd.ie*

### **MARIA VERONICA IELMINI**

University of Alberta, Biological Sciences, Edmonton,  
AB, Canada  
*ielmini@ualberta.ca*

### **SALIM ISLAM**

University of Guelph, Molecular and Cellular Biology,  
Guelph, Canada  
*islams@uoguelph.ca*

### **WOJCIECH JACHYMEK**

Institute of Immunology and experimental Therapy,  
Immunochemistry, WrocBaw, Poland  
*jachymek@iitd.pan.wroc.pl*

### **DETLEF JENSEN**

Dionex (Europe) Management AG, EU Support and  
Laboratory Managment, Olten, Switzerland  
*Detlef.Jensen@Dionex.com*

## Participant list – 4th BMMC

### WIESLAW KACA

The Jan Kochanowski University of Humanities and Sciences, Department of Microbiology, Kielce, Poland  
*wieslaw.kaca@ujk.edu.pl*

### ZBIGNIEW KACZYNSKI

University of Gdansk, Faculty of Chemistry, Gdansk, Poland  
*zbyszek@chem.univ.gda.pl*

### ELISA KALLIO

University of Helsinki, Institute of Dentistry,  
University of Helsinki, Finland  
*elisa.kallio@helsinki.fi*

### KATARZYNA KASPERKIEWICZ

University of Silesia, Katowice, Poland, Department of Microbiology, Faculty of Biology and Environmental Protection, Katowice, Poland  
*katarzyna.kasperkiewicz@us.edu.pl*

### TIM KEYS

Hannover Medical School, Glycobiology, Hannover, Germany  
*keys.tim@mh-hannover.de*

### YURIY KNIREL

N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Laboratory of Carbohydrate Chemistry, Moscow, Russia  
*yknirel@gmail.com*

### IWONA KOMANIECKA

Maria Curie-Sklodowska University, Department of Genetics and Microbiology, Lublin, Poland  
*ikoma@hektor.umcs.lublin.pl*

### BOZHENA KOMAROVA

N.D. Zelinsky Institute of Organic Chemistry Russian Academy of Sciences, Laboratory of Glycoconjugate Synthesis, Moscow, Russia  
*kbozhena@gmail.com*

### NIKOLAY KONDAKOV

N.D. Zelinsky Institute of Organic Chemistry RAS, N.K. Kochetkov Laboratory of Carbohydrate Chemistry, Moscow, Russian Federation  
*nkondak@ioc.ac.ru*

### LEONID KONONOV

N.D. Zelinsky Institute of Organic Chemistry, N.K. Kochetkov Laboratory of Carbohydrate Chemistry, Moscow, Russian Federation  
*leonid.kononov@gmail.com*

### TIMO KORHONEN

University of Helsinki, Department of Biosciences, University of Helsinki, Finland  
*timo.korhonen@helsinki.fi*

### MICHAEL KOWARIK

GlycoVaxyn AG, Research, Schlieren, Switzerland  
*michael.kowarik@glycovaxyn.com*

### VADIM KRYLOV

N.D. Zelinsky Institute of Organic Chemistry, Laboratory of Glycoconjugate Chemistry, Moscow, Russia  
*vadimkrilov@yandex.ru*

### WANDA KUFLOWSKA (Accompanying person)

Institute of Immunology and Experimental Therapy, Department of Immunology of Infectious Diseases, Wrocław, Poland

### JOSEPH LAM

University of Guelph, Molecular and Cellular Biology, Guelph, ON, CANADA  
*jlam@uoguelph.ca*

### BEATA LIBEREK

University of Gdańsk, Department of Chemistry, Gdańsk, Poland  
*beatal@chem.univ.gda.pl*

### BUKO LINDNER

Research Center Borstel, Molecular Infection Biology, Borstel, Germany  
*blindner@fz-borstel.de*

### PAVEL LUKYANOV

Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of Russian Academy of Sciences, Vladivostok, Russia  
*paluk@mail.ru*

### KAARINA LÄHTEENMÄKI

Finnish Red Cross Blood Service, Research and Development, Helsinki, Finland  
*Kaarina.Lahteenmaki@veripalvelu.fi*

## **Participant list – 4th BMMC**

### **ANTONIO MOLINARO**

Universita' di Napoli Federico II, Dipartimento di  
Chimica Organica e Biochimica, Napoli, Italy  
*molinaro@unina.it*

### **RENATO MORONA**

University of Adelaide, School of Molecular and  
Biomedical Science, Adelaide, Australia  
*renato.morona@adelaide.edu.au*

### **JUDY MORONA** (*Accompanying person*)

University of Adelaide, Public Health HTA, Adelaide,  
Australia  
*judy.morona@adelaide.edu.au*

### **LAURENCE MULARD**

Institut Pasteur, Unité de Chimie des Biomolécules,  
Paris Cedex 15, France  
*laurence.mulard@pasteur.fr*

### **THOMAS NEU**

Helmholtz Centre for Environmental Research, River  
Ecology, Magdeburg, Germany  
*thomas.neu@ufz.de*

### **JEMIINA NEUVONEN**

Turku University, Medical Microbiology and  
Immunology, Turku, Finland  
*jemiina.neuvonen@utu.fi*

### **NIKOLAY NIFANTIEV**

N.D. Zelinsky Institute of Organic Chemistry, Russian  
Academy of Sciences, Laboratory of Glycoconjugate  
Chemistry, Moscow, Russia  
*nen@ioc.ac.ru*

### **STEFAN OSCARSON**

University College Dublin, Centre for Synthesis and  
Chemical Biology, Dublin 4, Ireland  
*stefan.oscarson@ucd.ie*

### **OLGA OVCHINNIKOVA**

N.D. Zelinsky Institute of Organic Chemistry, Russian  
Academy of Sciences, Laboratory of Carbohydrate  
Chemistry, Moscow, Russia  
*olga.ovchinnikova@gmail.com*

### **NURIA PARERA PERA**

Utrecht University, Department of Medicinal  
Chemistry & Chemical Biology, Utrecht, The  
Netherlands  
*N.PareraPera@uu.nl*

### **GERALD PIER**

Harvard Medical School, Channing  
Laboratory/Brigham and Women's Hospital, Boston,  
MA, USA  
*gpier@rics.bwh.harvard.edu*

### **ELISE PINTA**

University of Turku, Medical Biochemistry and  
Genetics, Turku, Finland  
*elise.pinta@utu.fi*

### **NICOLA POHL**

Iowa State University, Chemistry and the  
Interdepartmental Graduate Program in Microbiology,  
Ames, IA, USA  
*npohl@iastate.edu*

### **VEIJO PORKKA**

Mediq Suomi, Laboratory dept., Espoo, Finland  
*veijo.porkka@oriola-kd.fi*

### **NATALIAY POTEKHINA**

M. V. Lomonosov Moscow State University, School  
of Biology, Moscow, Russian Federation  
*potekhina56@mail.ru*

### **KAMILA RABSZTYN**

University of Silesia, Department of Microbiology,  
Katowice, Poland  
*kamila.rabsztyn@us.edu.pl*

### **ANGELA ROMANOW**

Medizinische Hochschule Hannover, Zelluläre  
Chemie, Hannover, Germany  
*Romanow.Angela@mh-hannover.de*

### **MIRKO ROSSI**

University of Helsinki, Faculty of Veterinary  
Medicine, Dep. of Food Hygiene and Environmental  
Health, Helsinki, Finland  
*mirko.rossi@helsinki.fi*



## **Participant list – 4th BMMC**

### **JUSTYNA SAMASZKO**

University of Gdańsk, Chair of Sugar Chemistry,  
Gdańsk, Poland  
*samaszk@chem.univ.gda.pl*

### **ELKE SCHWEDA**

Södertörn University & Karolinska Institutet, Clinical  
Research centre, Huddinge, Sweden  
*elke.schweda@ki.se*

### **ANDREW SCOTT**

Defence Science and Technology Laboratory,  
Biomedical Sciences, Salisbury, United Kingdom  
*aescott2@dstl.gov.uk*

### **SOFYA SENCHENKOVA**

N.D. Zelinsky Institute of Organic Chemistry, Russian  
Academy of Sciences, Laboratory of Carbohydrate  
Chemistry, Moscow, Russia  
*sen@ioc.ac.ru*

### **KRISTIINA SIROLA**

University of Helsinki, Finland, Department of Food  
and Environmental Sciences, Helsinki, Finland  
*kristiina.sirola@helsinki.fi*

### **MIKAEL SKURNIK**

University of Helsinki, Haartman Institute, Helsinki,  
Finland  
*mikael.skurnik@helsinki.fi*

### **MONA SVENSSON**

Stockholm University, Organic chemistry, Stockholm,  
Sweden  
*mona@organ.su.se*

### **MONIKA SZULC**

Faculty of Chemistry, University of Gdańsk, Chair of  
Sugar Chemistry, Gdańsk, Poland  
*monikas@chem.univ.gda.pl*

### **PHILIP TOUKACH**

N.D. Zelinsky Institute of Organic Chemistry,  
Carbohydrate Chemistry, Moscow, Russia  
*\_never@inbox.ru*

### **ELENA TULSKAYA**

M.V. Lomonosov Moscow State University, School of  
Biology, Moscow, Russian Federation  
*em\_tulskaya@mail.ru*

### **ANNA TURSKA-SZEWCZUK**

Maria Curie-Sklodowska University, Department of  
Genetics and Microbiology, Lublin, Poland  
*aturska@hektor.umcs.lublin.pl*

### **BRIGITTE TWELKMEYER**

Karolinska Institutet and University College of South  
of Stockholm, Clinical Research Centre, Huddinge,  
Sweden  
*Brigitte.Twelkmeyer@ki.se*

### **REBECCA ULC**

University College Dublin, School of Chemistry and  
Chemical Biology, Dublin, Ireland  
*Rebecca.Ulc@ucd.ie*

### **TERESA URBANIK-SYPNIEWSKA**

Maria Curie-Sklodowska University, Genetics and  
Microbiology, Lublin, Poland  
*turbanik@hektor.umcs.lublin.pl*

### **OLGA VALUEVA**

N.D. Zelinsky Institute of Organic Chemistry,  
Laboratory of Carbohydrate Chemistry, Moscow,  
Russia  
*olga.valueva@gmail.com*

### **MIGUEL VALVANO**

University of Western Ontario, Microbiology &  
Immunology, London, Ontario, Canada  
*mvalvano@uwo.ca*

### **CHRIS WHITFIELD**

University of Guelph, Molecular and Cellular Biology,  
Guelph, Canada  
*cwhitfie@uoguelph.ca*

### **GÖRAN WIDMALM**

Stockholm University, Department of Organic  
Chemistry, Stockholm, Sweden  
*gw@organ.su.se*

### **VARVARA VITIAZEVA**

Karolinska Institutet, Laboratory Medicine,  
Stockholm, Sweden  
*varvara.vitiazeva@ki.se*

## ***Participant list – 4th BMCC***

### **CHRISTIAN VOGEL**

Institute of Chemistry /University of Rostock, Organic  
Chemistry, Rostock, GERMANY  
*christian.vogel@uni-rostock.de*

### **EVELINA ZDOROVENKO**

N.D. Zelinsky Institute of Organic Chemistry, Lab 21,  
Moscow, Russia  
*zdorovenkoe@mail.ru* ,

### **ELZBIETA ZAJAC** (*Accompanying person*)

Jan Kochanowski University in Kielce, Institute of  
Mathematics, Kielce, Poland  
*ezajac@ujk.edu.pl*

# 4th Baltic Meeting on Microbial Carbohydrates

*Hyytiälä Forest Station*

*September 19-22, 2010*

*Home page: <http://www.hi.helsinki.fi/4bmmc/>*

## FINAL PROGRAMME

### *Sunday, September 19*

Time	Name	Affiliation	Topic or title
14.00 – 18.00	Registration. Entrance to Building A		
18.00 – 19.00	Dinner. Building A dining room		
Session 1	<b>OPENING SESSION</b> Chairperson <b>Mikael Skurnik</b>		
19.00 – 19.10	<b>Mikael Skurnik</b>	University of Helsinki, Finland	Opening
19.10 – 20.00	<b>Göran Widmalm</b>	Arrhenius Laboratory, Stockholm University	Structure and dynamics of microbial polysaccharides using NMR spectroscopy
	Get together. Old Dining Room		

# Monday, September 20

Time	Name	Affiliation	Topic or title
07.00 – 08.00	Breakfast. Building A dining room		
<b>Session 2</b>			
<b>BIOLOGICAL ROLE OF MICROBIAL CARBOHYDRATES</b> Chairperson <b>Otto Holst</b>			
08.20 – 09.10	<b>Antonio Molinaro</b>	Università di Napoli Federico II, Napoli, Italy	Microbial cell surface glycoconjugates and elicitation of plant innate immunity
09.10 – 09.30	<b>Jemiina Neuvonen</b>	University of Turku, Finland	Decorin binding of <i>Borrelia burgdorferi</i> s.s., <i>Borrelia garinii</i> and <i>Borrelia afzelii</i>
09.30 – 10.00	<b>Marta Biedzka-Sarek</b>	Natl Inst of Health and Welfare, Finland	ApoA-I C-terminal domain exerts bactericidal activity
10.00 – 10.15	<b>Antti Uotila</b>	University of Helsinki, Finland	Introduction of Hyytiälä Forestry Filed Station
10.15 – 10.35	Coffee break outside lecture hall		
<b>Session 3</b>			
<b>SYNTHESIS OF MICROBIAL CARBOHYDRATES 1</b> Chairperson <b>Stefan Oscarsson</b>			
10.40 – 11.30	<b>Nicola J. Pohl</b>	Iowa State University, USA	Successes and challenges in the automated synthesis of microbial carbohydrates
11.30 – 11.50	<b>Katalin Daragics</b>	University College Dublin, Ireland	Synthesis of <i>Neisseria meningitidis</i> lipopolysaccharide structures based on orthogonal protecting group strategy
11.50 – 12.10	<b>Rebecca Ulc</b>	University College Dublin	Towards the development of glycoconjugate vaccines: Synthesis of capsular polysaccharide structures of <i>Cryptococcus neoformans</i>
12.10 – 13.00	Lunch. Building A dining room		
<b>Session 4</b>			
<b>CHARACTERIZATION OF MICROBIAL CARBOHYDRATES</b> Chairperson <b>Timo Korhonen</b>			
13.00 – 13.50	<b>Thomas Neu</b>	Helmholtz Centre for Environmental Research – UFZ, Magdeburg, Germany	Extracellular polymeric substances in biofilm systems – <i>In situ</i> imaging and functionality
13.50 – 14.10	<b>Varvara Vitiazeva</b>	Karolinska Institute, Sweden	Structural studies of O-antigen from <i>Haemophilus parainfluenzae</i> strain Hpi20
14.10 – 14.30	<b>Katarzyna Anna Duda</b>	Research Center Borstel, Germany	<i>Escherichia coli</i> 1303 mastitis isolate strain carries a novel O-antigen and the rare K-12 core type in its lipopolysaccharide

Session 5 **BIOSYNTHESIS AND GENETICS OF MICROBIAL CARBOHYDRATES**  
Chairperson **Andrzej Gamian**

14.30 – 14.50 **Olga Ovchinnikova** N.D.Zelinsky Institute, RAS, Russia Genetic studies of *Providencia* O-antigens

14.50 – 15.20 Coffee break. Building A dining room

15.20 – 16.10 **Rita Gerardy-Schahn** Hannover Medical School, Germany Capsule biosynthesis in *Neisseria meningitis*  
16.10 – 16.30 **Salim Islam** University of Guelph, Canada Characterization of the proposed charged-pore structure of the O-antigen flippase Wzx from *Pseudomonas aeruginosa* PAO1

**POSTER SESSION 1**  
Chairpersons

16.30 – 18.00 Posters 1-21

18.00 – 19.00 Dinner. Building A dining room  
19.30 – Sauna etc Networking in Old Dining Room

# Tuesday, September 21

Time	Name	Affiliation	Topic or title
07.00 – 08.00	Breakfast. Building A dining room		
<b>Session 6</b>			
<b>SYNTHESIS OF MICROBIAL CARBOHYDRATES 2</b>			
Chairperson <b>Nikolay Nifantiev</b>			
08.20 – 09.10	<b>Núria Parera Pera</b>	Utrecht University, Netherlands	Synthesis of carbohydrates for bacterial adhesion inhibition and detection
09.10 – 09.35	<b>Bozhena Komarova</b>	N.D.Zelinsky Institute, RAS, Russia	First Synthesis of the pentasaccharides of the outer core region of <i>Pseudomonas aeruginosa</i> lipopolysaccharide
09.35 – 09.50	<b>Nikolay Kondakov</b>	N.D.Zelinsky Institute, RAS, Russia	4-(2-Chloroethoxy)phenyl glycosides – novel intermediates suitable both for block synthesis of oligosaccharides and preparation of neoglycoconjugates
09.50 – 10.10	<b>Nataliya Potekhina</b>	Moscow State University, Russia	Anionic phosphorus-containing cell wall polymers of bacilli
10.10 – 10.30	Coffee break outside lecture hall		
<b>Session 7</b>			
<b>MICROBIAL CARBOHYDRATES IN VACCINES</b>			
Chairperson <b>Yuriy Knirel</b>			
10.30 – 11.20	<b>Paolo Costantino</b>	Novartis Vaccines & Diagnostics Research Center, Siena, Italy	Chemistry and characterization of glycoconjugate vaccines
11.20 – 12.10	<b>Laurence Mulard</b>	Institut Pasteur, Paris, France	From LPS to synthetic oligosaccharide-based vaccines against endemic shigellosis: a multidisciplinary approach
12.10 – 13.00	Lunch. Building A dining room		
13.00 – 13.20	<b>Michael Kowarik</b>	GlycoVaxyn AG, Switzerland	A novel bioconjugate vaccine to prevent <i>Staphylococcus aureus</i> infection
<b>Session 8</b>			
<b>CHARACTERIZATION OF MICROBIAL CARBOHYDRATES</b>			
Chairperson <b>Elke Schweda</b>			
13.20 – 14.10	<b>Joseph Lam</b>	University of Guelph, Canada	Glycosyltransferases associated with outer-core oligosaccharide biosynthesis in <i>Pseudomonas aeruginosa</i>
14.10 – 14.40	<b>Buko Lindner</b>	Research Center Borstel, Germany	High resolution FT-ICR MS/MS and MS <sup>3</sup> of phosphoinositides (PIP <sub>x</sub> )
14.40 – 15.00	<b>Detlef Jensen</b>	Dionex Management AG, Olten, Switzerland	Structure characterization of carbohydrates by anion exchange chromatography – application of pulsed amperometric detection and mass spectrometry

15.00 – 15.30 Coffee break. Building A dining room

15.30 – 16.20 **Miguel Valvano** University of Western Ontario, Canada Biogenesis of O antigen by the Wzy-mediated pathway

**STUDENT GRANT DELIVERY CEREMONY**

16.20 – 16.30 **Mikael Skurnik** University of Helsinki, Finland

**POSTER SESSION 2**  
Chairpersons

16.30 – 18.00 Posters 22-42

19.00 – 21.00 Conference Buffet Dinner. Old Dining Room

21.00 – 23.00 Dr. Helander blues band. Old Dining Room

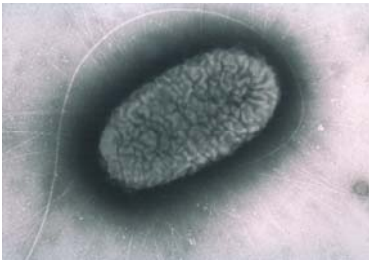
# Wednesday, September 22

Time	Name	Affiliation	Topic or title
07.00 – 08.00	Breakfast. Building A dining room		
Session 9	<b>POLYSACCHARIDE BIOSYNTHESIS MACHINERY</b> Chairperson <b>Jukka Finne</b>		
08.10 – 09.00	<b>Mario Feldman</b>	University of Alberta, Canada	Evolutionary connections between LPS and protein glycosylation biosynthetic pathways in bacteria
09.00 – 09.50	<b>Renato Morona</b>	University of Adelaide, Australia	Making bacterial cell surfaces smooth: structure and function of polysaccharide co-polymerases
09.50 – 10.10	Coffee break outside lecture hall		
10.10 – 10.30	<b>Elise Pinta</b>	University of Turku, Finland	Identification of <i>Yersinia enterocolitica</i> serotype O:3 lipopolysaccharide ligases
Session 10	<b>CLOSING SESSION</b> Chairperson <b>Mikael Skurnik</b>		
10.30 – 11.20	<b>Gerald Pier</b>	Harvard Medical School, Boston, MA, USA	Synthetic oligoglucosamine conjugate vaccine targeting multiple bacterial pathogens
11.20 – 12.10	Closing, lunch at building A dining room and departure		

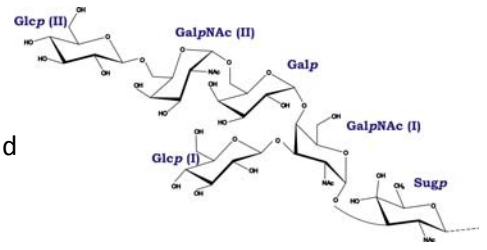


No	Last Name	First Name	Title of poster
<b>SYNTHESIS 1</b>			
1	Gening	Marina	Labeled synthetic beta(1-3)-glucans and their binding to dendritic cells
2	Krylov	Vadim	Synthesis of large fucoidan fragments - potential inhibitors of microbial adhesion
3	Bonaccorsi	Filippo	<i>Neisseria meningitidis</i> serotype A: towards the optimisation of the synthesis of C-oligosaccharide analogues
4	Horan	Heather	Synthesis of glycoconjugate vaccine candidates against <i>Moraxella catarrhalis</i>
5	Black	Alan	Synthesis of structures corresponding to the capsular polysaccharide of <i>Neisseria meningitidis</i> group A
6	Dmochowska	Barbara	Synthesis and biological activities of (2-ammoniummethyl) beta-D-glycopyranoside bromide
<b>BIOLOGY 1</b>			
7	Holst	Otto	Separation of mycobacterial phosphoinositol mannosides
8	Edgar	Chris	Chemical synthesis of Leishmania phosphoglycan structures conjugated to a biotin moiety
9	Kaca	Wieslaw	Serotyping clinical isolates of <i>Proteus mirabilis</i> strains
10	Feodorova	Valentina	Critical role of the LpxM of <i>Yersinia pestis</i> in immunogenesis and pathogenesis of plague
11	Ielmini	Maria Veronica	<i>Desulfovibrio desulfuricans</i> PglB: the most "eukaryotic" of the bacterial N-oligosaccharyltransferases
12	Rabsztyn	Kamila	Characterization of the anti-ECA antibodies in rabbit antiserum against <i>Yersinia enterocolitica</i> O:3 Rc mutant bacteria
<b>STRUCTURAL ANALYSIS 1</b>			
13	Kononov	Leonid	What useful information can a carbohydrate chemist gain from using optical rotation?
14	Komaniecka	Iwona	Studies on the core region from <i>Bradyrhizobium elkanii</i> lipopolysaccharide
15	Fontana	Carolina	Structural studies of <i>Vibrio parahaemolyticus</i> AN-1600 lipopolysaccharide by NMR spectroscopy.
16	Svensson	Mona	Structural elucidation of the capsular polysaccharide repeating unit from <i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i> PIA2
17	Zdorovenko	Evelina	Structure of the O-polysaccharides obtained from new species of Enterobacteriaceae: <i>Pragia fontium</i> and <i>Budvicia aquatica</i>
18	Kaczynski	Zbigniew	The structure of the O-specific polysaccharide from the lipopolysaccharide of <i>Pectobacterium atrosepticum</i> SCRI1039
19	Twelkmeyer	Brigitte	A new LC-MS method for the analysis of O-deacylated lipopolysaccharides
20	Børud	Bente	Glycan diversity in O-linked protein glycosylation of <i>Neisseria</i> species
21	Urbanik-Sypniewska	Teresa	Characteristics of Lipopolysaccharides of <i>pssB-pssA</i> and <i>rosR</i> Mutants of <i>Rhizobium leguminosarum</i> bv. <i>trifolii</i>
22	Beczala	Agnieszka	<i>Yersinia pseudotuberculosis</i> O:9 lipopolysaccharide reactivity with phage $\phi$ R1-37 and anti-YeO3-c-R1 antiserum

No	Last Name	First Name	Title of poster
<b>SYNTHESIS 2</b>			
23	Nifantiev	Nikolay	Binding of fluorescein labeled synthetic $^{2}(1\rightarrow 3)$ -glucans to human dendritic cells
24	Szulc	Monika	Synthesis of new analogs of vancomycin with potential bactericidal properties
25	Samaszko	Justyna	Chemical synthesis of vancomycin derivatives modified with sugar and peptide fragments
26	Liberek	Beata	In search of new inhibitors of GlcN-6-P synthase. Synthesis of benzyl and thiophenyl 2-deoxy-6-O-diethylphosphonato-2-hydroxyimino-D-hexopyranosides
27	Vogel	Christian	Efficient synthesis of $\beta$ -allyl C-glycosides of D-ribofuranose and 2-deoxy-D-ribofuranose and their use for the preparation of potential antimicrobial agents
<b>BIOLOGY 2</b>			
28	Rossi	Mirko	Identification and characterization of a <i>Helicobacter s</i> ialyltransferase from GT-42
29	Dziadziuszko	Halina	Immunological studies of <i>Salmonella</i> Telaviv somatic antigen epitopes
30	Scott	Andrew	Flagellar glycosylation in the biothreat agent <i>Burkholderia pseudomallei</i>
31	Kasperkiewicz	Katarzyna	Mannan-binding lectin (MBL) interaction with lipopolysaccharides (LPS) from rough mutants of <i>Yersinia enterocolitica</i> O:3 serotype of various chemotypes
32	Arabski	Michal	The correlation of anti-LPS <i>Proteus mirabilis</i> antibodies level with tlr4 (Thr399Ile) gene polymorphism in rheumatoid arthritis
33	Kallio	Elisa	The effect of VLDL-associated LPS on macrophages in periodontitis patients
<b>STRUCTURAL ANALYSIS 2</b>			
34	Fedonenko	Yulia	Structural and cross-serological analyses of the O-, C- and H-antigens of <i>Azospirillum brasilense</i> type strain Sp7
35	Valueva	Olga	Structural investigation of the O-polysaccharide of <i>Azorhizobium caulinodans</i> HAMBI 216 consisting of rhamnose, 2-O-methylrhamnose and 3-O-methylrhamnose
36	Toukach	Philip	Bacterial carbohydrate structure database 3: novel version
37	Gamian	Andrzej	Diversity of exopolysaccharide structure isolated from <i>Lactobacillus species</i> (colitis "+") present in murine intestinal microflora
38	Bychowska	Anna	Chemical structure of the polysaccharidic O-antigen of <i>Cronobacter turicensis</i>
39	Czerwicka	Malgorzata	Structure of the O-polysaccharide isolated from <i>Cronobacter sakazakii</i> 767
40	Tul'skaya	Elena	Cell wall teichuronic and teichulosonic acids of gram-positive bacteria
41	Knirel	Yuriy A.	Structures of the O-polysaccharides of <i>Photorhabdus asymbiotica</i> subsp. <i>asymbiotica</i> and subsp. <i>australis</i> resembling that of <i>Francisella tularensis</i>
42	Senchenkova	Sofya	The complete <i>Salmonella</i> O-antigen structure elucidation
43	Choma	Adam	Structural characterization of the lipid A from <i>Bradyrhizobium yuanmingense</i>
44	Turska-Szewczuk	Anna	Structural characterization of the O-specific polysaccharide from the lipopolysaccharide of the fish pathogen <i>Aeromonas bestiarum</i> strain P1S
45	Jachymek	Wojciech	Immunochemical studies of the <i>Hafnia alvei</i> 2670 lipopolysaccharide

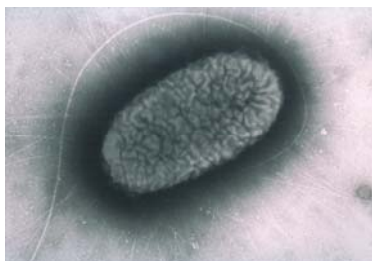


**4<sup>th</sup> Baltic meeting on  
Microbial Carbohydrates**  
Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



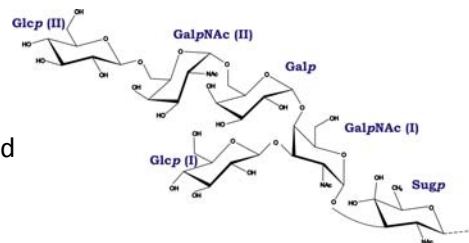
# ABSTRACTS FOR ORAL PRESENTATIONS

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## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT

#### ApoA-I C-terminal domain exerts bactericidal activity

MARTA BIEDZKA-SAREK<sup>1</sup>, JARI METSO<sup>1</sup>, SAKARI JOKIRANTA<sup>2</sup>, JOANNA RADZIEJEWSKA-LEBRECHT<sup>3</sup>,  
VASSILIS ZANNIS<sup>4</sup>, MIKAEL SKURNIK<sup>2</sup>, AND MATTI JAUHIAINEN<sup>1</sup>

<sup>1</sup> National Institute for Health and Welfare, and FIMM, Institute for Molecular Medicine, Biomedicum, Helsinki, Finland

<sup>2</sup> Haartman Institute, Helsinki, Finland

<sup>3</sup> University of Silesia, Katowice, Poland

<sup>4</sup> Boston University School of Medicine, Boston, USA

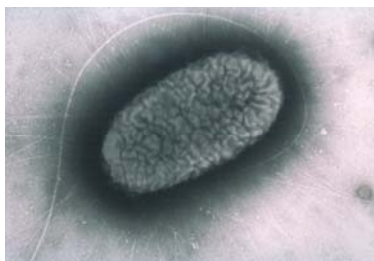
The purpose of this study was to investigate the role of lipoproteins, especially high-density lipoproteins (HDL), in serum killing of a model Gram-negative bacterium *Yersinia enterocolitica*. The bacterium resists bactericidal action of human serum and causes food-borne disease with a broad range of intestinal and extraintestinal manifestations.

Blocking of a major structural component of HDL, apolipoprotein A-I (apoA-I), in human serum increased survival of *Y. enterocolitica* strains expressing lipopolysaccharide (LPS) O-antigen. Addition of purified LPS to human serum increased survival of O-antigen-expressing bacteria. This effect was due to the blocking of apoA-I by LPS since addition of exogenous apoA-I, HDL2, and HDL3 to serum nullified the LPS-mediated effect and the bacterial survival dropped to a control level. In contrast, addition of LDL or VLDL to the LPS-treated serum did not improve the serum killing potential.

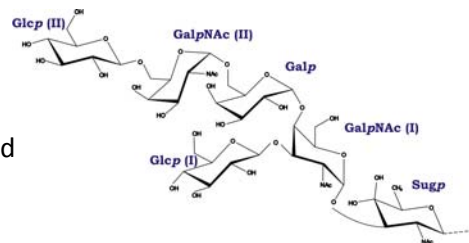
To map bioactive apoA-I domain we used apoA-I monoclonal antibodies with well defined epitopes. Domain mapping analyses revealed that the C-terminus of apoA-I provides the bactericidal potential. This finding was confirmed using apoA-I mutants carrying C-terminal deletions or point substitutions.

To investigate whether apoA-I action involves the complement system, the three known complement activation pathways were blocked in LPS-treated serum using a monoclonal antibody against C5. Addition of apoA-I to LPS-treated serum restored serum killing potential only in the absence of the complement-blocking antibody. This suggests that the active complement is an absolute requirement for the bactericidal action of apoA-I in human serum.

Our in vitro results demonstrate that high-density lipoproteins (HDL) are involved in the complement-mediated killing of *Y. enterocolitica*. ApoA-I C-terminal domain is necessary for the apoA-I mediated bactericidal activity of human serum. The data further suggest that LPS O-antigen is the apoA-I target on the bacterial surface.



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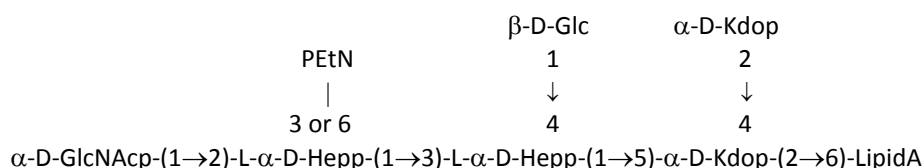
## ABSTRACT

### Synthesis of *Neisseria meningitidis* Lipopolysaccharide Structures Based On Orthogonal Protecting Group Strategy

K. DARAGICS, J. D. M. OLSSON, J.-L. BOUISSIERE, H. HORAN, S. OSCARSON

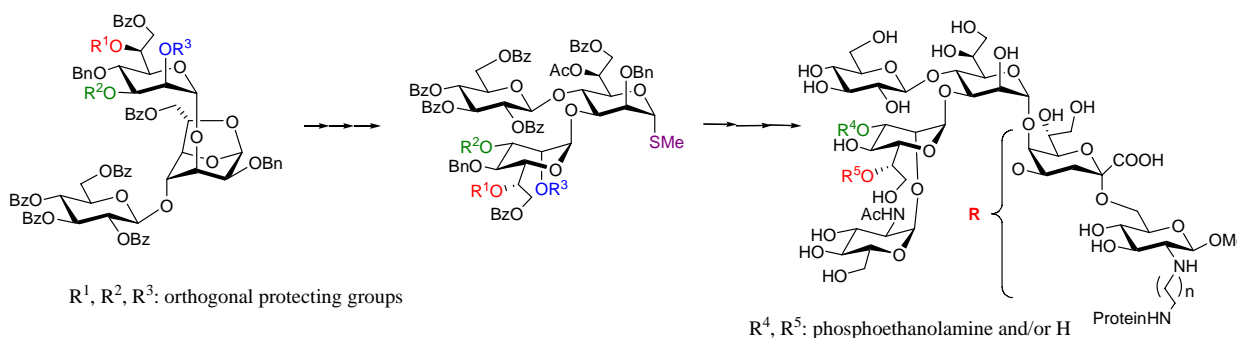
*Centre for Synthesis and Chemical Biology, UCD, Belfield, Dublin 4, Ireland; e-mail: katalin.daragics@ucd.ie*

*Neisseria meningitidis* is the major cause of bacterial meningitis, and mainly five serogroups (A, B, C, W and Y-135) are associated with meningococcal meningitis. All the different serogroups of *N. meningitidis* contain the same LPS-structures, which lacks the polysaccharide O-antigen, thus, contain only the core and the Lipid A part. The structure of the conserved inner core part has been identified (Fig. 1) [1]. In our ongoing programme we aim to synthesize a glycoconjugate vaccine against *N. meningitidis* based on LPS structures.



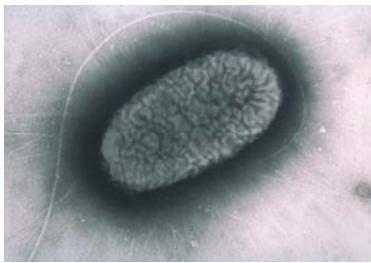
**Figure 1.** Structure of the conserved inner core of *N. meningitidis* LPS.

We now report on the preparation of the heptose-containing trisaccharide thioglycoside donor containing orthogonal protecting groups at positions O-2, O-3 and O-6 of the second heptose residue. We present efforts towards glycosylation of the acceptor containing di-Kdo and lipidA parts using the orthogonally protected thioglycoside donor.



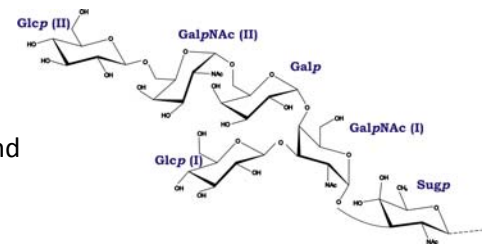
**Figure 2.** Structures of the orthogonally protected trisaccharide precursor and donor, and the target compounds

[1] Cox, A. D.; Wright, J. C.; Gidney, M. A. J.; Lacelle, S.; Plested, J. S.; Martin, A.; Moxon, E.; Richards, J. C. *Eur. J. Chem.* **2003**, 270, 1759-1766.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT

#### ***Escherichia coli* 1303 mastitis isolate strain carries a novel O-antigen and the rare K-12 core type in its lipopolysaccharide**

KATARZYNA ANNA DUDA<sup>1</sup>, BUKO LINDNER<sup>2</sup>, HELMUT BRADE<sup>3</sup>, ANDREAS LEIMBACH<sup>4,5</sup>, ELZBIETA BRZUSZKIEWICZ<sup>5</sup>, ULRICH DOBRINDT<sup>4</sup>, OTTO HOLST<sup>1</sup>

##### *Divisions of*

<sup>1</sup>Structural Biochemistry,

<sup>2</sup>Immunochemistry

<sup>3</sup>Medical and Biochemical Microbiology, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, D-23845 Borstel, Germany

<sup>4</sup>Institute for Molecular Infection Biology, Julius-Maximilians-University of Würzburg, D-97070 Würzburg, Germany

<sup>5</sup>Göttingen Genomics Laboratory, Institute of Microbiology and Genetics, Georg-August-University of Göttingen, D-37077 Göttingen, Germany...

*Escherichia coli* is an important etiologic agent of mastitis. Mastitis, namely infection and inflammation of the udder, has a tremendous economic importance for the dairy industry. A specific set of virulence-associated genes could not be identified for *E. coli* mastitis isolates. Thus, pathogen-associated molecular patterns (PAMPs), e.g. lipopolysaccharides (LPS), are thought to play a significant role during the cause of infection. More than 180 different O-antigens (OPS), defining different serogroups, have been described for *E. coli*, however, none has so far been characterized of LPS from a strain causing mastitis. There are five core region types in *E. coli*, namely R1, R2, R3, R4 and K-12, the latter of which is rarely detected in environmental isolates. The chemical structures of all five core types have been published. In this study, *E. coli* strain 1303 (O5:H<sup>-</sup>) isolated from udder secretions of cows with clinical mastitis was investigated. The LPS were isolated utilizing hot phenol/water extraction and subjected to immunochemical, mass spectrometrical as well as NMR spectroscopical analyses.

The O-repeating unit was characterized as  $\rightarrow 3$ - $\alpha$ -L-FucpOAc(1 $\rightarrow$ 4)- $\beta$ -D-Galp(1 $\rightarrow$ 3)- $\alpha$ -D-GalpNAc(1 $\rightarrow$ 4)- $\beta$ -D-Quip3NAc(1 $\rightarrow$

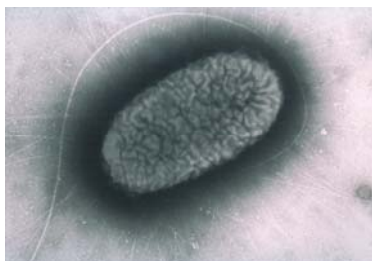
To date, there are two existing subtypes of serotype O:5, designated as O5ab and O5ac. The OPS of strain 1303 differs from these subtypes by the presence of FucpOAc instead of Ribf.

The nucleotide sequence of the O-antigen gene cluster of *E. coli* strain 1303 has been determined. In large parts, this cluster located between the *gnd* and *galF* genes comprised a presently unknown DNA sequence with 13 putative open reading frames.

Interestingly, Western blot performed with monoclonal antibodies against different *E. coli* core types together with MS and NMR analyzes revealed the presence of the K-12 core type substituted by the OPS at position 7 of the terminal L,D-heptose. The K-12 core oligosaccharide genes could be detected in 12.3% of the mastitis isolates, whereas this core type appeared only in 4.2% of the fecal isolates from healthy cows.

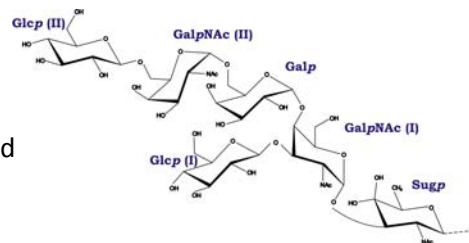
Thus, *E. coli* 1303 represents a new subtype of serotype O:5 and contains in its LPS the rare K-12 core type which expression may correlate with the ability of an *E. coli* strain to cause bovine mastitis.





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT

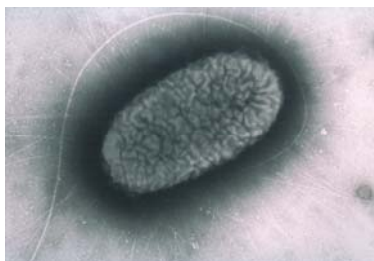
#### Characterization of the proposed charged-pore structure of the O-antigen flippase Wzx from *Pseudomonas aeruginosa* PAO1.

SALIM TIMO ISLAM<sup>1</sup>, VÉRONIQUE LOUISE TAYLOR<sup>1</sup>, ROBERT CURTIS FORD<sup>2</sup>, JOSEPH SUI-LUNG LAM<sup>1</sup>

<sup>1</sup> Dept. of Molecular and Cellular Biology, University of Guelph, Canada

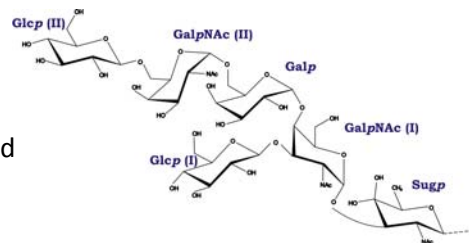
<sup>2</sup> Faculty of Life Sciences, University of Manchester, United Kingdom

The Wzy-dependent pathway model for the biosynthesis and assembly of cell-surface polysaccharides is applicable to a wide range of Gram-negative bacteria, yet it remains poorly understood. Research in our laboratory focuses on the cell surface of *Pseudomonas aeruginosa*, an important opportunistic Gram-negative bacterial pathogen that can cause fatal infections in compromised patients. Two forms of lipopolysaccharide (LPS) are produced by the bacterium, termed A band and B band, the latter being the immunodominant cell-surface antigen synthesized via the Wzy-dependent pathway; this stepwise pathway results in LPS capped by a negatively-charged heteropolymeric O antigen (O-Ag). Essential to this pathway is the O-Ag flippase Wzx, an integral inner membrane (IM) protein that is believed to mediate translocation of trisaccharide repeat units bound to undecaprenyl pyrophosphate from the inner leaflet to the outer leaflet of the IM. While Wzx proteins are found in a wide range of bacteria, structural data to explain their purported function was non-existent until a recent investigation by our group in which the topology of Wzx from *P. aeruginosa* PAO1 was mapped. This study revealed the presence of 12 transmembrane (TM) helices containing a range of charged amino acids within the membrane-embedded portion of the protein. Analysis of potential helix-helix and helix-membrane contact points of the TM domains suggested the presence of a charged channel running down the length of the protein, providing a plausible explanation for the mechanism of the flippase function. To gain a better understanding of the structure and function of Wzx, we have initiated biophysical studies. Optimal overexpression and detergent solubilization conditions have been developed by expressing Wzx with a cleavable C-terminal His-tagged green fluorescent protein (GFP-His<sub>8</sub>) fusion, allowing for measurement of *in vivo* and *in vitro* GFP fluorescence. This fusion construct also facilitated the determination of optimal detergent conditions for solubilization once purified. Negative staining followed by transmission electron microscopy (TEM) of monodisperse populations of the protein is currently being carried out using the EMAN software suite to gain preliminary tertiary structural insights. Furthermore, Wzx-GFP-His<sub>8</sub> fusions have been reconstituted in membrane vesicles and verified by TEM as well as in-gel fluorescence scanning of SDS-PAGE gels loaded with reconstituted vesicles. By performing protease protection assays of vesicle-reconstituted Wzx-GFP-His<sub>8</sub>, we found that trypsin digestion of the vesicles did not yield mass ions that would correspond to either Wzx or GFP when the enzymatic digests were analyzed by MALDI-TOF MS. The lack of detection of trypsin-cleaved GFP products suggested a preferred orientation of the construct within the vesicle, with the C-terminus of Wzx (and the associated GFP tag) localized within the interior of the vesicle, and the periplasmic face of Wzx exposed on the outside of the vesicle. The observation that no Wzx-specific products were detected was consistent with the topology map generated for the protein, in which no periplasmic loop regions containing the requisite trypsin-cleavage sites were identified. To gain a better understanding of the structure and oligomeric state of Wzx, work is underway to generate 2D crystals and to examine these by cryo-electron microscopy. Taken together, the abovementioned analyses will advance our understanding of the structure and function of the flippase Wzx.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT

#### Structure characterization of carbohydrates by anion exchange chromatography – application of pulsed amperometric detection and mass spectrometry

DETLEF JENSEN<sup>1</sup>, CEES BRUGGINK<sup>2</sup>

<sup>1</sup> *Dionex (Europe) Management AG, Olten, Switzerland*

<sup>2</sup> *Dionex Benelux BV, Amsterdam, Netherlands*

Carbohydrates in their variety and complex chemical composition play an important role in chemical processes, in pharmaceutical industry, Food and Beverage and many others. The mentioned market segments share a common necessity. This is to analyze the carbohydrates for both quantitative and structural information in research and quality control.

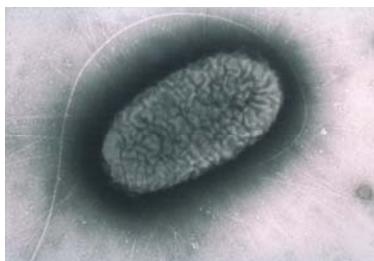
Modern liquid chromatographic techniques allow for the direct analysis of native and derivatized carbohydrates. High Performance Anion Exchange Chromatography (HPAEC) followed by Pulsed Amperometric Detection (PAD) is a well established technology to determine the monosaccharide composition as well as to profile the oligo- and polysaccharides. The chromatographic characteristics of homologue polysaccharides are often predictable, and PAD serves as a rugged and reliable detection technique, so that the polysaccharides can be identified by retention time. For a mixture of heterogeneous oligosaccharides, however, their retention behaviour can be quite unpredictable. The lack of additional molecular size information complicates the interpretation of chromatograms and makes their identification more difficult.

Therefore combining HPAEC-PAD with mass spectrometry (MS) is very helpful to gather information about size and the monosaccharide type-composition of the oligo- and polysaccharides. Collision induced fragmentation allows drawing conclusions on the order of the monosugar units and often reveals information on their linkage. These experimental data very often relates with literature, simplifying the identification of carbohydrates in complex matrices.

More detailed and specific information can be obtained cleaving monosaccharides off, using enzymatic digestions applying exo-cleaving enzymes. Chromatographic analyses executed after every digestion step serves not only as a control for the enzymatic efficiency, but it also allows identification of the oligosaccharide from which the exo-monosaccharide was cleaved off.

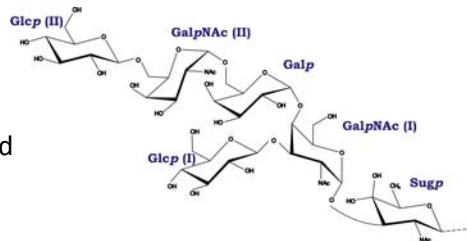
This presentation will focus on chromatographic separations, suitable stationary phases for the mentioned task. Experimental data and results from the HPAEC-PAD/MS hyphenation will be discussed.





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT

#### First Synthesis of Pentasaccharide Glycoforms I and II of the Outer Core Region of the *Pseudomonas aeruginosa* Lipopolysaccharide

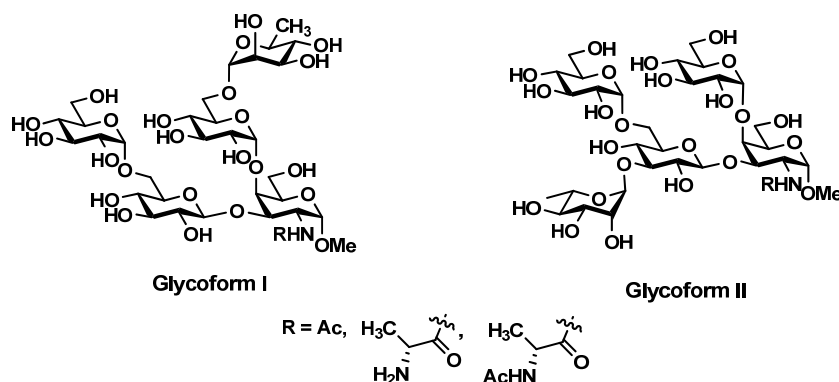
B. S. KOMAROVA<sup>1</sup>, YU. E. TSVETKOV<sup>1</sup>, G. B. PIER<sup>2</sup>, N. E. NIFANTIEV<sup>1</sup>

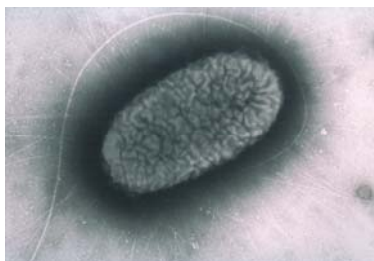
<sup>1</sup> Laboratory of Glycoconjugate Chemistry, N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospect 47, 119991 Moscow, Russia

<sup>2</sup> Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115

Cystic Fibrosis (CF) is a congenital disease, which affects one out of 2500-3000 Caucasians. The main cause of morbidity and mortality among CF patients is chronic lung infection with *Pseudomonas aeruginosa*. CF patients have a mutant version of a protein called the Cystic Fibrosis Transmembrane Conductance Regulator, a wild type version of which normally is responsible for an appropriate immune response leading to bacterial clearance. The outer core region of *P. aeruginosa* lipopolysaccharide (LPS) is a bacterial ligand for wild type CFTR. The LPS is found on the cell wall of the bacteria in two isomeric glycoforms, differing in the position of the attachment of a rhamnose residue.

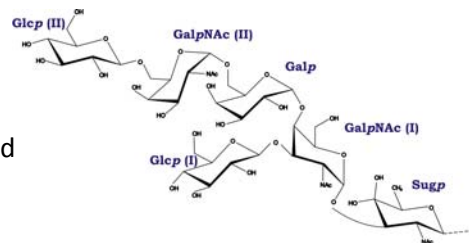
In this communication we report on the synthesis of a series of pentasaccharide methyl glycosides corresponding to the glycoforms I and II of the outer core region of the *P. aeruginosa* LPS. They were prepared as glycoforms bearing acetyl, alanyl and N-acetylalanyl substituents. This permits the use of obtained compounds as probes for investigating the mechanism of CFTR-LPS outer core interaction and to evaluate the role of the amino group on the alanine substituent of the LPS-core galactose residue in this interaction.





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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September 19-22, 2010



### ABSTRACT

#### 4-(2-Chloroethoxy)phenyl glycosides – novel intermediates suitable both for block synthesis of oligosaccharides and preparation of neoglycoconjugates

NIKOLAY N. KONDAKOV, ALEXANDER I. ZININ, ANNA M. SHPIRT, POLINA I. ABRONINA,  
VLADIMIR I. TORGOV, ALEXANDER O. CHIZHOV, LEONID O. KONONOV

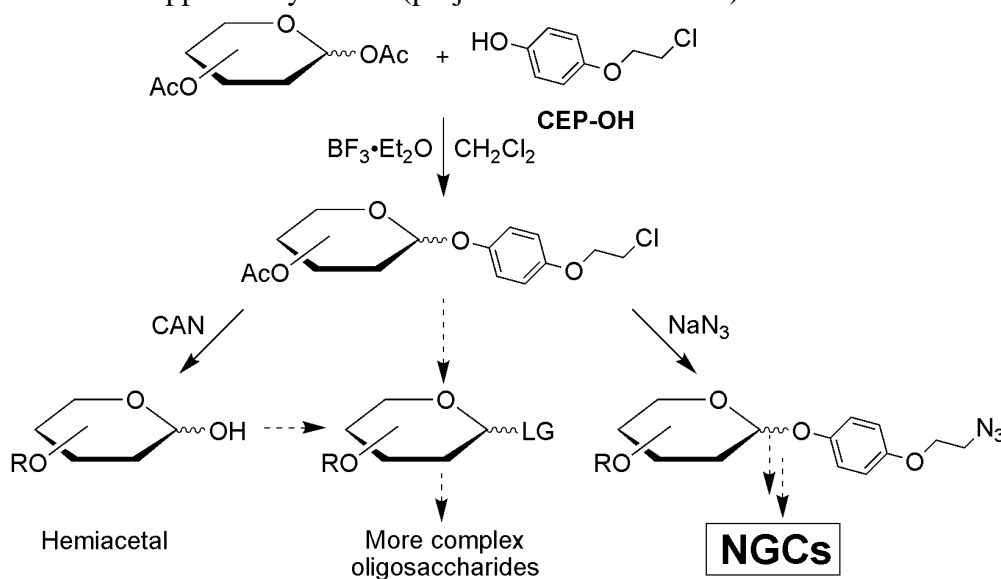
*N.D. Zelinsky Institute of Organic Chemistry of RAS, Leninsky prospect, 47, 119991, Moscow, Russian Federation  
kononov@ioc.ac.ru*

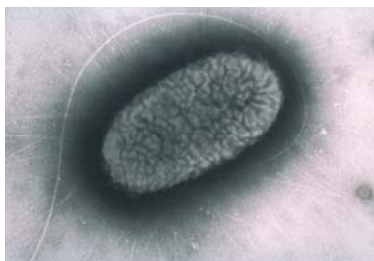
A choice of anomeric protecting groups is often of decisive importance for the successful outcome of an oligosaccharide synthesis. Furthermore, conversion of the assembled saccharide into a functional derivative, such as a spacers glycoside, is a necessary prerequisite for the synthesis of neoglycoconjugates (NGCs). Introduction of aglycon with a functional group at early steps of oligosaccharide synthesis gives opportunity to build NGCs easily, but complicates further use of these functionalized glycosides in block synthesis of more complex oligosaccharides. Use of temporary protective groups at the anomeric center, such as 4-methoxyphenyl group (MP), gives opportunity for the block synthesis of oligosaccharides, but their conversion to NGCs becomes more complicated.

In this work we suggest 4-(2-chloroethoxy)phenyl (CEP) as new “universal” protective group for the anomeric center. It consists of two parts: the first part is similar to MP group and provide us with methods of introduction and cleavage of this group, the second part is 2-chloroethoxy group, in which chlorine can easily be substituted with azido group to give 4-(2-azidoethoxy)phenyl (AEP) glycosides. The latter can further be used for preparation of NGCs either directly or via amine.

We have synthesized CEP glycosides of glucose, galactose, mannose, rhamnose, fucose, arabinofuranose, glucosamine and lactose from the corresponding anomeric acetates. We showed possibility of cleavage of CEP group with cerium-ammonium nitrate (CAN) and its conversion to AEP group.

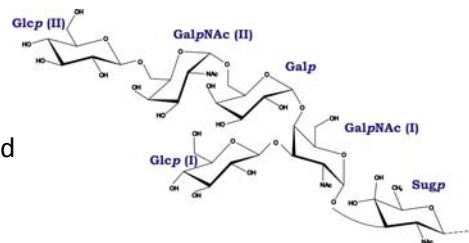
This work was supported by RFBR (project No. 10-03-01019).





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT

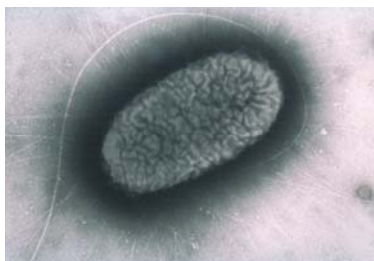
#### A Novel Bioconjugate Vaccine to Prevent *Staphylococcus aureus* Infection

MICHAEL KOWARIK<sup>1</sup>, JEAN C. LEE<sup>2</sup>, KATHRYN A. KELLEY<sup>2</sup>, KELLY SHIELDS<sup>2</sup>, VERONICA GAMBILLARA<sup>1</sup>, GERD LIPOWSKY<sup>1</sup> AND MICHAEL WACKER<sup>1</sup>.

<sup>1</sup> GlycoVaxyn AG, Schlieren, Switzerland.

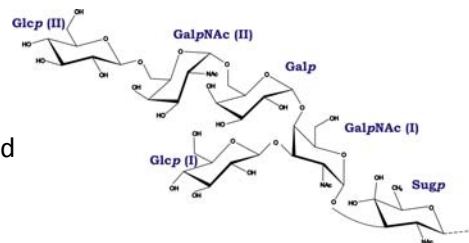
<sup>2</sup> Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.

Conjugate vaccines have helped extinction of several different infectious diseases. We have developed a proprietary technology that enables the manufacture of bioconjugate vaccines based on complex polysaccharide structures. The *Campylobacter jejuni* enzyme PglB is able to transfer an oligosaccharide to a protein consensus sequence, thereby allowing the production of glycoproteins in bacterial cells. This protein glycosylation system has been functionally transferred into *Escherichia coli*, and it allows the conjugation of an antigenic polysaccharide to a designer protein of choice through an N-glycosidic linkage. Conjugate vaccines have been produced in *E. coli* by co-expression of PglB, a protein carrier, and an antigenic polysaccharide cluster. In this work we present the production and preclinical evaluation of a divalent bioconjugate vaccine to prevent *Staphylococcus aureus* infection. Recombinant production of the carbohydrate in *E. coli* was achieved by complementing the truncated *Pseudomonas aeruginosa* PA103 Serovar O11 O-antigen cluster with genes from *S. aureus* capsular polysaccharide biosynthesis cluster from Serotype CP5 and CP8. The proteins encoded by the chimeric DNA cluster are responsible for the production a recombinant polysaccharide of *S. aureus* CP5 and CPP8, respectively. The polysaccharide were conjugated to the protein carrier protein EPA (nontoxic *P. aeruginosa* exotoxin A) by PglB. Preclinical evaluation of the bioconjugate showed efficacy in opsonophagocytosis, passive and active immunization studies. This reports shows for the first time that a immunogenic and protective bioconjugate containing a polysaccharide of a Gram-positive bacterium is synthesized in *E. coli*. The bivalent *S. aureus* bioconjugate will be evaluated in clinical trials.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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September 19-22, 2010



### ABSTRACT

#### Mass spectrometric structural analysis of phosphatidylinositol phosphates (PIP<sub>x</sub>) by high resolution IRMPD FT-MS/MS and MS<sup>3</sup>

BUKO LINDNER<sup>1</sup>, TOM SCIOR<sup>2</sup>, NICOLE ZEHETHOFER<sup>1</sup>

<sup>1</sup> Divisions of Immunochemistry, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, D-23845 Borstel, Germany

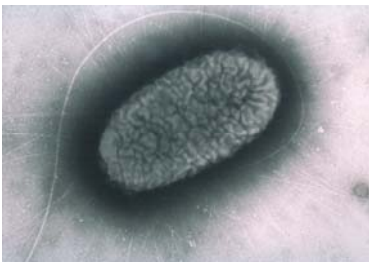
<sup>2</sup> Departamento de Farmacia, Facultad De Ciencias Químicas, Benemerita Universidad Autonoma de Puebla, 72570 Colonia San Manuel, Puebla, PUE., Mexico

Phosphoinositides (PIP<sub>x</sub>) are derived through various metabolic routes from diacylphosphatidylinositol (PI) by one-, two- or threefold phosphorylation of the inositol ring at position three, four or five, leading to the formation of three monophosphorylated, three biposphorylated and one triphosphorylated phosphoinositide species. Although PIPs account for only 0.5 – 1% of total phospholipids in mammalian cells, they are highly bioactive and play important roles in the regulation of membrane trafficking and intracellular signaling. Furthermore, PIP metabolism is a key early event in phagocytosis of pathogenic bacteria (e.g. *Legionella pneumophila*). The specific function of PIPs has been shown to depend on the rate and the site of phosphorylation. The existence of region isomers and the low amounts of PIPs available from biological isolates makes their structural identification challenging.

In order to develop in the long term a sensitive LC-MS/MS method to monitor PIPs we investigated their fragmentation behaviour using a high resolution hybrid Apex-Qe FT-MS system (Bruker Daltonics, Bremen, Germany) equipped with a 7 Tesla actively shielded superconducting magnet and an Apollo Dual ESI/ MALDI ion source. In the negative ion mode MS/MS and MS<sup>3</sup> spectra were generated by infrared multiphoton dissociation (IRMPD) or by collision induced dissociation (CID). The high resolution of the mass spectrometer allowed the unequivocal determination of the number of phosphates as well as the fatty acids residues of the phosphoinositides. However, no product ions corresponding to inner ring cleavages of the inositol ring were observed, which are required for straight forward determination of the linkage site of the phosphate groups.

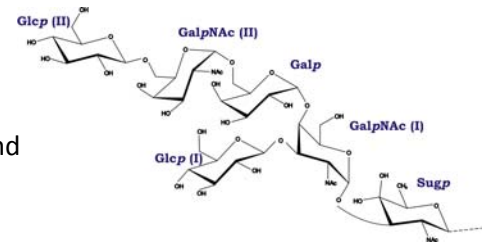
The fragmentation behaviour of the most polycationic diacylphosphatidylinositol-tri-phosphates was intensively studied, since unexpectedly an abundant loss of inositol-bi-phosphate was observed. By IRMPD MS<sup>3</sup> experiments it could be shown that this neutral loss occurred after the loss of water from the precursor ion indicating phosphate migration along the inositol ring to the glycerol backbone. So far, phosphate migration had been demonstrated for LPS containing Kdo-P. Further fragmentation of PIP<sub>3</sub>P-(inositol-bi-phosphate) generated an abundant product ion at  $m/z$  214.9515 which is in excellent agreement with an elemental composition consisting of C<sub>3</sub>H<sub>5</sub>O<sub>7</sub>P<sub>2</sub> (calculated mass 214.9516 u). Using different theoretical approaches possible structures were modeled and revealed one stable consensus structure which is consistent with the proposed cyclic phosphate structure linked to the glycerol backbone.

The financial support by the DFG (SFB TR22 Z01 and DFG LI-448/4) is gratefully acknowledged.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT

#### Decorin binding of *Borrelia burgdorferi* s.s., *Borrelia garinii* and *Borrelia afzelii*

JEMIINA NEUVONEN<sup>1,3</sup>, VUOKKO LOIMARANTA<sup>2</sup>, MATTI K. VILJANEN<sup>1</sup> AND JUKKA HYTÖNEN<sup>1</sup>

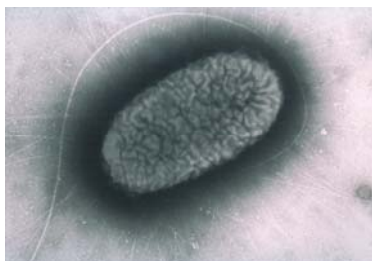
<sup>1</sup>Department of Medical Microbiology and Immunology, University of Turku, Finland,

<sup>2</sup>Department of Medical Biochemistry and Genetics, University of Turku, Finland,

<sup>3</sup>Turku Graduate School for Biomedical Sciences, Finland

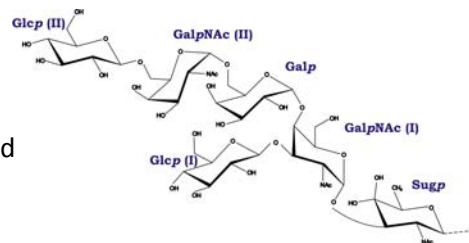
Lyme borreliosis is a tick born infectious disease which is caused by *Borrelia burgdorferi* sensu lato bacteria. There are three major borrelia genospecies, *B. burgdorferi* sensu stricto (Bbss), *B. garinii* (Bg) and *B. afzelii* (Ba), which are known to cause disease in humans. Decorin binding proteins A and B (DbpA and B) are two borrelial adhesins that are expressed during mammalian infection. Decorin is widely expressed throughout the body, and highest concentrations are detected in the skin and joints. Decorin binding proteins of the three borrelia genospecies differ in their amino acid sequence, but little attention has been paid to the potential difference in their biological activity. We have expressed recombinant Dbps and constructed recombinant borrelia strains to study this matter. DbpA and B of Bbss, Bg and Ba were expressed in *E.coli*, and the binding of decorin to recombinant Dbps was studied by Western blot and Surface Plasmon Resonance assay. Three recombinant borrelia strains were constructed through cloning of *dbpAB* operons from the three genospecies to the non-infective Bbss B313, which lacks several major surface proteins including DbpA and B. The binding of biotinylated decorin to the recombinant borrelia strains was studied by Western blot and Dot blot assays. In cell adhesion studies, the binding of fluorescent recombinant borrelia strains to fibroblasts was studied. DbpA from Bg and DbpB from Bbss and Bg bound to decorin as individual recombinant proteins, while DbpA from Bbss and Ba and DbpB from Ba showed only little or no adherence to decorin. Parallel results were obtained when using recombinant borrelia strains. The strains expressing Dbps of Bbss and Bg bound to decorin and decorin expressing cells, while the strain with Dbps of Ba showed only background level of adherence. Thus, the Dbps from different borrelia genospecies have different decorin binding properties. The DbpB but not DbpA from Bbss mediated decorin binding, while both DbpA and DbpB from Bg bound strongly to decorin in all tested conditions but the Dbps from Ba showed no decorin binding in our in vitro assays.





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
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### ABSTRACT

#### Genetic studies of *Providencia* O-antigens

OLGA G. OVCHINNIKOVA,<sup>1</sup> NINA A. KOCHAROVA,<sup>1</sup> BIN LIU,<sup>2</sup> LU FENG,<sup>2</sup> ANTONI ROZALSKI<sup>3</sup>,  
LEI WANG,<sup>2</sup> YURIY A. KNIREL<sup>1</sup>

<sup>1</sup> N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia

<sup>2</sup> TEDA School of Biological Sciences and Biotechnology, Nankai University, Tianjin, People's Republic of China

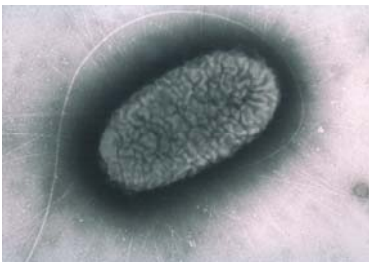
<sup>3</sup> Institute of Microbiology and Immunology, University of Lodz, Lodz, Poland

Bacteria *Providencia* are opportunistic human pathogens associated with urinary tract and wound infections and enteric diseases. Based on the lipopolysaccharide O-antigens (O-polysaccharides), three *Providencia* species, viz. *P. alcalifaciens*, *P. rustigianii* and *P. stuartii*, are classified into 63 O-serogroups. The O-antigen structures of these species have been extensively studied by the authors and a number of rarely occurring monosaccharides, including 6-deoxyhexoses, 6-deoxyhexosamines and higher aldulosonic acids, as well as unusual non-sugar residues have been identified as their components. However, no data on the location and organization of the O-antigen gene clusters have been reported for *Providencia*.

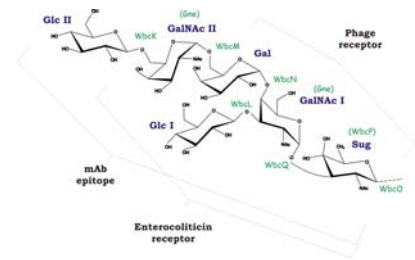
In this study, we found that in *P. alcalifaciens* O19, O36, O40 and *P. stuartii* O47 genes for the O-antigen synthesis are clustered between the *cpxA* and *yibK* housekeeping genes on the chromosome. The O-antigen gene clusters of *P. alcalifaciens* O36 and O40 were sequenced and putative genes for the synthesis of nucleotide precursors of the constituent monosaccharides as well as genes encoding glycosyl transferases, O-unit flippase and O-antigen polymerase were identified based on their similarities with ORFs in Genbank and taking account of the O-antigen structures. In addition, homologues of *wza*, *wzb* and *wzc* genes required for the surface expression of capsular polysaccharide were found upstream *yibK* in all four bacteria studied. Their location within the O-antigen gene cluster is reported for the first time and suggests that these genes may be involved in the transport and regulation of the O-antigens in *Providencia*.

The data obtained open a way for elucidation of biosynthetic pathways of unusual sugar components and mechanisms of diversification of the *Providencia* O-antigens.

This work was supported by the Russian Foundation for Basic Research (Project 08-04-92221-NNSF).



4<sup>th</sup> Baltic meeting on  
Microbial Carbohydrates  
Hyttiälä Forest Field Station, Finland  
September 19-22, 2010



## ABSTRACT

### Identification of *Yersinia enterocolitica* serotype O:3 lipopolysaccharide ligases

ELISE PINTA<sup>1,2</sup>, ZHILIN LI<sup>1</sup>, JULIA BATZILLA<sup>3</sup>, MARIA PAJUNEN<sup>4</sup>, ALEXANDER RAKIN<sup>3</sup> AND MIKAEL SKURNIK<sup>1,5</sup>

<sup>1</sup> Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Finland

<sup>2</sup> Department of Medical Biochemistry and Genetics, University of Turku, Finland

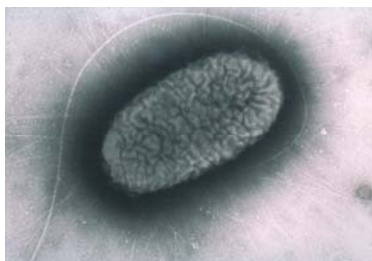
<sup>3</sup> Max von Pettenkofer-Institut, Muenchen, Germany

<sup>4</sup> Department of Genetics, Univ of Turku, Finland

<sup>5</sup> Helsinki Univ Central Hospital Laboratory Diagnostics, Helsinki, Finland

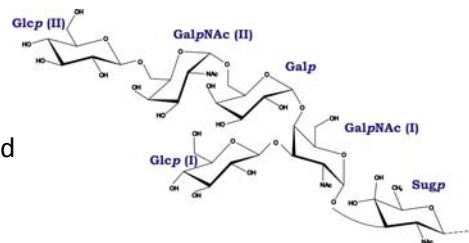
*Yersinia enterocolitica* O:3 (YeO3) is a Gram-negative bacterium expressing lipopolysaccharide (LPS) with a hexasaccharide branch known as the outer core (OC). The biosynthesis of the OC hexasaccharide is directed by the chromosomally located OC gene cluster that contains nine genes including genes responsible for biosynthesis of nucleotide sugar precursors (*gne*, *wbcP*), glycosyltransferases (*wbcKLMNOQ*) and flippase (*wzx*). However, the ligase that would transfer and ligate OC from undecaprenyl-phosphate-carrier to lipid A – inner core is not encoded by the OC gene cluster. In addition to OC, YeO3 also expresses O-specific polysaccharide (OPS or O-antigen) linked to lipid A – inner core. The OPS ligase gene is also unknown. In *Salmonella* and *Escherichia coli* the OPS-ligase is encoded by the *waaL* gene.

In this work we set out to identify the YeO3 OC and OPS ligase(s). We identified a putative OC-ligase (Lig1727) encoding gene by screening a transposon insertion library of YeO3 for OC-non-expressing mutants. Database search using the Lig1727 amino acid sequence and membrane topology prediction identified two other ligase candidates encoded in the genome of *Y. enterocolitica* O:8 that were named Lig532 and Lig777 (50 % and <20% identity to Lig1727). Interestingly, *Y. pestis* and *Y. pseudotuberculosis* have only the Lig532 homologue, while one or both of the other ligases is additionally present in other *Yersinia* species. All three ligases showed ligase activity in a complementation assay using an *E. coli waaL* mutant, indicating that the ligases are promiscuous with regard to the transferred oligo- or polysaccharide unit. Using single, double and triple ligase mutants we have data showing that Lig1727 is the major OC-ligase and Lig532 the OPS-ligase in YeO3. The role of Lig777 remains unresolved.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT

#### Anionic phosphorus-containing cell wall polymers of bacilli

N.V. POTEKHINA<sup>1</sup>, A.S. SHASHKOV<sup>2</sup>, S.N. SENCHENKOVA<sup>2</sup>, G.M. STRESHINSKAYA<sup>1</sup>, YU.I. KOZLOVA<sup>1</sup>, E.M. TUL'SKAYA<sup>1</sup>, AND E.B. KUDRYASHOVA<sup>3</sup>

<sup>1</sup>School of Biology, M. V. Lomonosov Moscow State University, Moscow, Russia;

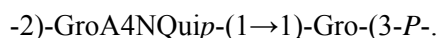
<sup>2</sup>N. D. Zelinsky Institute of Organic Chemistry RAS, Moscow, Russia;

<sup>3</sup>All-Russian Collection of Microorganisms (VKM), G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms RAS, Pushchino, Russia

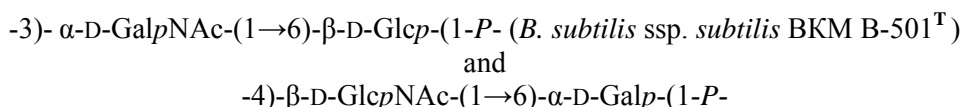
Teichoic acids, the cell wall anionic polymers of Gram-positive bacteria, manifest structural diversity due to variation of the type of the phosphodiester bonds in the polymer core and the nature and the combination of the constituents. To date, teichoic acids of a wide group of bacteria, predominantly of the order *Actinomycetales*, have been investigated. On the other hand, teichoic acids of bacilli cell walls where these polymers were discovered in 1958 are explored to a much lesser extent. Here we present the results of structural studies of teichoic acids and sugar 1-phosphate polymers of 22 VKM strains of the *Bacillus subtilis* group. The cell walls of the strains investigated, contain poly(polyol phosphate) and poly(glycosylpolyol phosphate) (the type I and type II teichoic acids, respectively). Their structures were established by chemical and NMR spectroscopic methods. Structural variations in teichoic acids of the type I were revealed, viz., the presence of 1,3-poly(glycerol phosphate) with  $\alpha$ - and  $\beta$ -D-Glcp,  $\alpha$ - and  $\beta$ -D-GlcpNac substituents and 1,5-poly(ribitol phosphate) with  $\alpha$ - and  $\beta$ -D-Glcp and  $\beta$ -D-GlcpNac substituents. The  $\alpha$ -linked glucose constituent is identified for the first time in ribitol teichoic acids. The core of the type II teichoic acids was represented by one general structure, viz., glycosyl-(1 $\rightarrow$ 1/2)glycerol linked by phosphodiester bonds between the hydroxyl groups at C(3) of glycerol and C(6) of a sugar component ( $\alpha$ - and  $\beta$ -D-Galp,  $\alpha$ -D-Glcp and  $\alpha$ -D-GlcpNac). A new structure with a disubstituted core glycerol residue,



was found in *B. subtilis* VKM B-764. A new structural type of teichoic acids was identified in *B. subtilis* VKM B-762. The phosphodiester bond links the hydroxyl groups at C(3) of glycerol and C(2) of glyceric acid, which N-acylates the 4-aminoquinovose residue of the chain:

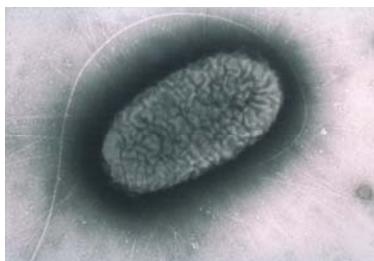


Teichoic acids of all the bacilli studied contain O-linked, partially N-acetylated D-alanine residues. Cell walls of four strains of *B. subtilis* were found to contain, in addition to teichoic acids, disaccharide 1-phosphate polymers of the following structures:



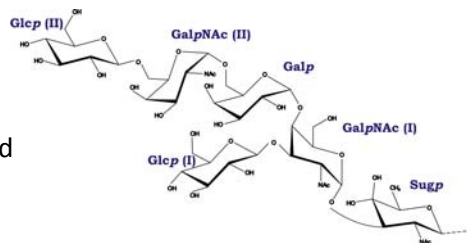
with the GlcpNac residue partially O-acetylated at O(3) and O(6) (*B. subtilis* ssp. *spizizenii* VKM B-720, VKM B-916 and *B. subtilis* VKM B-761). Thus, in our studies of cell wall polymers of 22 VKM strains of bacilli a variety of teichoic acids and sugar 1-phosphate polymers were revealed. The results obtained can be of value in the taxonomy of *B. subtilis* for the differentiation, based on the composition and structure of cell wall polymers, of closely related strains.





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### ABSTRACT

#### Towards the development of glycoconjugate vaccines: Synthesis of capsular polysaccharide structures of *Cryptococcus neoformans*

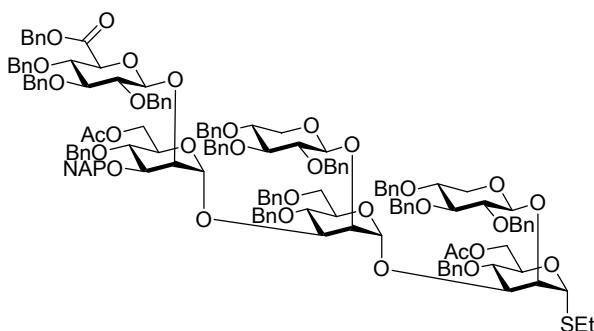
REBECCA ULC<sup>1</sup>, LINA RYDNER<sup>2</sup>, STEFAN OSCARSON<sup>1</sup>

<sup>1</sup> Centre for Synthesis and Chemical Biology, School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Dublin, Ireland

<sup>2</sup> Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

*Cryptococcus neoformans* is an opportunistic fungal pathogen that causes severe diseases primarily in immunocompromised individuals (e.g. HIV positive patients).<sup>[1]</sup> *C. neoformans* is surrounded by a thick layer of capsular polysaccharides (CPS), which is an important virulence factor. In order to investigate the immunobiological properties of the fungal CPS, and thereby aid glycoconjugate vaccine development, we are synthesising part structures of the fungal CPS.

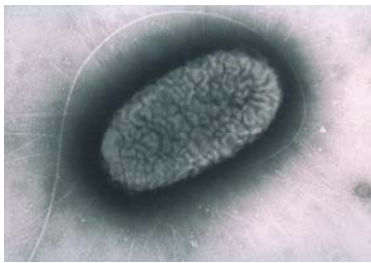
Currently, we are focusing on the synthesis of a thioglycoside hexasaccharide building block containing  $\alpha$ -Manp,  $\beta$ -Xylp,  $\beta$ -GlcA and 6-*O*-acetyl motifs. The hexasaccharide corresponds to the serotype A structure of *C. neoformans*. Our aim is to use the hexasaccharide as a building block in the construction of larger polysaccharide structures (dodecasaccharides) by using a block approach.



The hexasaccharide may be obtained by glycosylation of a  $\beta$ -linked Glcp-Manp or a  $\beta$ -linked GlcpA-Manp disaccharide with a (Xylp-Manp)<sub>2</sub> tetrasaccharide. A reliable methodology to access related thioglycoside building blocks has been developed previously in our group.<sup>[2,3,4]</sup> We present an improved synthetic pathway to the glucuronic acid-containing disaccharide. Synthetic aspects will be discussed, such as the formation of a  $\beta$ -Glc glycosidic linkage, the regioselective introduction of 6-*O*-acetyl groups and the oxidation of the primary position of the glucose moiety to the glucuronic acid motif followed by benzylation to give the benzyl ester protected GlcpA-Manp disaccharide donor.

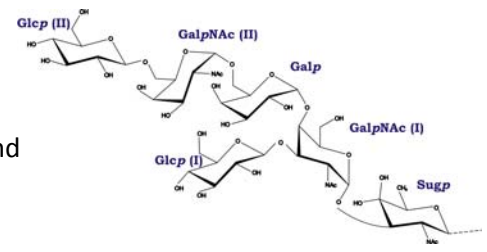
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- [2] M. Alpe, P. Svahnberg, S. Oscarson, *J. Carbohydr. Chem.* **2003**, *22*, 565-577.
- [3] M. Alpe, P. Svahnberg, S. Oscarson, *J. Carbohydr. Chem.* **2004**, *23*, 403-416.
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## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT

#### Structural studies of *O*-antigen from *Haemophilus parainfluenzae* strain *Hpi20*

VARVARA VITIAZEVA<sup>1</sup>, DEREK W. HOOD<sup>2</sup>, ELKE K. H. SCHWEDA<sup>1</sup>

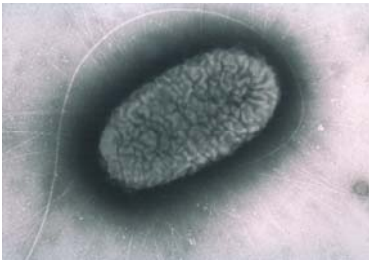
<sup>1</sup> Clinical Research Centre, Karolinska Institutet and University College of South Stockholm, NOVUM, S-141 86 Huddinge, Sweden;

<sup>2</sup> Molecular Infectious Diseases Group, University of Oxford Department of Paediatrics, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, OX3 9DS, United Kingdom

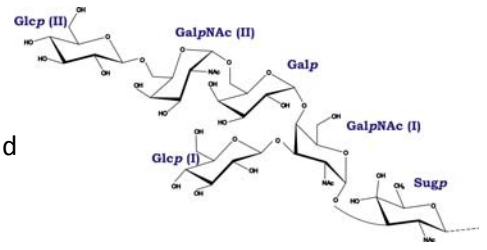
*Haemophilus parainfluenzae* is a gram-negative bacterium that colonizes the upper respiratory tract of humans. Almost all people carry *H. parainfluenzae*. Despite their close relatedness to *H. influenzae* and abundance in the nasopharynx they very rarely cause disease. Lipopolysaccharide (LPS) is essential to the commensal and disease-causing behaviors of *H. influenzae*. While, our genetic and structural studies show that LPS of *H. influenzae* and *H. parainfluenzae* share some identical features, there are also major differences. Interestingly, *H. parainfluenzae* is capable of elaborating LPS consisting of both core and *O*-polysaccharide chains.

SDS-PAGE analysis of LPS from *H. parainfluenzae* strain 20 (*Hpi20*) indicated this strain to express *O*-antigen. Structural details for LPS from *Hpi20* were obtained using NMR spectroscopy on LPS, core oligosaccharide material and lipid A. LPS from *Hpi20* comprises the glucose-substituted triheptosyl inner-core moiety L- $\alpha$ -D-Hepp-(1 $\rightarrow$ 2)-[PEtn $\rightarrow$ 6]-L- $\alpha$ -D-Hepp-(1 $\rightarrow$ 3)-[ $\beta$ -D-Glcp-(1 $\rightarrow$ 4)]-L- $\alpha$ -D-Hepp linked to lipid A via Kdo 4-phosphate as observed for *H. influenzae*.

The *O*-antigen biological repeating unit has the following structure:  $\rightarrow$ 4)- $\alpha$ -D-GalpNac-(1 $\rightarrow$ P $\rightarrow$ 6)- $\beta$ -D-Glcp-(1 $\rightarrow$ 3)- $\alpha$ -D-FucpNac4N-(1 $\rightarrow$ , where D-FucpNac4N is 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose. This residue was found to be  $\beta$ -(1 $\rightarrow$ 4) linked to the Glc residue linked to proximal heptose of the triheptosyl inner core moiety.

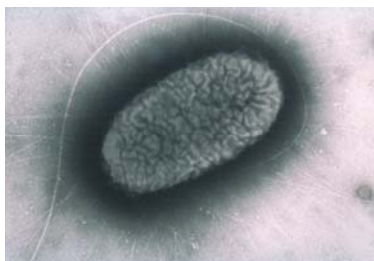


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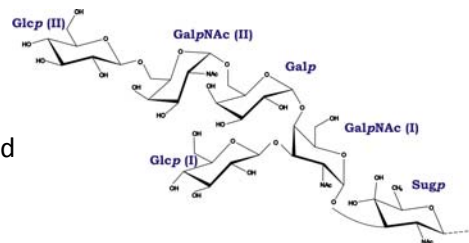


# INVITED SPEAKER ABSTRACTS

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## ABSTRACT

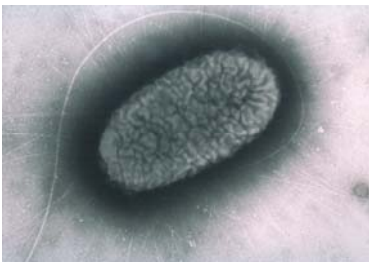
### Chemistry and Characterization of Glycoconjugate Vaccines,

PAOLO COSTANTINO

*Novartis Vaccines and Diagnostics Research Center. Via Fiorentina, 1-53100 Siena (Italy).*

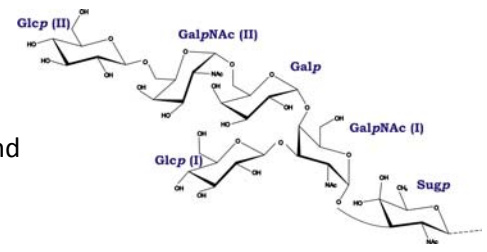
Carbohydrate-protein conjugates are among the most successful vaccines developed during the last 20 years. They have been the solution to the lack of immunogenicity of bacterial polysaccharide vaccines in young children and infants, the age group at highest risk of infections from encapsulated micro-organisms. Covalent conjugation to protein carriers changes polysaccharides from T-cell independent to T-cell dependent antigens resulting in improved immunogenicity, particularly in infants as expressed by higher antibody titers, predominantly of the IgG class, and immunological memory (1). Glycoconjugate vaccines can be prepared using different procedures, however two main approaches have been traditionally employed: one based on random chemical activation of polysaccharide chains followed by conjugation, the other based on oligosaccharides coupled to the protein through their terminal groups (2, 3). In some cases the conjugation chemistry requires prior derivatization of the protein carriers. Synthetic biology now offers new opportunities to obtain well-defined glycoconjugates through the introduction of unnatural amino acids into the carrier protein, which can then selectively react with functionalized carbohydrates (4). Technologies to obtain glycoconjugates from engineered bacteria are also being developed (5). Glycoconjugate vaccines need to be carefully characterized and controlled in order to consistently produce them with optimal immunogenicity. To achieve this aim, a variety of physicochemical methodologies including spectrophotometric methods, HPLC, Mass Spectrometry and NMR, have been applied (6). After the success of the first generation of conjugate vaccines, a number of vaccines against other infectious diseases are in development and the concept has been extended to target tumour cells (7). Progress in carbohydrate chemistry is permitting the development of conjugate vaccines based on synthetic oligosaccharides; this represents a powerful tool for elucidating the structural features required for optimal immunogenicity of glycoconjugate vaccines (8,9,10).

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- 3) M. Bröker, P.M.Dull, R. Rappuoli, P.Costantino. *Vaccine*; 2009, 27, 5574-5580.
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- 10) V. Pozsgay, C. Chu, L. Pannell, J. Wolfe, J.B. Robbins and R. Schneerson. Protein conjugates of synthetic saccharides elicit higher levels of serum IgG lipopolysaccharide antibodies in mice than do those of the O-specific polysaccharide from *Shigella dysenteriae* type 1 *PNAS*; 1999, 96, 5194-5197.



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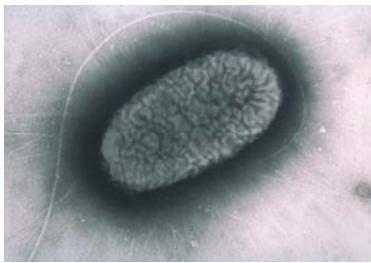
### ABSTRACT

#### Evolutionary connections between LPS and protein glycosylation biosynthetic pathways in bacteria

MARIO FELDMAN

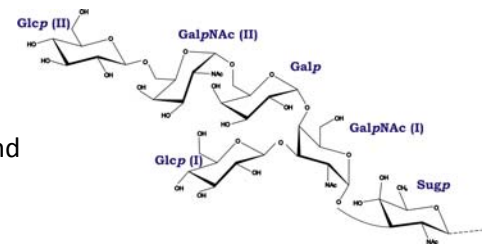
*Department of Biological Sciences, Alberta Ingenuity Centre for Carbohydrate Science, University of Alberta, Edmonton, Alberta T6G 2E9, Canada*

Bacteria generate and attach countless glycan structures to diverse macromolecules. Despite this diversity, the mechanisms of glycoconjugate biosynthesis are often surprisingly similar. My laboratory focuses on the synthesis of LPS and glycoproteins in bacteria. Three steps which are essential for both pathways are completed by membrane proteins. These consist of the initiation of glycan assembly through the attachment of a first sugar residue onto the lipid carrier undecaprenyl pyrophosphate; the translocation across the plasma membrane; and the final transfer onto proteins or lipid A-core. These membrane proteins are not only responsible for analogous biosynthetic steps, but they are also evolutionarily related to enzymes in other pathways. In this presentation, I will discuss our recent advances in the study of the *Helicobacter pylori* Wzk flippase, and *Neisseria meningitidis* PglL O-oligosaccharyltransferase. Because many translocation and conjugation enzymes display relaxed substrate specificity, these bacterial enzymes could be exploited in engineered living bacteria for customized glycoconjugate production, generating potential vaccines and therapeutics.



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### ABSTRACT

#### Capsule biosynthesis in *Neisseria meningitidis*

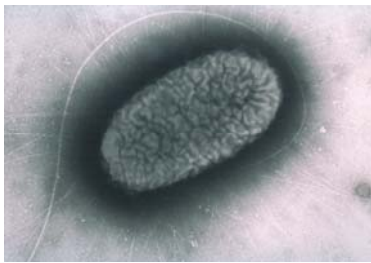
RITA GERARDY-SCHAHN, ANGELA ROMANOW, TIM KEYS, TIMM FIEBIG, KATHARINA STUMMEYER,  
AND FRIEDRICH FREIBERGER

Hannover Medical School, Institute for Cellular Chemistry, Carl-Neuberg-Strasse 1, 30625 Hannover

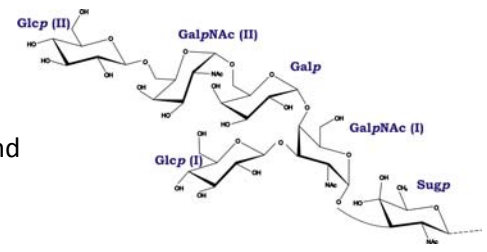
Bacterial meningitis remains a serious threat to global health, accounting for an estimated annual 170,000 deaths worldwide (WHO, <http://www.who.int/nuvi/meningitidis/en/>). *Neisseria meningitidis* (*Nm*) is one of the most important causative agents of bacterial meningitis because of its potential to spread in epidemic waves. Crucial virulence determinants of disease causing *Nm* species are their extracellular polysaccharide capsules that are essential for meningococcal survival in human serum. Based on antigenic variation of these polysaccharides at least twelve different serogroups of *Nm* have been identified (A, B, C, E29, H, I, K, L, W-135, X, Y and Z), of which only six (A, B, C, W-135, Y and X) account for virtually all cases of disease. The capsular polysaccharides (CPS) of all serogroups are negatively charged linear polymers. Serogroup B and C are encapsulated in homopolymeric CPSs composed of sialic acid (Neu5Ac) moieties that are linked by either  $\alpha$ -2 $\rightarrow$ 8 glycosidic linkages in serogroup B or by  $\alpha$ -2 $\rightarrow$ 9 linkages in serogroup C. CPS of serogroups W-135 and Y are heteropolymers, composed of galactose/Neu5Ac repeating units [ $\rightarrow$ 6)- $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -Neu5Ac-(2 $\rightarrow$ )]<sub>n</sub> and glucose/Neu5Ac repeating units [ $\rightarrow$ 6)- $\alpha$ -D-Galp-(1 $\rightarrow$ 4)- $\alpha$ -Neu5Ac-(2 $\rightarrow$ )]<sub>n</sub>, respectively. The CPS of *NmA* and *NmX* do not contain Neu5Ac moieties, but are instead built from N-Acetyl-mannosamine 1-phosphate [ $\rightarrow$ 6)- $\alpha$ -D-ManpNac-(1 $\rightarrow$ OPO<sub>3</sub> $\rightarrow$ )]<sub>n</sub> or N-Acetyl-glucosamine 1-phosphate [ $\rightarrow$ 6)- $\alpha$ -D-GlcpNac-(1 $\rightarrow$ OPO<sub>3</sub> $\rightarrow$ )]<sub>n</sub> repeating units.

The key enzymes in the CPS biosynthesis are the membrane associated capsule polymerases. Candidate genes have been identified for all six disease causing serogroups, but knowledge of enzymology and/or structure-function relations are very limited. To mechanistically explore these enzymes, the genes encoding the capsule polymerases of serogroups A, B, C, W-135, Y and X have been cloned, introduced into expression vectors and expressed as recombinant proteins. Assay systems have been established and are currently used to accompany attempts aimed at defining the minimal active enzymes as well as functional domains determining substrate and linkage specificities of these enzymes. In the 4<sup>th</sup> BMCC meeting we will give a state of the art report on the obtained results.





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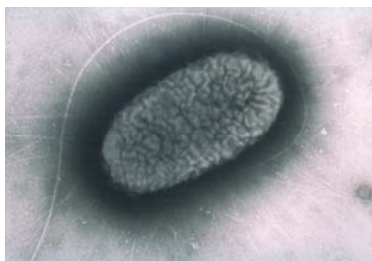
## ABSTRACT

### Glycosyltransferases associated with outer-core oligosaccharide biosynthesis in *Pseudomonas aeruginosa*

JOSEPH S. LAM, DANA KOCÍNCOVÁ, YOUAI HAO, ARMEN CHARCHOGLYAN, AND DYANNE BREWER

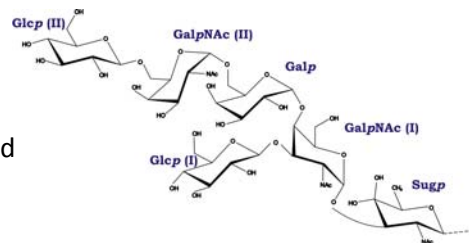
Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, N1G 2W1, Canada

*Pseudomonas aeruginosa* is an important opportunistic human pathogen infecting debilitated individuals including those with cystic fibrosis, cancer and burn wounds. One of the major virulence factors elaborated by this bacterial species is lipopolysaccharide (LPS). *P. aeruginosa* LPS is composed of lipid A, core oligosaccharide (OS) and O antigen. Differences in the O antigen polysaccharide segregate *P. aeruginosa* into 20 serotypes. The core OS is conceptually divided into inner and outer regions. Although the structure of the outer core OS, composed of glucose, rhamnose and galactosamine residues, has been elucidated, the functions and mechanisms of the glycosyltransferases involved in core OS biogenesis is currently unknown. One of these transferases is MigA, previously shown by our group to transfer a rhamnose residue to the outercore OS. Using the sequence of *migA* to perform a BLAST search in the *P. aeruginosa* PAO1 genome, we have identified a novel gene, *PA1014*, which encodes a protein belonging to the GT2 family of glycosyltransferases and shows the highest homology to MigA (57% identity). We hypothesized that *PA1014* is involved in outer core OS biosynthesis of PAO1. To test this, we constructed a gentamycin-cassette insertion mutant of *PA1014* and observed that the mutant has an altered outer core OS structure, as LPS from the mutant did not react with the outer core-specific monoclonal antibody (mAb) 5C-101 in Western immunoblots. The LPS defect in this mutant was restored by complementation with *PA1014* *in trans* showing that *PA1014* is indeed involved in core OS biosynthesis. LPS from the *PA1014* mutant was analyzed by MALDI-TOF mass spectrometry and the results showed a reduction in molecular mass that is consistent with the mass of a single glucose (Glc) residue when compared to LPS from wildtype PAO1 strain. The outer-core-specific mAb 5C-101 was previously shown to recognize Glc IV, the terminal  $\beta$ 1 $\rightarrow$ 2 linked residue of the outer core OS found in PAO1 and other serotype O5 strains. Glc IV is absent in the outer core of serotype O6, which explains why O6 LPS is non-reactive to mAb 5C-101. After transforming an O6 strain with a plasmid carrying *PA1014*, LPS from the transformant became reactive to 5C-101 in Western immunoblots. Interestingly, although the wt O6 core OS lacked the terminal Glc IV, we were able to PCR amplify and clone a *PA1014* homologue from this serotype (designated as *PA1014*<sub>O6</sub>). When *PA1014*<sub>O6</sub> was used to complement the *PA1014*<sub>PAO1</sub> mutant, LPS biosynthesis was restored to the wildtype level. This indicates that the O6 homologue is fully functional; however, *PA1014*<sub>O6</sub> is likely not expressed efficiently or repressed *in vivo* in the O6 strain. Indeed, using RT-PCR, we were only able to detect *PA1014* transcript in PAO1, but not in an O6 strain. The house-keeping gene *rpoD* was used as an internal control when examining gene expression of both strains. To explain the low expression level of *PA1014* in O6, promoter region of the gene in both strains were compared and a deletion in *PA1014*<sub>O6</sub> promoter was found within a region that is similar to a -10 hexamer normally recognized by RpoD ( $\sigma$ 70). We performed 5' RACE (rapid amplification cDNA ends) of the *PA1014*<sub>PAO1</sub> promoter and revealed that the +1 transcription site is located 9 bp downstream of the predicted -10 hexamer. Furthermore, we tested the presence of *PA1014* in all O1-O20 serotype type strains. A majority of the strains not producing the terminal Glc IV residue was found to possess full-length *PA1014* gene, and in each case identical change within the *PA1014* promoter region as that found in the O6 strain was observed. In conclusion, our data showed that firstly, *PA1014* encodes a 1 $\rightarrow$ 2 glucosyltransferase involved in outer core OS biosynthesis of *P. aeruginosa* PAO1. Secondly, *PA1014* is present within different *P. aeruginosa* genomes. Thirdly, absence of the terminal Glc residue in core OS of some serotypes is a consequence of DNA rearrangements within the *PA1014* promoter region.



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### ABSTRACT

#### Cell wall microbial glyco-conjugates as elicitors or suppressors of plant innate immunity

ANTONIO MOLINARO

*Dipartimento di Chimica Organica e Biochimica, Università di Napoli Federico II, via Cintia 4, I-80126, Napoli, Italy*

Innate immunity is the first line of defense against invading microorganisms in vertebrates and the only line of defense in invertebrates and plants and therefore plays a crucial role in the early recognition and subsequent triggering of a pro-inflammatory response to invading pathogens.

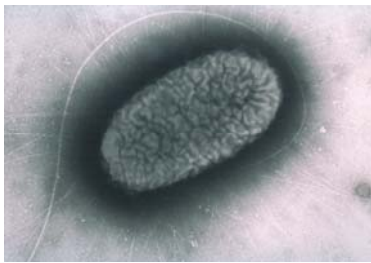
This mechanism relies on recognition of evolutionarily conserved structures on pathogens, termed microbe-associated molecular patterns (MAMPs), through a limited number of germ line-encoded pattern recognition receptors. MAMPs are characterized by being invariant among entire classes of pathogens, essential for the survival of the pathogen, and distinguishable from "self".

The unravelling of the components involved in plant innate immunity is important for understanding the complex interactions between plants and bacterial pathogens, and perhaps, between bacterial pathogens and mammals.

Gram negative lipopolysaccharide and peptidoglycan are two very important cell wall glyco-conjugates and act as MAMPs also in plant/bacteria interactions. Besides their general architectural principle, a number of subtle chemical variations are at the basis of the dynamic host-guest recognition that in case of pathogens is followed by the innate response and in case of symbiosis is followed by its suppression. Therefore, the structural study of such glyco-conjugates involved as virulence factors in animal or plant infections is a pivotal pre-requisite for the comprehension at molecular level of the innate immunity mechanisms.

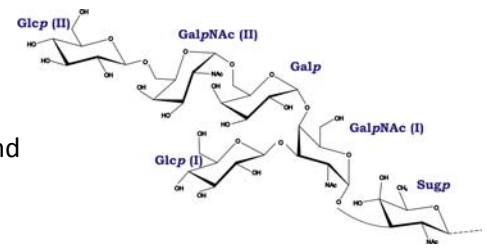
In this communication I will show some examples of isolation, structure determination and elicitation and/or suppression of plant innate immunity by peptidoglycan and lipopolysaccharides from plant pathogen and symbiotic Gram negative bacteria.





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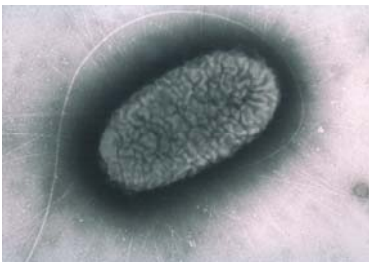
### ABSTRACT

#### **Making bacterial cell surfaces smooth: structure and function of polysaccharide co-polymerases**

RENATO MORONA

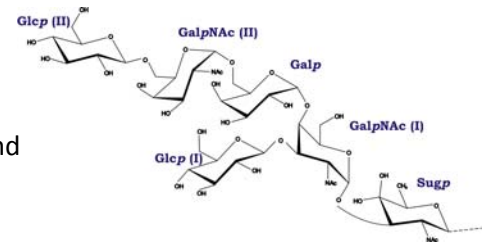
*School of Molecular and Biomedical Science, University of Adelaide, Adelaide, Australia*

The lipopolysaccharides (LPSs) of bacteria such as *Shigella flexneri* and *Salmonella enterica* sv Typhimurium are important virulence factors, and the length of the LPS O antigen (Oag) chains influences cell invasion and complement resistance. The LPS molecules on the cell surface exist as distinct populations: rough LPS (R-LPS) which are not capped by Oag chains, and smooth LPS (S-LPS) which have a bi-modal distribution as two populations with different Oag modal chain lengths are present. During synthesis, the modal chain length is determined by the Wzz proteins which are polysaccharide co-polymerase (PCP) class 1 proteins. PCPs are associated with both Wzy-dependent and Wzy-independent polysaccharide biosynthesis systems, and have various functions including: synthesis, chain length determination, export, and cell wall ligation. Wzz proteins are anchored into the inner membrane by two transmembrane (TM) regions, have a hydrophilic region between the TM regions that is located in the periplasm, and they are also able to form oligomers. By an unknown mechanism, the Wzz influences polymerisation of the Oag repeat units by Wzy, the Oag polysaccharide polymerase, such that the chains have a modal length distribution that is characteristic of the Wzz protein(s) expressed by the bacterial cell. The properties of the Wzz and related PCP proteins will be described. Both structure-function studies, and structure determination have been undertaken. Wzz proteins form oligomeric, bell-shaped structures, however the number of monomers in each oligomer formed by different Wzz proteins is controversial. The results of recent random linker insertion mutagenesis, and also previous site-directed mutagenesis studies, suggest that the residues throughout the Wzz protein influence its function. We have evidence that Wzz oligomerisation and stability is related to its function. We have also investigated the interaction between different Wzz proteins expressed within the same cell and uncover a potential mechanism whereby bi-modality of LPS Oag is achieved. A model for Wzz activity will be also discussed.



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### ABSTRACT

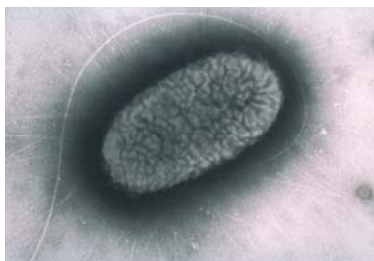
#### Extracellular polymeric substances in biofilm systems – In situ imaging and functionality

THOMAS R. NEU<sup>1</sup> JOHN R. LAWRENCE<sup>2</sup>

<sup>1</sup> *Helmholtz Centre for Environmental Research – UFZ, Magdeburg, Germany*

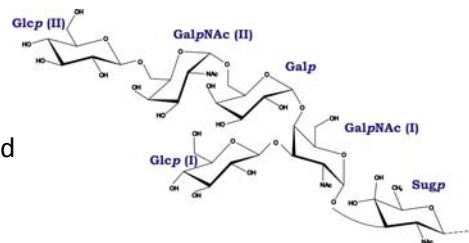
<sup>2</sup> *Environment Canada, Saskatoon, Saskatchewan, Canada*

The microenvironment of interfacial microbial communities is composed of extracellular polymeric substances (EPS) produced by the microbial species present. In fact EPS represents a unifying term for extracellular polymers including: polysaccharides, proteins, nucleic acids, amphiphilic polymers and refractory polymers derived from microorganisms. As a result EPS compounds may be involved in multiple functions and therefore represent an essential constituent of biofilm systems. However, due to the complexity of the polymer mixture, EPS characterisation in environmental biofilms and bioaggregates remains a big challenge. In the first part of this presentation in situ imaging by means of fluorescence techniques in combination with advanced laser scanning microscopy is described as an approach to study the native EPS matrix. In the second part a novel concept of EPS functionality and chemical activity is provided suggesting EPS as a component which is: 1) constructive, 2) adsorptive, 3) active, 4) surface-active, 5) informative, 6) nutritive, 7) locomotive, 8) conductive and 9) redox-active. In conclusion, EPS maybe considered as highly versatile polymers which have key functions within wanted and unwanted microbial films and aggregates.



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### ABSTRACT

#### Synthesis of carbohydrates for bacterial adhesion inhibition and detection

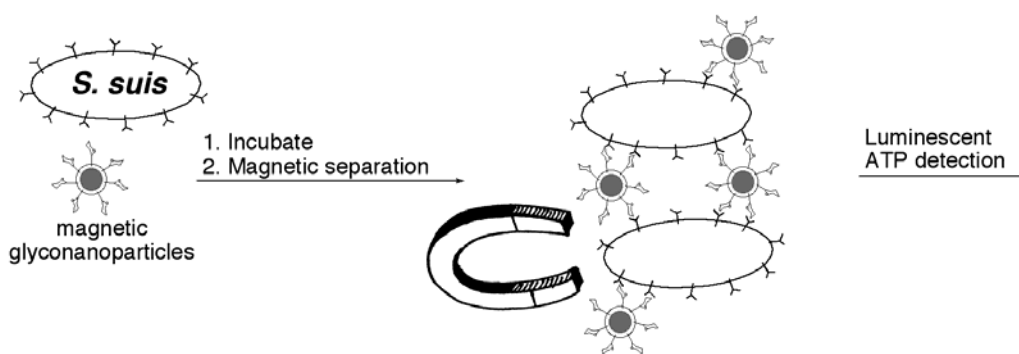
N. PARERA PERA, H. M. BRANDERHORST, R. J. PIETERS

Utrecht University, Utrecht, 3508 TB, The Netherlands.

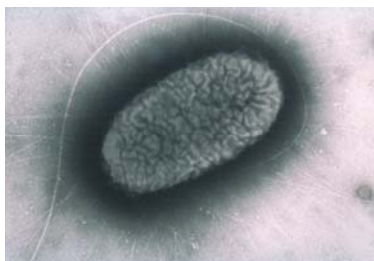
Bacteria carry adhesion proteins on their cell surface that are involved in the recognition of carbohydrate components of cell membranes. Inhibition of bacterial adhesion is a method to prevent the colonization or invasion that usually follows it and thus may prevent the subsequent infection.

Carbohydrate sequences have been synthesized and tested as inhibitors of bacterial adhesion. In our laboratories the *Pseudomonas aeruginosa* target sequence of GalNAc $\beta$ 1,4Gal was prepared and evaluated in monovalent and multivalent form for this purpose.<sup>1</sup> More recently ligands for the *P. aeruginosa* lecA were identified.<sup>2</sup> Gram-positive zoonotic pathogen *Streptococcus suis* binding to Gal $\alpha$ 1,4Gal (Galabiose) was also studied as the target of our adhesion inhibition studies.

Detection and characterization of the bacterial pathogens has been approached as well using magnetic glyconanoparticles as an alternative to generally time-consuming overnight culturing (see below). This method was applied to detect *S. suis* for the first time.<sup>3</sup> To this end galabiose-displaying magnetic particles were synthesized. After incubation of the glyconanoparticles with the pathogen and magnetic concentration, the amount of bound bacteria could be quantified down to 10<sup>4</sup> CFU. Sub-microparticles were used in the range of 250 nm. The size of the glyconanoparticles proved to be crucial, since larger microparticles did not succeed.

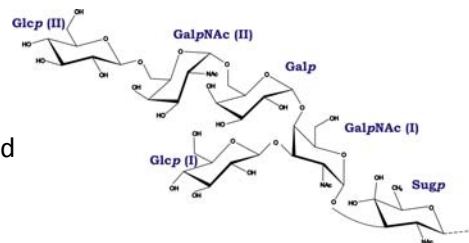


1. Autar R., Liskamp R. M., Pieters R. J. *Carbohydr. Res.*, **2005**, 340, 2436–2442.
2. Parera Pera, N., Branderhorst, H. M., Kooij, R., Maierhofer, C., van der Kaaden, M., Liskamp, R. M. J., Wittmann, V., Ruijtenbeek, R. and Pieters, R. J. **2010**, *in press. ChemBioChem*, n/a. doi: 10.1002/cbic.201000340.
3. Parera Pera, N., Kouki A., Haataja S., Branderhorst, H.M., Liskamp R. M. J., Visser, G.M., Finne, J., Pieters. R. J., *Org. Biomol. Chem.*, **2010**, 8, 2425 – 2429.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
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### ABSTRACT

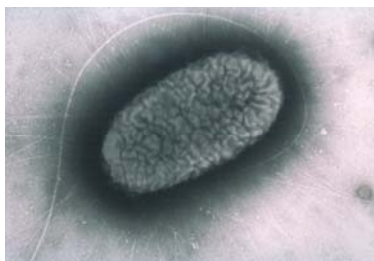
#### Synthetic oligosaccharide conjugate vaccines: a strong future for chemistry, microbiology, infectious diseases and immunology

MARINA L. GENING,<sup>1</sup> DAVID SKURNIK<sup>2</sup>, TOMAS MAIRA-LITRAN,<sup>2</sup> COLETTE CYWES-BENTLEY,<sup>2</sup> CLARRISSA POZZI,<sup>2</sup> YURY E. TSVETKOV,<sup>1</sup> NIKOLAY E. NIFANTIEV,<sup>1</sup> GERALD B. PIER,<sup>2</sup>

<sup>1</sup> N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky prospect 47, 119991 Moscow, Russia

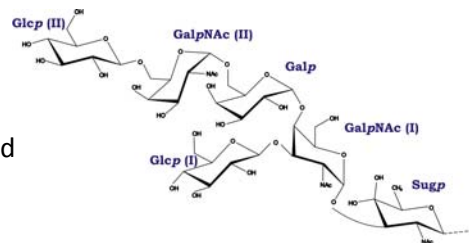
<sup>2</sup> Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, 181 Longwood Avenue, 02115 Boston, Massachusetts

Conjugate vaccines containing a bacterial surface or capsular polysaccharide linked to a protein carrier have yielded tremendous success for reducing disease caused by *S. pneumoniae*, *H. influenzae* and *N. meningitidis*. However, difficulties with this approach include the high cost of production, high variability of the structural, and hence immunological, properties of surface polysaccharides, and variations in the fine chemical substituents that impact the quality of the antibodies induced. Notably, many years of observations show that one mechanism bacteria use to avoid host immunity is to produce a wide variety of surface polysaccharides that then engender a requirement for specific immunity to each one. However, within the past 10 years it has become apparent that there is a highly conserved, immunologically invariant surface polysaccharide produced by many bacterial species, including some of the most important human pathogens, that is a potential vaccine target. This is poly- $\beta$ (1 $\rightarrow$ 6)-*N*-acetylglucosamine (PNAG) whose production by organisms such as *E. coli*, *S. aureus*, *Y. pestis*, *B. pertussis*, *A. baumannii* and many others has been established. Published work has shown that the native, highly acetylated (>80% *N*-acetyl substituents) glycoform of PNAG induces antibodies that fail to mediate in vitro opsonophagocytic killing, the major laboratory correlate of protective antibody. These antibodies are not protective in experimentally infected animals. However, by reducing the *N*-acetylation level to <40%, conjugate vaccines can be produced that elicit high titers of opsonic, protective antibodies. Problematically, the deacetylated PNAG glycoform prepared from bacterially-derived native PNAG is difficult to work with due to solubility issues, so synthetic oligosaccharides representing 5, 7, 9 and 11  $\beta$ -1-6-linked *N*-acetyl glucosamines (GlcNAc) or  $\beta$ -1-6-linked glucosamines (GlcNH<sub>2</sub>) have been made with terminal sulphhydryl groups on some of these for conjugation to carrier proteins. Confirming results derived with either the highly or poorly acetylated native PNAG glycoform, the synthetic 5GlcNH<sub>2</sub> or 9GlcNH<sub>2</sub> oligosaccharides conjugated to carrier proteins such as tetanus toxoid (TT) elicited opsonic, protective antibodies whereas fully acetylated 5GlcNAc or 9GlcNAc did not. The 9GlcNH<sub>2</sub> oligomer could be conjugated to a variety of carrier proteins representing toxins or virulence factors from different pathogens and elicited the desired opsonic antibodies to PNAG. Protection against infections caused by *S. aureus*, including methicillin-resistant *S. aureus* and *E. coli* was achieved by antibodies raised to 9GlcNH<sub>2</sub>-conjugate vaccines. More recent studies show the 5GlcNH<sub>2</sub>-TT conjugate vaccine is also effective at inducing opsonic, and likely protective, antibodies. Overall, drawing on synthetic carbohydrate chemistry, modern immunology and molecular microbiology, synthetic GlcNH<sub>2</sub> as an oligosaccharide component of a conjugate vaccine has the potential to provide protective immunity against the broad range of bacterial pathogens that express PNAG, potentially addressing a critical problem in infectious diseases, namely the need for new prophylactic and therapeutic approaches to multi-drug resistant pathogens.



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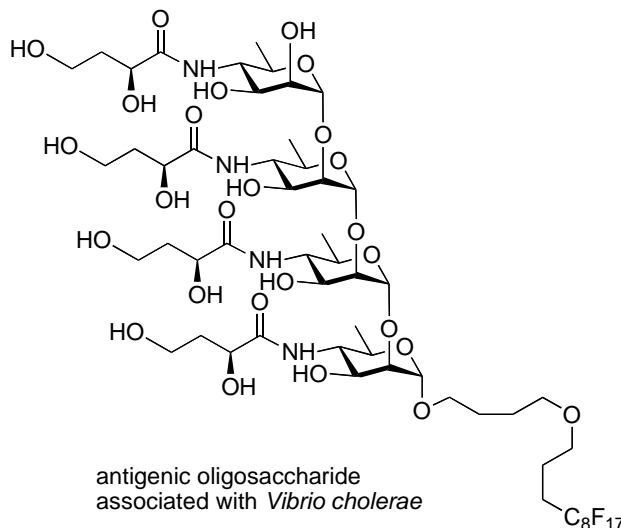
### ABSTRACT

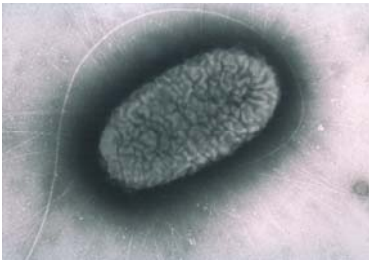
#### Successes and Challenges in the Automated Synthesis of Microbial Carbohydrates

NICOLA L. B. POHL<sup>1</sup>.

<sup>1</sup> Department of Chemistry and the Interdepartmental Graduate Program in Microbiology, Iowa State University, Ames, IA 50011 USA

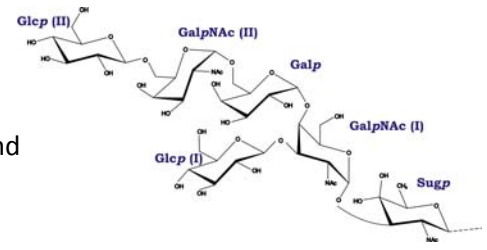
Procurement and structural identification of pure microbial carbohydrates/oligosaccharides is a major limitation in the study of these biomolecules. The covalent attachment of molecules to solid-phases has allowed the automated syntheses of a range of bioactive molecules. Unfortunately, solid-phase approaches usually expend large excesses of building blocks to achieve the high yields required when intermediates cannot be purified and therefore are practically limited to building blocks that are made in a few steps and to reactions that proceed with very high stereochemical fidelity and yield. This talk will discuss an alternative solution-phase automation strategy to iterative synthesis based on noncovalent fluorocarbon interactions. The challenges in implementing such an automated approach based on noncovalent interactions will be presented in the context of the synthesis of a range of microbe-associated antigenic oligosaccharides including complex structures from *Staph. aureus* and cholera. Microbial carbohydrate synthesis is particularly challenging because of the diverse range of unusual monosaccharide building blocks, too. Some applications of these synthetic oligosaccharides, for example in microarrays, will also be presented.





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### ABSTRACT

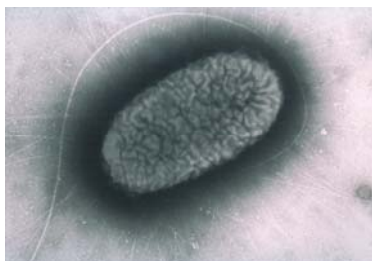
#### Biogenesis of O antigen by the Wzy-mediated pathway

MIGUEL VALVANO

*Microbiology and Immunology, University of Western Ontario, London, ON, Canada, N6A 5C1*

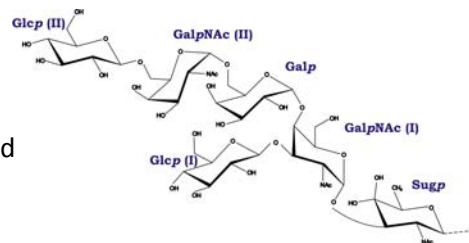
Lipopolysaccharide (LPS) is a major constituent of the outer leaflet of the Gram-negative bacterial outer membrane, and consists of lipid A, core oligosaccharide (OS), and O-specific polysaccharide or O antigen. The biogenesis of LPS is a complex process involving various steps that occur at the bacterial inner membrane followed by the translocation of LPS molecules to the bacterial cell surface. The lipid A-core OS is assembled independently from the O antigen and both biosynthesis pathways converge by the ligation of the O antigen onto outer core domain of the lipid A-core OS acceptor. I will review the current mechanisms strictly operating in the biogenesis of the O-specific LPS, with a special emphasis on the polymerase (Wzy)-dependent pathway. I will describe ongoing research in my laboratory dealing with structure and function of membrane proteins involved in: (i) initiation of O antigen biosynthesis, focused particularly in the membrane protein WecA and WbaP; (ii) membrane translocation of O repeating subunits mediated by the Wzx protein; and (iii) the ligation reaction of the O unit with the lipid A-core OS.





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### ABSTRACT

#### Structure and dynamics of microbial polysaccharides using NMR spectroscopy

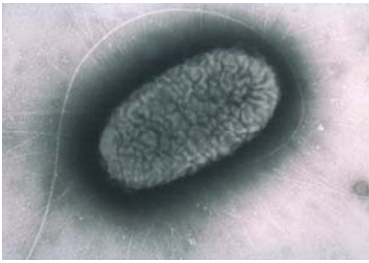
GÖRAN WIDMALM

*Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*

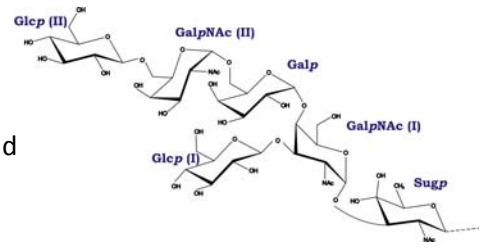
A large variety of sugars are found in capsular and O-antigen polysaccharides of bacteria. Furthermore, the sugars are of different complexity and include pentoses, deoxyhexoses, lactyl substituted hexoses, heptoses and nonuloses. The number of sugar residues in the repeating units of these polysaccharides varies most often from two to seven. The topology of the repeats can be described as linear, branched or double branched. We have analyzed the topology of *E. coli* O-antigens based on the number of sugar residues in the backbone. The most common topology contains four sugars in the backbone, being linear or containing a single residue in the side-chain. Using NMR spectroscopy we have been able to identify the terminal sugar residues in several O-antigen polysaccharides. Consequently, these NMR analyses indicated the biological repeating units in different strains. Our NMR analyses show that structures anticipated to be synthesized via the Wzy-dependent pathway have as the first sugar residue either D-GlcNAc or D-GalNAc in the case of *E. coli* O-antigens. In addition, in all but a few cases, these residues are 3-substituted [Stenutz, R.; Weintraub, A.; Widmalm, G. *FEMS Microbiol. Rev.* 2006, 30, 382-403].

The computer program CASPER, Computer Assisted SPectrum Evaluation of Regular polysaccharides, is used for the prediction of NMR chemical shifts and for structure determination [Stenutz, R.; Jansson, P.-E.; Widmalm, G. *Carbohydr. Res.* 2006, 341, 1003-1010]. All structures consistent with data from chemical analyses (if present) are generated, their <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts calculated and finally ranked according to their agreement with the experimental spectra. The computation of the chemical shifts is based on schemes with substituent-induced chemical shift changes. The application can make use of one-dimensional <sup>1</sup>H and <sup>13</sup>C NMR spectra as well as two-dimensional <sup>1</sup>H, <sup>13</sup>C-HSQC NMR spectra. Matching simulated 2D NMR chemical shifts with the experimental spectrum is more complicated than for 1D spectra but an algorithm has been developed to this end. Chemical shift predictions of oligo- and polysaccharides with conserved backbone structures but with structural modifications will be presented to underscore its potential. In cases when the NMR chemical shift overlap is extensive, additional methodology is needed. Reducing the dimensionality of NMR experiments is an efficient approach to obtaining additional information without acquiring large data sets. Instead of employing a full 3D NMR spectrum, it is possible to record a tilted projection where e.g. <sup>13</sup>C and <sup>1</sup>H evolutions are linked together. Thus, small contributions from <sup>13</sup>C chemical shifts are utilized, which result in 'mixed' proton-carbon frequencies in a tilted plane, using e.g. a <sup>1</sup>H, <sup>1</sup>H-NOESY-<sup>1</sup>H, <sup>13</sup>C-HSQC TILT experiment [Kupče, E.; Nishida, T.; Widmalm, G.; Freeman, R. *Magn. Reson. Chem.* 2005, 43, 791-794].





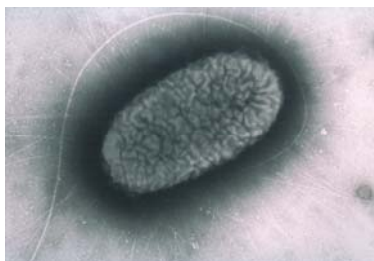
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# POSTER SESSION 1

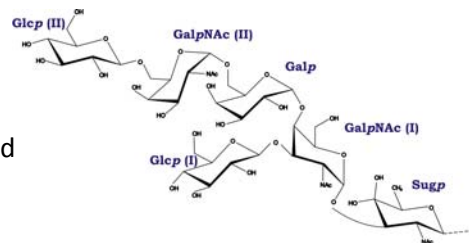
## ABSTRACTS 1-22

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### ABSTRACT - POSTER 1

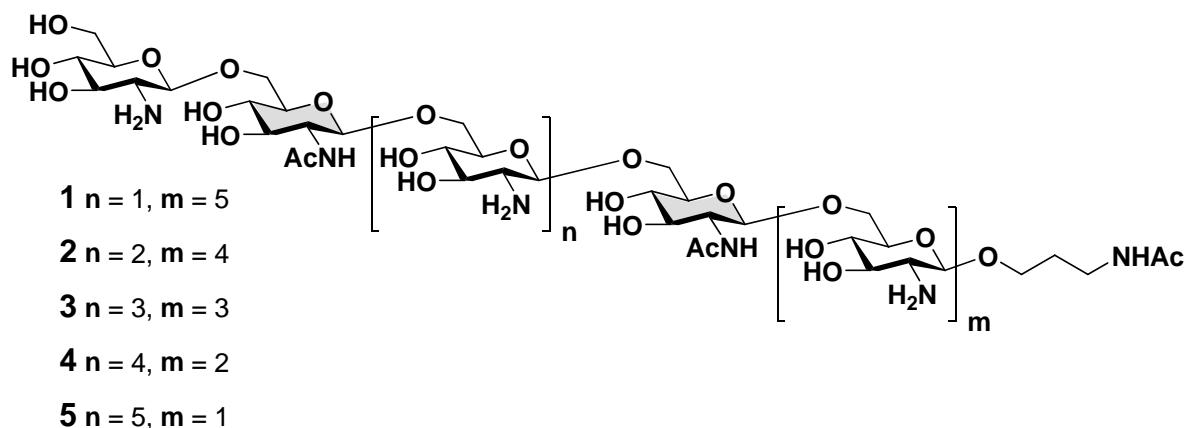
#### Synthetic nona- $\beta(1\rightarrow6)$ -glucosamines with various patterns of N-acetylation representing fragments of an exopolysaccharide produced by *Staphylococcus aureus*

MARINA L. GENING,<sup>1</sup> OLGA N. YUDINA,<sup>1</sup> YURY E. TSVETKOV,<sup>1</sup> GERALD B. PIER,<sup>2</sup> NIKOLAY E. NIFANTIEV<sup>1</sup>

<sup>1</sup> N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky prospect 47, 119991 Moscow, Russia

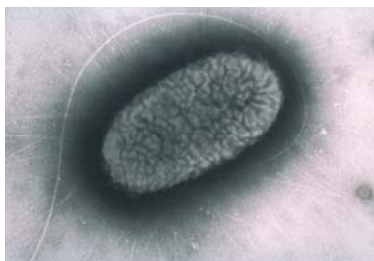
<sup>2</sup> Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, 181 Longwood Avenue, 02115 Boston, Massachusetts

Poly- $\beta(1\rightarrow6)$ -*N*-acetylglucosamine (PNAG) is an exopolysaccharide produced by *S. aureus* and many other pathogenic bacteria. Chemical de-*N*-acetylation of PNAG results in the formation of dPNAG, containing 15-30% of residual *N*-acetyl groups. dPNAG is a promising vaccine candidate, but its precise composition is unknown and presumably it is a statistical combination of D-glucosamine and *N*-acetyl-D-glucosamine residues. Prior work has shown that antibodies to synthetic nona- $\beta(1\rightarrow6)$ -D-glucosamine conjugated to a protein carrier can mediate *in vitro* opsonic killing and protect mice from *S. aureus* and *E. coli* infections.[1] As a next stage we have synthesized a set of five nonaglycosamines (**1-5**) containing two *N*-acetylglucosamine residues separated by a different number of intervening glucosamine units.



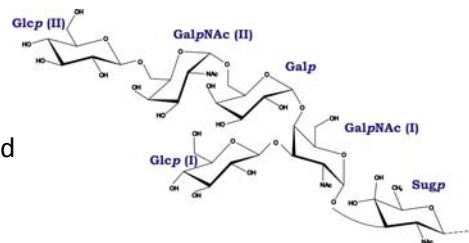
The key step in the synthesis of these compounds was an appropriate choice of *N*-protecting groups and rational oligosaccharide block construction. All compounds were derivatized with a spacer arm for further conjugation to carrier proteins. Initial ELISA experiments revealed that compound **3** has a higher affinity of binding to protective monoclonal antibodies than do compounds **1**, **2**, **4** and **5**. The lower affinity was observed for compound **5**.

[1] M.L. Gening, T. Maira-Litran, A. Kropec, D. Skurnik, M. Grout, Y.E. Tsvetkov, N.E. Nifantiev, G.B. Pier «Synthetic beta-(1-6)-linked *N*-acetylated and non-acetylated oligoglucosamines to produce conjugate vaccines for bacterial pathogens.» *Infect. Immun.*, 78 (2010) 764-772.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 2

#### Synthesis of large fucoidan fragments - potential inhibitors of microbial adhesion

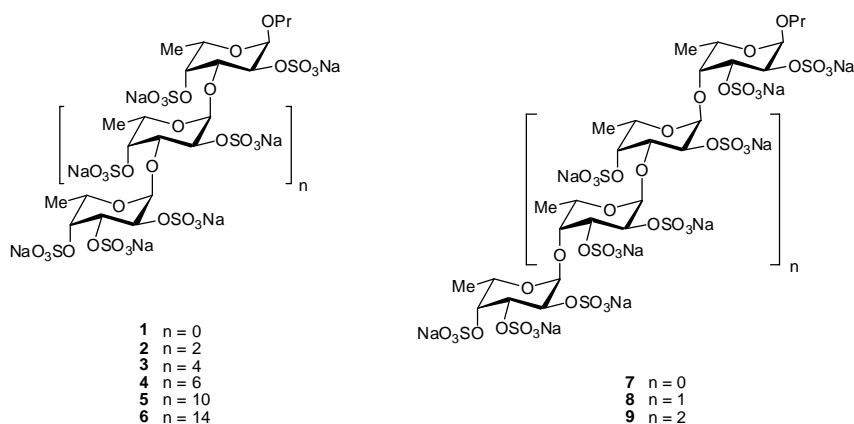
VADIM B. KRYLOV, NADEZHDA E. USTYUZHANINA, ALEXEY A. GRACHEV, NIKOLAY E. NIFANTIEV

Laboratory of Glycoconjugate Chemistry, N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky prospect 47, 119991, Moscow, Russia. E-mail: nen@ioc.ac.ru

Fucoidans are highly sulfated polysaccharides consisting essentially of  $\alpha$ -L-fucopyranose residues. They were found in many species of brown seaweeds and in some echinoderms. These biopolymers effectively inhibit microbial and viral adhesion to host cells, angiogenesis development, P- and L-selectin mediated inflammation, blood coagulation and some other biological processes.

In this communication we report on the synthesis of totally sulfated oligosaccharides related to fucoidan chains. The first series of compounds consist of di-, tetra-, hexa-, octa-, dodeca-, and hexadecasaccharides **1-6** built up of (1 $\rightarrow$ 3)-linked  $\alpha$ -L-fucopyranose residues, which correspond to polysaccharides isolated from several seaweeds including *Saccharina latissima* (previous name *Laminaria saccharina*), *Chorda filum*. The second series of compounds is presented by di-, tetra-, and hexasaccharide **7-9** built up of alternating (1 $\rightarrow$ 3)- and (1 $\rightarrow$ 4)-linked  $\alpha$ -L-fucopyranose residues, related to fucoidans from *Fucus evanescense*, *F. distihus* and some others.

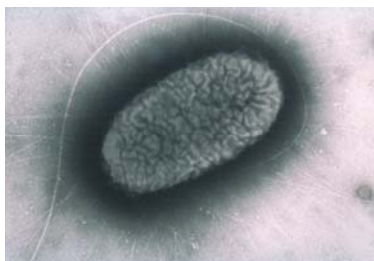
Oligosaccharide chains of target compounds **1-9** were assembled by stereocontrolled blockwise strategy with the use of optimally protected precursors following by exhaustive acid-promoted O-sulfation [1] of corresponding deprotected oligosaccharide.



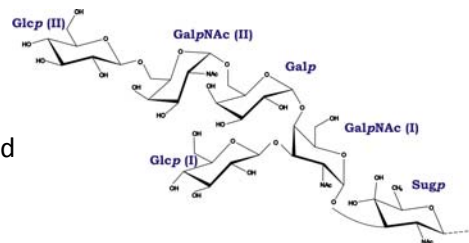
Ability of sulfated oligosaccharides **1-9** to inhibit of microbial adhesion is under investigation.

**Acknowledgments:** This work was supported by the Russian Foundation for Basic Research (Grants 08-04-00812-a, 10-03-00980-a) and Grant of President of Russian Federation (MK-3901.2009.4).

[1] Krylov, V.; Ustyuzhanina, N.; Grachev, A.; Nifantiev, N. *Tetrahedron Lett.* **2008**, *49*, 5877-5879.



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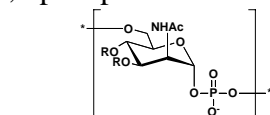
ABSTRACT - POSTER 3

*Neisseria meningitidis* Serotype A: towards the optimization  
of the synthesis of C-oligosaccharide analogues

FILIPPO BONACCORSI, STEFAN OSCARSON

Centre For Synthesis And Chemical Biology, University College Dublin, Belfield Dublin 4, Dublin Ireland

*Neisseria meningitidis* Type A is a pathogenic bacterium that causes several diseases such as meningitis, bacteremia and septicemia. It is surrounded by a polysaccharide capsule which protects the bacterium from osmotic lysis, phagocytosis and is responsible for being the main cause of pathogenicity<sup>1</sup>. The repeating unit (Figure 1), is composed of a single unit of *N*-acetylmannosamine connected to the next unit by a 1,6 phosphodiester linkage.



*Neisseria Meningitidis* Type A CPS Repeating Unit Figure 1

A glycoconjugate vaccine has been prepared by harvesting the natural cell capsule. This vaccine is prone to degradation due to the instability of the phosphodiesteric linkage between the two units, making its administration difficult. In order to solve this problem the synthesis of more stable C-oligosaccharide analogues has been performed, replacing the anomeric oxygen with a methylene moiety (Figure 2).

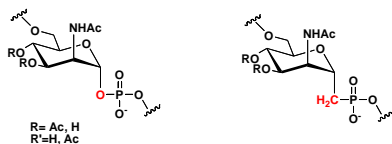


Figure 2

The synthesis of a C-oligosaccharide of *Neisseria meningitidis* Type A tetrasaccharide has been already reported<sup>2</sup> (Figure 3).

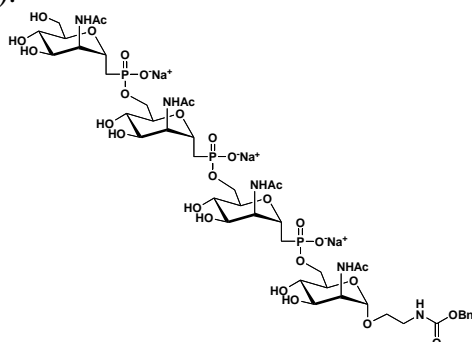
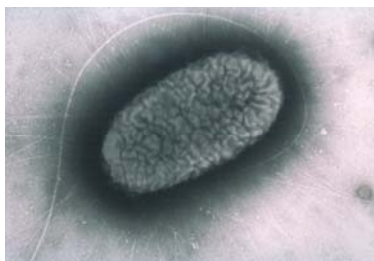


Figure 3

In this work we will present attempts to optimize the synthesis of these C-phosphonate structures for efficient vaccine candidate production.

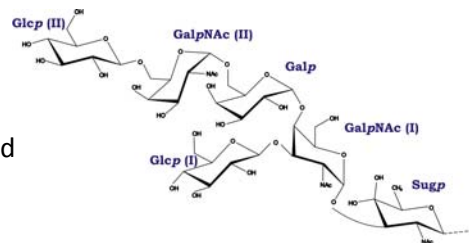
1-Morley, S. L.; Pollard, A. J. *Vaccine*, **2002**, *20*, 666-687

2-Teodorovic, P.; Slättegård, R.; Oscarson, S. *Org. Biomol. Chem.* **2006**, *4*, 4485.



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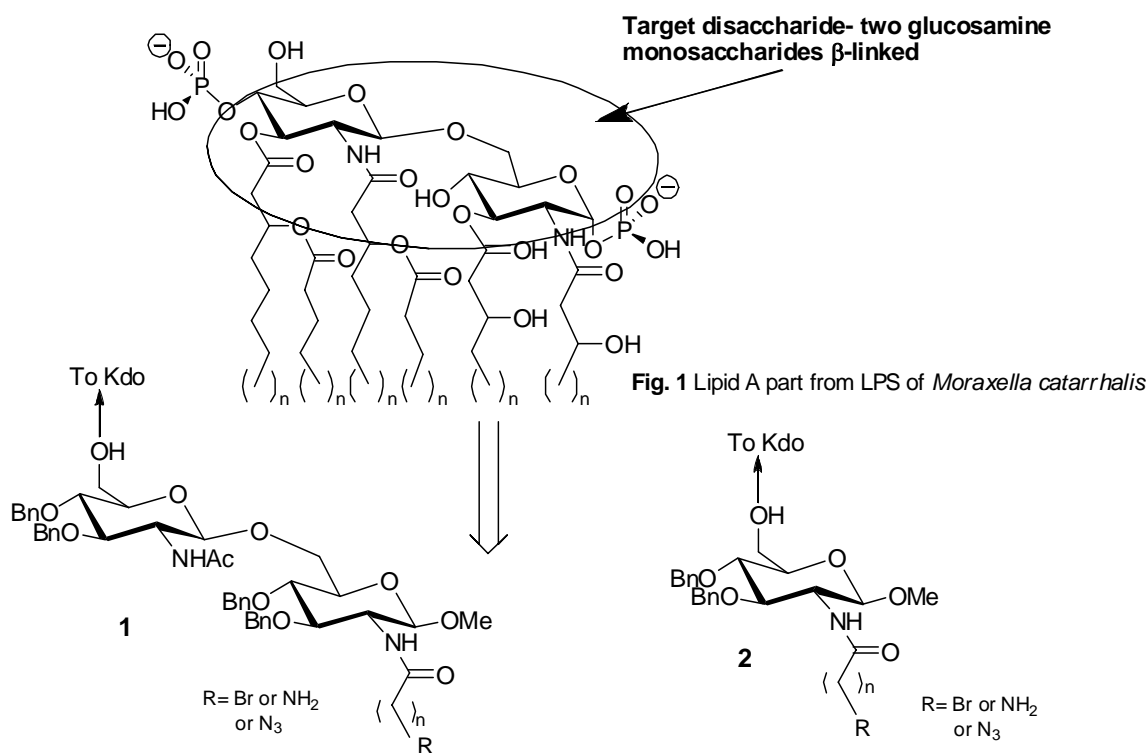
### ABSTRACT - POSTER 4

#### SYNTHESIS OF GLYCOCONJUGATE VACCINE CANDIDATES AGAINST *Moraxella catarrhalis*

STEFAN OSCARSON<sup>1</sup> and HEATHER HORAN<sup>1</sup>

<sup>1</sup> Centre for Synthesis and Chemical Biology, School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland.

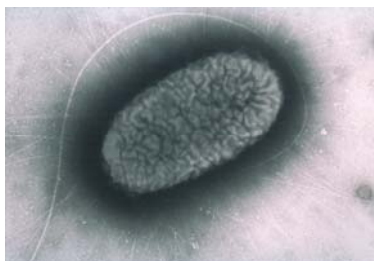
*Moraxella catarrhalis* is a Gram negative bacterium responsible for upper respiratory tract infections; including otitis media.<sup>1</sup> Our interest lies in synthesising lipopolysaccharide (LPS) structures of these bacteria (Fig. 1). Segments of the core LPS region have been synthesised earlier.<sup>2,3</sup> In nature, the LPS is anchored in the outer bacterial membrane by the fatty acids of the Lipid A part.<sup>4</sup> These acidic residues give the native structure its characteristic toxicity. We hope to preserve the function of this structure in a non-toxic manner by producing synthetic analogues.



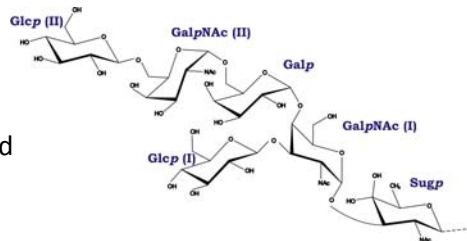
Structures **1** and **2** (Fig.2) could function as synthetic mimics for our target glucosamine disaccharide (Fig. 1). These in turn will be glycosylated to Kdo residues of the inner core. Spacers of varying length and function will be attached to the amino group of **1** and **2**, to support and conserve the natural 3D presentation of the core unit in conjugate vaccine candidates.

#### References:

- [1] B.W. Catlin, *Clin. Microbiol. Rev.* 1990, 28, 126..
- [2] K. Ekelof, S. Oscarson, *J. Org. Chem.*, 1996, 61, 7711.
- [3] K. Ekelof, S. Oscarson, *Carbohydr. Res.*, 1995, 278, 289.
- [4] J. M. Braun, J. Beuth, C. C. Blackwell, *Vaccine* 2004, 7, 898.



4<sup>th</sup> Baltic meeting on  
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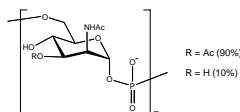
**ABSTRACT - POSTER 5**

**Synthesis of structures corresponding to the capsular polysaccharide of *Neisseria meningitidis* group A**

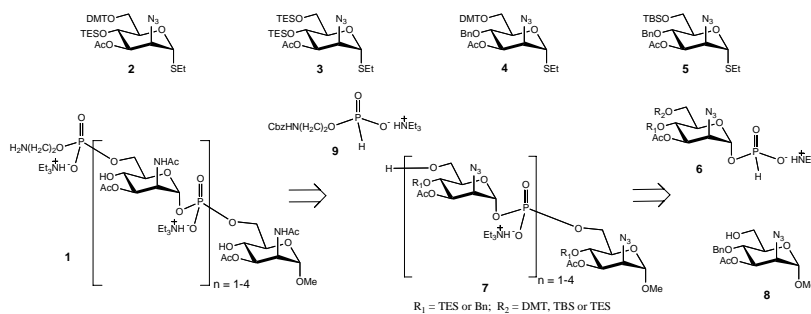
A. BLACK, A.V. NIKOLAEV

*Division of Biological Chemistry & Drug Discovery, College of Life Sciences, University of Dundee, Dundee, DD1 5EH.*

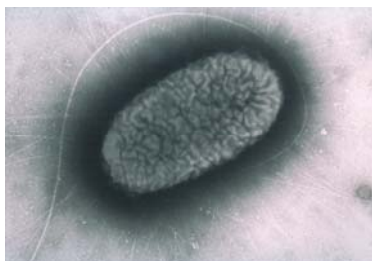
One of the causative agents of meningitis is the gram-negative bacteria *Neisseria meningitidis*. In the region known as the African meningitis belt, *N. meningitidis* serotype A is endemic in the population and is also known to cause large scale epidemics. An important virulence factor of this bacterium is the presence of a capsular polysaccharide (CPS) and apart from being an important surface antigen, also protects the bacteria cells against phagocytosis by the host's immune defense. The CPS structure of the serotype A is the (1-6)-linked poly(3-*O*-acetyl-2-acetamido-2-deoxy- $\alpha$ -D-mannosyl phosphate):



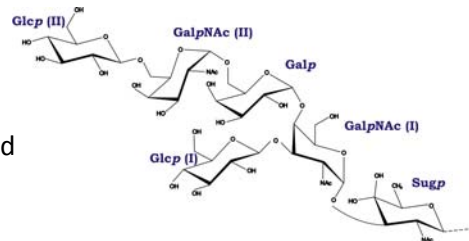
A cost effective approach to a vaccine would be to synthetically produce fragments of the CPS. In order to synthesise these CPS structures (**1**), the monosaccharide precursors **2-5** must be prepared first with temporary protection of the O-6 position and a permanent protection at the O-4 position. Formation and elongation of the CPS oligomers proceeds through the condensation of anomeric H-phosphonates **6**. In order to accommodate this, compounds **2-5** allow for hydrolysis at the position-1 to the corresponding hemi-acetals. These are then converted to the H-phosphonates **6** before coupling to a monohydroxyl unit (structures **8**) to form a phosphodiester linkage. Removal of the temporary protection at O-6 then gives the free hydroxyl (structures **7**) needed for the chain elongation to continue. The fragments then will be capped with ethanolamine H-phosphonate **9** that will allow for conjugation to a protein carrier.







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ABSTRACT - POSTER 6

Synthesis and biological activities of (2-ammoniumethyl)  $\beta$ -D-glucopyranoside bromide

BARBARA DMOCHOWSKA<sup>1</sup>, AGATA KUBIŃSKA<sup>1</sup>, ANNA WOZIWODZKA<sup>2</sup>, JACEK PIOSIK<sup>2</sup>

<sup>1</sup>University of Gdańsk, Faculty of Chemistry, Sugar Chemistry Group, J. Sobieskiego 18, 80-952 Gdańsk, Poland

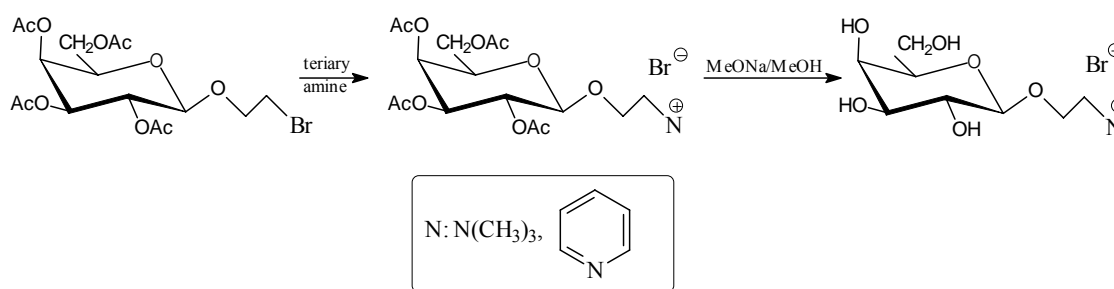
<sup>2</sup>Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Poland

The glycosides play the great role in biological activity. Some of them constitute structural parts of many antibiotics or vitamins. The chemists are currently interested in D-glucopyranosides as potential anti-HIV agents.

Quaternary ammonium salts (QACs) constitute a huge, very interesting and widely used group of organic compounds. Their antibacterial, antiviral and antifungal activities are known well. The activity of many biological agents depends on the quaternary ammonium group presence. Many of them demonstrate antistatic and anticorrosive activity. Numerous QACs exhibit also surface activity, good detergency and low toxicity. [1]

While examining biological activity of *N*-(2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)trimethylammonium bromide and *N*-(2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)-pyridinium bromide, it has been found that both compounds exhibit noncompetitive type of inhibition to the AMP-Deaminase (AMP-DA) isolated from the rat skeletal muscle [2]. Observed activity can lead to a further toxicological consequences due to the significant role of the studied enzyme involved in a number of physiological processes such as metabolism of the purine nucleotide cycle.

A new series of quaternary ammonium bromides have been synthesized in reaction of (2-bromoethyl) 2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-galactopyranoside with tertiary amines: pyridine and trimethylamine (scheme 1). The structures of isolates were determined by spectral analysis including extensive 2D NMR analyses and X-ray crystallography. QACs were detected of mutagenic activity. *Vibrio harveyi* A16 (a *luxE* mutant) was used for the bioluminescence-based mutagenicity assay. [3]

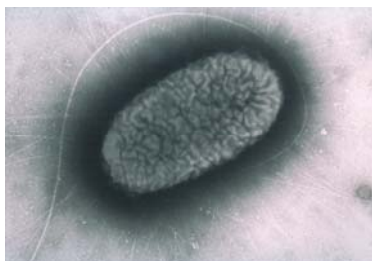


Scheme 1

**References:** [1] W. Śliwa, *N*-Substituted Salts of pyridine and related compounds, WSP, Częstochowa, Poland, ISBN 83-7098-198-4, 1996. [2] A. Składanowski, P. Stepnowski, K. Kleszczyński, B. Dmochowska, *Envir. Tox. Pharm.*, 2005, **19**(2), 291 [3] B. Podgorska, G. Węgrzyn, *Lett. Appl. Microbiol. Genet.*, 2007, **48**, 409

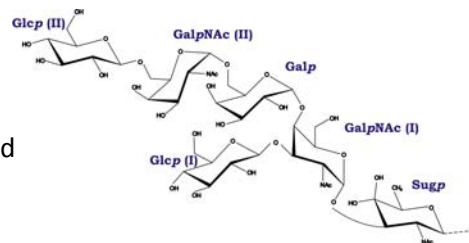
**Acknowledgements:** This work was partially financed by grant DS/8451-4-0134-0 and BW/8451-5-0452-0





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 7

#### Separation of mycobacterial phosphoinositol mannosides

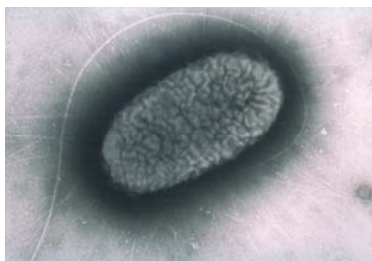
REGINA ENGEL<sup>1</sup>, BUKO LINDNER<sup>2</sup>, OTTO HOLST<sup>1</sup>

Divisions of<sup>1</sup>Structural Biochemistry and<sup>2</sup>Immunochemistry, Research Center Borstel, Leibniz Center for Medicine and Biosciences, Parkallee 1-40, D-23845 Borstel, Germany. oholstfz@borstel.de

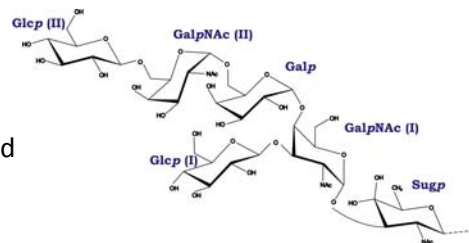
Mycobacteria comprising environmental and human- (*Mycobacterium tuberculosis*, *M. leprae*, *M. avium*) and animal-pathogenic species possess a unique and very complex cell envelope. This cell envelope contains a number of carbohydrate-containing constituents, of which the lipoarabinomannan (LAM) and mycoloyl-arabinogalactan-complex are the most important. LAM is composed of a GPI-anchor to which a mannan chain is linked, which in turn is substituted by a branched arabinan. Partial structures of LAM are the lipomannan (LM, GPI anchor plus the mannan) and the phosphoinositol mannosides (PIMs, GPI anchor plus 2 – 6 mannosides). LM and PIMs are thought to represent biosynthetic precursors of LAM.

Apart from the numerous immunological/immunomodulatory features that have been described for LAM, also the PIMs were reported to possess such properties, in particular to react with Toll-like receptor 2 and to play a role in the fusion of phagosomes and early endosomes. Since PIMs represent a heterogeneous mixture of molecules (2 – 4 fatty acids of different chain lengths, 2 – 6 mannosides), it remains unclear which of the molecules represent(s) the biological active compound(s). A prerequisite to investigate this issue is the isolation of single pure PIM molecules from the mixture. Here, we present a protocol by which pure PIM were isolated from *M. tuberculosis*.

Briefly, the mycobacteria were disrupted by French Press treatment and then extracted with CHCl<sub>3</sub>/CH<sub>3</sub>OH/H<sub>2</sub>O. The chloroform-phase was first purified by chromatography on Silica Gel 60, which yielded the PIM fraction. This was separated into single components by successive reversed-phase chromatography utilizing RP-18 and RP-30 columns. The purity of the compounds was confirmed by mass spectrometry.



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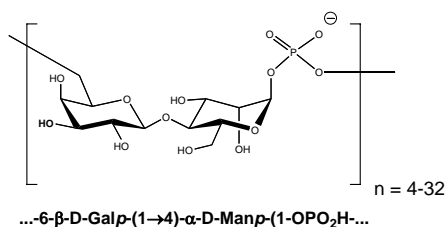
**ABSTRACT - POSTER 8**

**Chemical synthesis of *Leishmania* phosphoglycan structures conjugated to a biotin moiety**

C.W. EDGAR, M.A.J. FERGUSON, A.V. NIKOLAEV

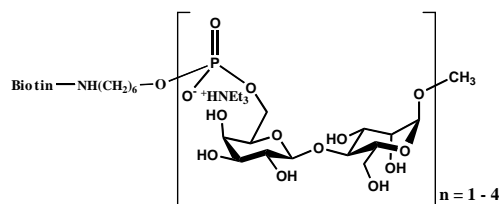
*Division of Biological Chemistry & Drug Discovery, College of Life Sciences, University of Dundee, Dundee, DD1 5EH.*

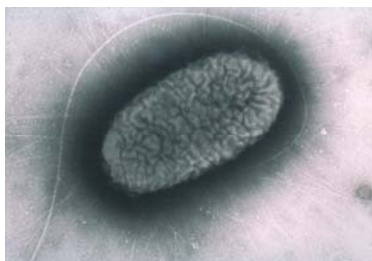
Leishmaniasis is the second largest parasitic killer in the world (after malaria) responsible for an estimated 500,000 cases each year worldwide. Visceral leishmaniasis infections (i.e. internal organs) are more complicated to diagnose due to the lack of visible symptoms. Current tests of the disease look for antibodies against the parasite, but these antibodies are expressed long after the infection. It has been found the *Leishmania* parasite excretes a unique phosphoglycan repeat unit;



By testing for this repeat unit, the diagnostic test will be looking for an active infection in the patient. It has been found that there are 4 monoclonal antibodies to detect this uniquely excreted phosphoglycan. This provides a basis for a test kit, the biomarker (i.e. the phosphoglycan repeats), and the way to detect the biomarker. For the construction of a dipstick style test or an ELISA format assay, a synthetic repeat unit phosphoglycan is needed to act as a positive control in a dipstick test or as a known comparison in a ELISA.

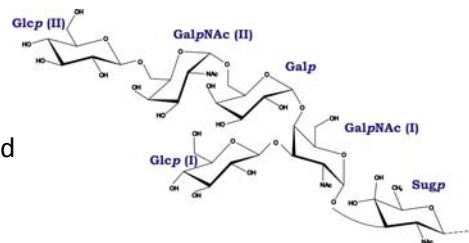
The synthetic structure must also be anchored to a solid surface for testing. Biotin will be used as the anchor, it has shown many different uses in biotechnology and its very high affinity to the avidin proteins make it very useful. A 6-aminohexanol spacer arm will also be included. After many attempts to introduce the biotin spacer moiety to the repeat unit at the D-mannose 1-phosphate, it was decided to change the strategy and integrate the biotin-spacer moiety as a phosphate at the D-galactose unit. This meant performing chain elongation from the reducing end, with the first disaccharide unit being capped at the D-mannose anomeric position with a methyl group. Below are examples of the targeted biotinylated phosphoglycan structures to be prepared in this project.





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### ABSTRACT - POSTER 9

#### Serotyping clinical isolates of *Proteus mirabilis* strains

WIESŁAW KACA<sup>1,2</sup>, JOANNA WAWSZCZYK<sup>1</sup>, MAREK KWINKOWSKI<sup>1</sup>, NORBERT MAJCHER<sup>1</sup>,  
SEBASTIAN GRABOWSKI<sup>2</sup>, YURIY KNIREL<sup>3</sup>

<sup>1</sup> Department of Microbiology, Institute of Biology, Jan Kochanowski University, ul. Świętokrzyska, Kielce, Poland

<sup>2</sup> Department of General Microbiology, Institute of Microbiology and Immunology, University of Łódź, Banacha, Łódź, Poland

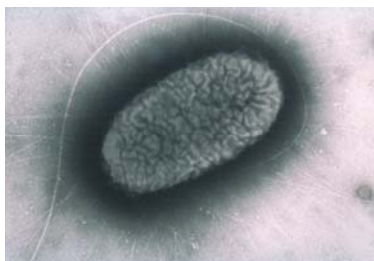
<sup>3</sup> N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia

*Proteus mirabilis* rods are common in normal human intestine microflora. However, certain pathogenic strains of *P. mirabilis* can cause lower and upper urinary tract infections (UTI). Bacteria from the genus *Proteus* are also involved in rheumatoid arthritis. Recently, structure and serology of the O-antigens (O-polysaccharide parts of the LPSs) of 76 *Proteus* O-serogroups have been summarized [1]. The frequency of the occurrence of different O-serogroups among UTI isolates of *P. mirabilis* is unknown. The aim of this work was serotyping of *P. mirabilis* UTI strains based on chemically defined O-antigens using two clinical collections from Sweden and Poland including 99 and 24 UTI strains, respectively. As a result, a relatively simple two-step scheme was proposed for serotyping of *Proteus* rods on the basis of ELISA with thermostable surface antigens of *Proteus* cells and immunoblotting with isolated LPSs. Using polyclonal anti-*P. mirabilis* rabbit antisera, 50 strains from the Swedish collection and 8 strains from the Polish collection were classified, and the occurrence of strains from serogroups O10, O38, O36, O30, O17, O23, O9, O40, O49, O27, O5, O13, O24, and O33 was demonstrated. From the Swedish strains, 10 belonged to serogroup O10 and 5 each to serogroups O38, O30, and O36. Therefore, none of the O-serogroups was predominant. The majority of serotyped clinical *P. mirabilis* strains possess acidic O-antigens containing uronic acids and various acidic non-carbohydrate substituents. In immunoblotting, antisera cross-reacted with both O-antigen and core parts of the LPSs. The core part of 20 LPSs bound single serum and 12 LPSs more than two sera. Bioinformatic analysis of available sequences allowed proposing a molecular approach for the determination of *Proteus* core oligosaccharide structures. Identification of the core oligosaccharide of *P. mirabilis* R110, derived from a serogroup O3 wild strain, by means of RLFP analysis of galacturonic acid transferases is shown as an example.

**Conclusions.** The most frequently occurring O-serogroups among *P. mirabilis* UTI strains were identified. The diversity of serological reactions of the LPSs is useful for serotyping of *P. mirabilis* clinical isolates. A role of acidic components of the O-antigens in UTI remains to be elucidated.

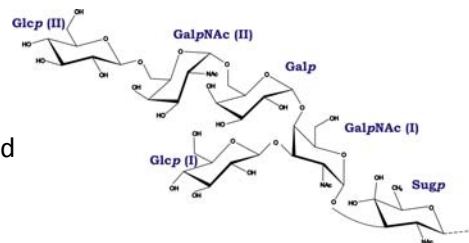
[1] Knirel Y. A., Perepelov A. V., Kondakova A. N., Senchenkova S. N., Sidorczyk Z., Rozalski A., Kaca W. Structure and serology of O-antigens as the basis for classification of *Proteus* strains. *Innate Immun.*, **2010**, doi:10.1177/1753425909360668

The work was supported by grant from Jan Kochanowski University, Kielce, Poland (BS 125/S).



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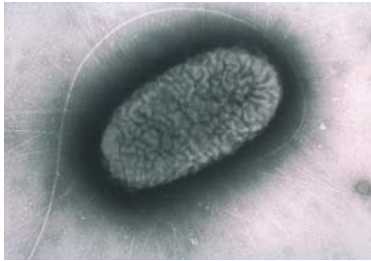
### ABSTRACT - POSTER 10

#### Critical role of the LpxM of *Yersinia pestis* in immunogenesis and pathogenesis of plague

VALENTINA A FEODOROVA<sup>1,2</sup>, LUDMILA N PAN'KINA<sup>1,2</sup>, ANNA N KONDAKOVA<sup>3,4</sup>, BUKO LINDNER<sup>4</sup>, OTTO HOLST<sup>4</sup>, GERALD B PIER<sup>5</sup>, YURIY A KNIREL<sup>3</sup>, ANDREY P ANISIMOV<sup>6</sup>, VLADIMIR L MOTIN<sup>7</sup>

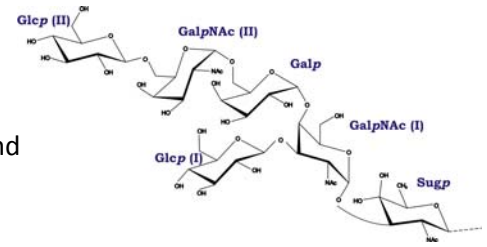
<sup>1</sup> Scientific and Research Institute for Medical and Veterinary Biotechnology, Branch-in-Saratov, Russia; <sup>2</sup> Russian State Anti-Plague Research Institute "Microbe", Saratov, Russia; <sup>3</sup> N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia; <sup>4</sup> Research Center Borstel, Leibniz Center for Medicine and Biosciences, Borstel, Germany; <sup>5</sup> Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; <sup>6</sup> State Research Center for Applied Microbiology and Biotechnology, Obolensk, Moscow Region, Russia; <sup>7</sup> Departments of Pathology/Microbiology & Immunology, University of Texas Medical Branch, Galveston, USA

Acyl transferase LpxM is involved in the biosynthesis of the lipid A portion of the *Y. pestis* lipopolysaccharide. In order to determine the role of the intact lipid A structure in immunogenesis and pathogenesis of plague, a deletion-insertion mutation in the *lpxM* gene was introduced into the vaccine strain *Y. pestis* EV line NIEG and the fully virulent wild-type strain *Y. pestis* 231. The generated mutants were designated as EV  $\Delta$ *lpxM* and 231  $\Delta$ *lpxM*, respectively. Mass spectrometric analysis of the mutant lipopolysaccharides showed that inactivation of LpxM resulted in synthesis of a pentaacyl form rather than the hexaacyl form of lipid A. Both mutants and parental strains were studied for residual virulence, dissemination into tissues and protective activity using outbred mice, inbred BALB/c mice and guinea pigs. The EV  $\Delta$ *lpxM* derivative was completely avirulent as there was no systemic bacterial spread or animal deaths among 100% of the inoculated animals. When the animals were treated with the parental vaccine strain EV, 28.6% of the animals died. A two-to-three fold swelling of the lymph nodes was observed in guinea pigs infected with EV  $\Delta$ *lpxM*, while no swelling was seen after infection with the EV strain. All animals inoculated with either *Y. pestis* 231  $\Delta$ *lpxM* or the parental 231 strain died, indicating that in this case the *lpxM* mutation did not reduce virulence. The death of animals from plague infection was verified bacteriologically. A single injection of  $10^3$ - $10^9$  CFU of the EV  $\Delta$ *lpxM* mutant protected 57.1-85.7% of outbred mice and 42.8% of BALB/c mice against s.c. challenge with 2,000 MLD as well as 25-50% guinea pigs from an infectious dose of 1,200 MLD of the wild-type *Y. pestis* strain 231. The EV vaccine was entirely ineffective (all animals died) against the same challenge dose of the virulent strain. Furthermore, a marked difference in the behavior was observed when the strains were cultured under specific conditions *in vitro* simulating key stages of the pathogen-host interaction, i.e. those of the bloodstream (S-1), early endosome (S-2) and phagolysosome (S-3). There was a good correlation between the proliferation of bacteria *in vivo* and *in vitro*. *Y. pestis* EV  $\Delta$ *lpxM* demonstrated a marked decrease in the ability to grow *in vitro* in each medium, i.e. under the S-1, S-2 and S-3 conditions. The coefficient of proliferation for the EV  $\Delta$ *lpxM* mutant was in the range of 0.61-0.95, whereas for the parental EV strain it varied depending on the condition used, decreasing to 0.86 in both S-1 and S-2 but increasing to 1.59 in S-3. These data suggest that LpxM play an important role in both plague pathogenesis and immunogenesis. The EV  $\Delta$ *lpxM* mutant might be a safer vaccine candidate than the parental strain EV.



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### ABSTRACT - POSTER 11

#### *Desulfovibrio desulfuricans* PglB: the most “eukaryotic” of the bacterial *N*-oligosaccharyltransferases

MARIA VERONICA IELMINI<sup>1</sup> and MARIO FELDMAN<sup>1</sup>

<sup>1</sup> Alberta Ingenuity Centre for Carbohydrate Science, Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.

Protein glycosylation is a widely distributed process among bacteria. Many different species are able to produce *O*- or *N*-glycoproteins by transferring a glycan previously assembled onto the lipid undecaprenylpyrophosphate. *N*-glycosylation has been most studied in *Campylobacter jejuni*, where the PglB oligosaccharyltransferase glycosylates more than 40 proteins.

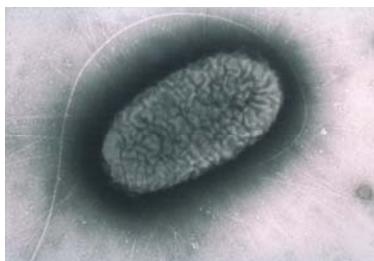
This enzyme can be functionally expressed in *Escherichia coli* and its ability to transfer a variety of polysaccharides to protein carriers *in vivo* has been demonstrated. PglB is homologous to Stt3, the main component of the eukaryotic OTase complex. Fully folded proteins displaying the D/E-X-N-Z-S/T sequence (where X and Z are any amino acid except proline) in a flexible loop in the periplasm can be recognized and glycosylated by *C. jejuni* PglB.

PglB homologous enzymes are also found in Archaea and in many  $\epsilon$ -proteobacteria, including pathogenic and environmental species. Outside the  $\epsilon$ -proteobacteria, PglB homologues have been only identified in the *Desulfovibrio* genus, which belongs to the  $\delta$ -proteobacteria. *Desulfovibrio* species are found in diverse environment, including soil, animal digestive tract, and sea and fresh water. At least one *N*-glycoprotein, the 16 heme cytochrome (HmcA) has been identified in *D. gigas*.

In this study we revised the DNA region adjacent to PglB in *D. desulfuricans* and found PglB is likely contained within an *N*-glycosylation locus. A phylogenetic analysis of the proposed OTase catalytic site showed this enzyme is evolutionarily closer to its eukaryotic counterparts than the *Campylobacter* enzymes. We functionally expressed *D. desulfuricans* PglB in *E. coli* and found that it can transfer mono- and oligosaccharides but not polysaccharides. Furthermore, using molecular biology techniques and mass spectrometry, we demonstrate that the *D. desulfuricans* PglB does not recognize the same acceptor consensus sequence than *C. jejuni* PglB.

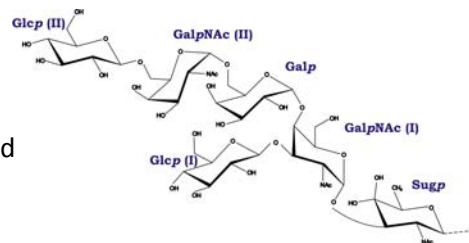
This results together with its evolutionarily proximity to the eukaryotic counterparts suggest that these two related enzymes may differ in their mechanism of action.





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### ABSTRACT - POSTER 12

#### Characterization of the anti-ECA antibodies in rabbit antiserum against *Yersinia enterocolitica* O:3 Rc mutant bacteria

KAMILA RABSZTYN<sup>1,2</sup>, KATARZYNA KASPERKIEWICZ<sup>1</sup>, CHUN-MEI LI<sup>2</sup>, MAGDALENA ŁUKASIK<sup>1</sup>,  
JOANNA RADZIEJEWSKA-LEBRECHT<sup>1</sup>, MIKAEL SKURNIK<sup>2</sup>

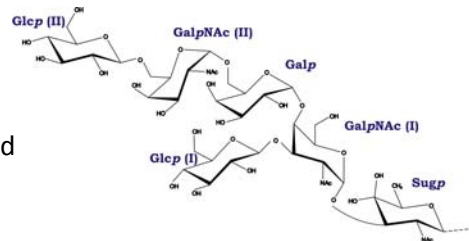
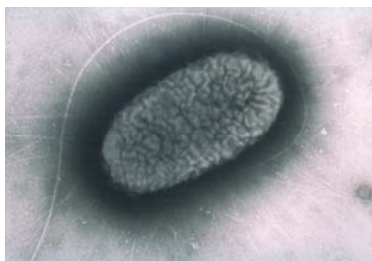
<sup>1</sup>Department of Microbiology, University of Silesia, Katowice, Poland

<sup>2</sup>Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland

Enterobacterial Common Antigen (ECA) is a major surface component of the outer membrane (OM) in bacteria belonging to *Enterobacteriaceae* family. ECA, a polymer built of trisaccharide repeating units, is anchored to the outer membrane either *via* its own glycerophospholipid (ECA<sub>PG</sub>) or *via* lipid A of lipopolysaccharide (ECA<sub>LPS</sub>). It was suggested that biosynthesis of ECA in *Yersinia enterocolitica* O:3 (YeO3) is temperature dependent. We assumed that studies using monovalent antisera against R mutants of YeO3 enriched with antibodies specific for ECA would elucidate the influence of the cultivation temperature on ECA biosynthesis and expression. The aim of this study was to remove anti-LPS antibodies from the polyvalent antiserum against YeO3-c-trs8-R by absorption with ECA-negative mutant bacteria. This would result in “monovalent” antiserum enriched with anti-ECA antibodies. First the ECA-negative mutant YeO3-c-OCR-ECA was constructed by allelic exchange. We then used both boiled and live ECA-negative bacteria for absorption.

In the first approach the ECA-mutant bacteria were boiled for 2.5 hr (the antigen used in immunization was prepared similarly) and then used for extensive absorptions. Absorbed and nonabsorbed antisera were analyzed by immunoblotting using as antigens ECA<sub>PG</sub> standard preparation from *S. montevideo* SH94 and LPS/PCP preparations from *Y. enterocolitica* strains Ye75S, Ye75R and YeO3-c-trs8-R. Immunoblotting using ECA<sub>PG</sub> preparation as antigen revealed a very strong immunostaining with a characteristic ladder like banding profile for nonabsorbed antiserum and very faint for absorbed antiserum. Immunoblotting using LPS/PCP preparations as antigens showed very strong immunostaining in the high and in the low molecular mass regions with absorbed antiserum. The results suggested that the used absorption method did not remove effectively all anti-LPS antibodies and also decreased the titer of ECA-specific antibodies.

When using live bacteria for absorptions we could see more efficient removal of anti-LPS antibodies. In addition to purified LPS of YeO3-c-trs8-R we used whole cell lysates from both YeO3-c-trs8-R and YeO3-c-OCR-ECA as antigens in immunoblotting. The nonabsorbed antiserum reacted strongly with both lysates and purified LPS while the absorbed serum showed weaker reaction and only with the YeO3-c-trs8-R LPS and lysate. No reaction with the ECA-negative lysate was observed. These results suggested that most anti-LPS antibodies were removed from the polyvalent antiserum against YeO3-c-trs8-R during the absorption.



## ABSTRACT - POSTER 13

### What useful information can a carbohydrate chemist gain from using optical rotation?

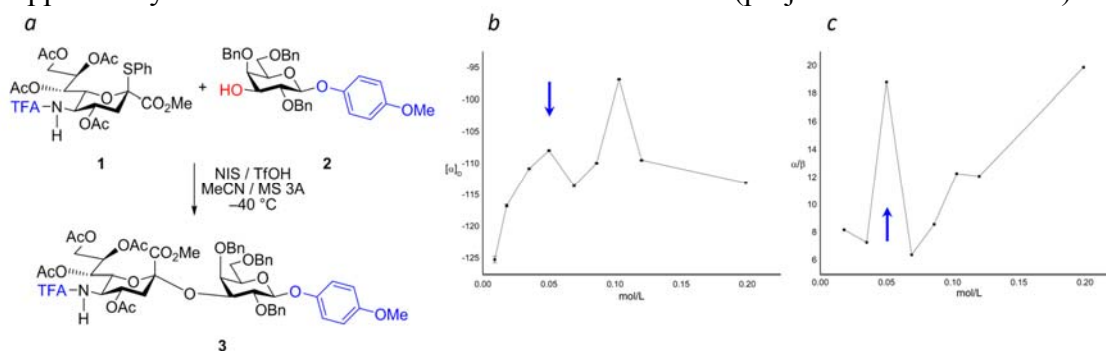
LEONID O. KONONOV<sup>1</sup>, ANNA V. ORLOVA<sup>1</sup>, NELLY N. MALYSHEVA<sup>1</sup>, TATIANA V. LAPTINSKAYA<sup>2</sup>

<sup>1</sup> N.D. Zelinsky Institute of Organic Chemistry of RAS, Leninsky prospect, 47, 119991, Moscow, Russian Federation

<sup>2</sup> Faculty of Physics, M.V. Lomonosov Moscow State University, Moscow, Russian Federation

E-mail: kononov@ioc.ac.ru, leonid.kononov@gmail.com

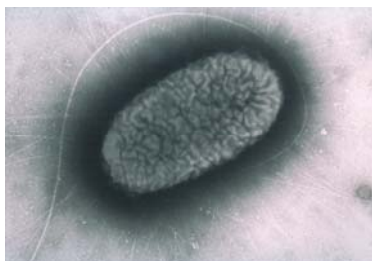
In this communication we wish to attract attention of carbohydrate audience to a novel application of polarimetry. Even subtle conformational changes are known to induce dramatic changes in specific rotation values [1]. If these changes are concentration dependent, they are apparently related to aggregation of solute molecules [2], which leads to the formation of supramers [3] comprising molecules differing in conformations or mode of their supramolecular arrangement [4]. The supramers, which have different structure or composition and hence specific rotation, are expected [3] to react differently. Thus, by studying concentration dependence of optical rotation of reagents one may find concentration areas, in which anomaly in chemical reactivity could be expected [3]. We performed a proof-of-principle study of concentration dependence of optical rotation of components of a model sialylation reaction (Fig. 1a) and their mixtures. Non-linear effects found by polarimetry (e.g., Fig. 1b) are well correlated with stereochemical outcome of the sialylation, which experiences dramatic changes upon small changes in concentration, ranging from moderate ( $\alpha : \beta = \sim 7:1$ ) to very high values ( $\alpha : \beta = \sim 20:1$ ) (Fig. 1c). The presence of homo- (**1**), (**2**) and hetero-supramers (**1 + 2**) in the solutions studied and their transformations upon concentration changes were confirmed by static and dynamic light scattering. This work was supported by the Russian Foundation for Basic Research (project No. 08-03-00839).



**Fig. 1.** (a) Model sialylation reaction; (b) specific optical rotation ( $[\alpha]_D$ ) of sialyl donor **1** in MeCN; (c) anomeric ratio ( $\alpha/\beta$ ) of sialylation product **3** obtained at different concentrations. The arrow is at 50 mM concentration, which is lower than the region of concentrations (above 69 mM) where mixed supramers **1 + 2** are formed, which would react differently than homo-supramers **1**.

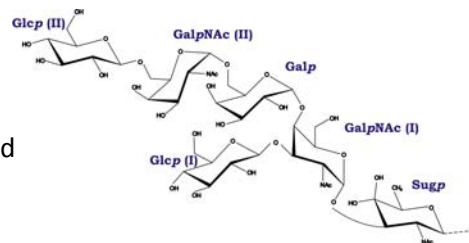
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## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT - POSTER 14

#### Studies on the core region from *Bradyrhizobium elkanii* lipopolysaccharide

IWONA KOMANIECKA<sup>1</sup>, ADAM CHOMA<sup>1</sup>, KATARZYNA ANNA DUDA<sup>2</sup>, ANNA TURSKA-SZEWCZUK<sup>1</sup>,  
BUKO LINDNER<sup>3</sup>, OTTO HOLST<sup>2</sup>

<sup>1</sup> Department of Genetics and Microbiology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin (Poland)

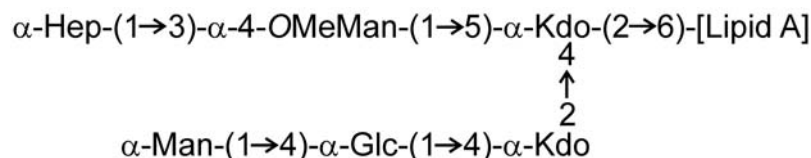
Divisions of: <sup>2</sup> Structural Biochemistry and <sup>3</sup> Immunochemistry, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, 23845 Borstel (Germany)

*Bradyrhizobium elkanii* is a member of the group of slow-growing rhizobia. These bacteria are able to induce the development of nitrogen-fixing nodules in the roots of *Glycine max* and also other wild-growing legumes. Lipopolysaccharide (LPS), as an integral component of the Gram-negative cell wall, plays an essential role in the symbiosis progression. This lipoglycan is usually composed of three domains: lipid A (a hydrophobic part that anchors the LPS molecule in outer membrane), core oligosaccharide and O-specific polysaccharide (O-antigen).

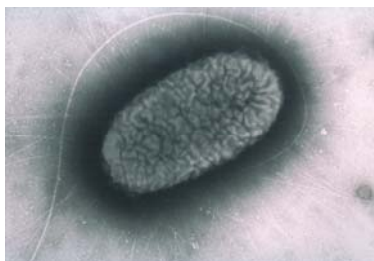
The core oligosaccharide was isolated from *B. elkanii* LPS by mild acid hydrolysis and fractionation by gel-permeation chromatography on Sephadex G50. The oligosaccharide-containing fraction was further separated by HPAEC (Dionex) under alkaline conditions. Isolated fragments of the core were characterized by 1D and 2D NMR spectroscopy, chemical composition analyses (including methylation analysis), as well as high-resolution mass spectrometry (ESI FT-ICR MS).

It was shown that the *B. elkanii* LPS core fraction was composed of methylated mannose (4-OMeMan), mannose (Man), glucose (Glc), D-glycero-D-manno-heptose (Hep) and Kdo residues. These components form two different oligosaccharides. The structure of the trisaccharide unit was defined as  $\alpha$ -Manp-(1→4)- $\alpha$ -Glc-(1→4)- $\alpha$ -Kdof. The second oligosaccharide was composed of 4-OMeMan  $\alpha$ -(1→5)-linked to Kdo, and this disaccharide was partially substituted with a heptose linked to 4-OMeMan *via* a (1→3) glycosidic bond.

We propose the structure of the *B. elkanii* LPS core region as

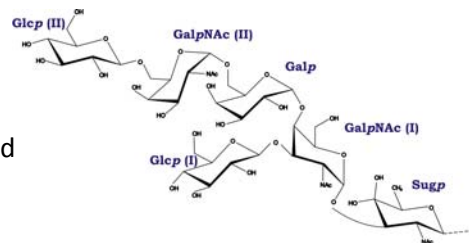


This work was supported by the Polish Ministry of Science and Higher Education (grant no. 303 109 32/3593).



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT - POSTER 15

#### Structural Studies of *Vibrio parahaemolyticus* AN-1600 lipopolysaccharide by NMR spectroscopy.

CAROLINA FONTANA<sup>1</sup>, MONA SVENSSON<sup>1</sup>, ANDREJ WEINTRAUB<sup>2</sup>, GÖRAN WIDMALM<sup>1</sup>

<sup>1</sup> Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden

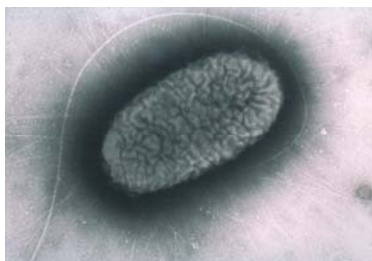
<sup>2</sup> Karolinska Institute, Department of Laboratory Medicine, Division of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden

*Vibrio parahaemolyticus* is a marine halophilic Gram-negative bacterium responsible for gastrointestinal poisoning in humans. This worldwide pathogen is primarily associated with the consumption of raw or inadequately cooked sea products, and is especially prevalent in Asian countries. Based on the serological specificities of its heat-stable somatic antigen it is presently divided into 11 or 13 O-serotypes. At the same time, based on the sugar composition of its lipopolysaccharides, it is also divided into 10 chemotypes.

In the present study the structure of the carbohydrate backbone of the lipopolysaccharide of *Vibrio parahaemolyticus* AN-1600 has been elucidated using nuclear magnetic resonance spectroscopy. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of the lipopolysaccharide revealed a glycerol residue and five monosaccharides. From these spectra we could also determine the presence of three N-Acetyl groups and two methyl groups belonging to 6-deoxysugars. The anomeric configurations of the glycosyl linkages were determined from the vicinal <sup>3</sup>J<sub>H,H</sub> coupling constants, and the one-bond <sup>1</sup>J<sub>C,H</sub> coupling constants obtained from the non-decoupled HSQC spectrum.

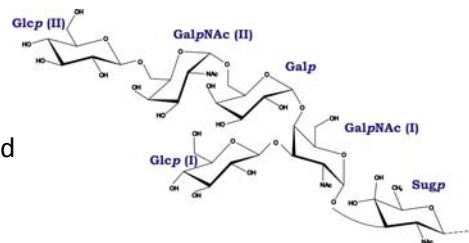
The sequence of sugar residues was elucidated by HMBC and NOESY experiments. The <sup>31</sup>P spectrum of the lipopolysaccharide showed a single peak at 1.35 ppm, which is in agreement with the chemical shift of a phosphodiester moiety. The proton-phosphorus connectivities over two and three bonds were determined by <sup>1</sup>H,<sup>31</sup>P-hetero-TOCSY experiments.

The results revealed a teichoic acid-like polysaccharide that consists of a penta-hexopyranose repeating unit. This repeating unit contains three aminosugars and consists of a lateral chain of two residues.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT - POSTER 16

#### Structural elucidation of the capsular polysaccharide repeating unit from *Leuconostoc mesenteroides* subsp. *cremoris* PIA2

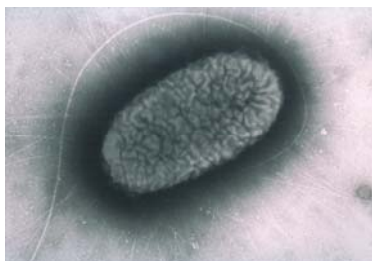
MONA V SVENSSON<sup>1</sup>, XUE ZHANG<sup>2</sup>, EINE HUTTUNEN<sup>2</sup> AND GÖRAN WIDMALM<sup>1</sup>

<sup>1</sup> Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, 106 91 Stockholm, Sweden

<sup>2</sup> Center of Agro-food Technology, Northeast Agricultural Research Center of China, 130124 Changchun, Jilin, People's Republic of China

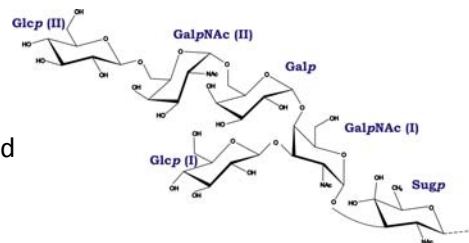
<sup>3</sup> Department of Food Technology, University of Helsinki, 00014 Helsinki, Finland

The traditional Finnish fermented milk viili is sour milk with a thick and slightly ropy consistency and mild acid and aromatic taste. It is inoculated with a culture of *Lactococcus* and *Leuconostoc* strains and on the surface of the product there is the mould *Geotricum candidum* which contributes to flavour formation and consumes lactate, thus lowering the acidity of the product. It is known that the production of exopolysaccharides (EPS) of the lactic acid bacteria has an effect on the consistency of fermented dairy products and the physical properties are related to the structure of the EPS. In this study transmission electron microscopy was used to show the strain *Leuconostoc mesenteroides* subsp. *cremoris* PIA2 (Valio Ltd., Finland) to produce capsular EPS (CPS). The strain PIA2 is one of potential probiotic strains and it was recently shown to have immunomodulatory effects. The primary molecular structure of the CPS was elucidated by NMR spectroscopy showing pentasaccharide repeating units constituted of one pyranose and four furanose galactosyl residues. Sequence information was obtained by two-dimensional <sup>1</sup>H,<sup>1</sup>H-NOESY and <sup>1</sup>H,<sup>13</sup>C-HMBC NMR experiments.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 17

#### Structure of the O-specific polysaccharides from some new species of the family *Enterobacteriaceae*

EVELINA L. ZDOROVENKO<sup>1</sup>, OLGA A. VALUEVA<sup>1</sup>, LYUDMILA D. VARBANETS<sup>1</sup>, VADIM V. KACHALA<sup>1</sup>,  
ALEXANDER S. SHASHKOV<sup>1</sup>, YURIY A. KNIREL<sup>1</sup>

<sup>1</sup> N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia

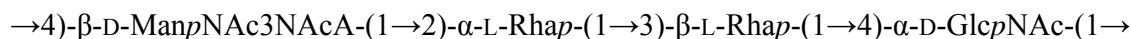
<sup>2</sup> D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kiev, Ukraine

O-specific polysaccharides (OPSs) of *Pragia fontium* and *Budvicia aquatica*, two new species in the family *Enterobacteriaceae*, were investigated. The OPSs were isolated by mild acid hydrolysis of the lipopolysaccharides and studied by sugar analysis, including determination of the absolute configurations of the monosaccharides, methylation analysis, Smith degradation and shift-correlated two-dimensional <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy.

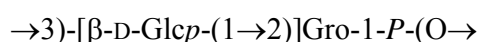
The OPSs of two *P. fontium* strains studied possess unique structures among known bacterial polysaccharide structures and show no similarity to each other. The OPS of *P. fontium* 97U116 is built up of linear pentasaccharide O-units, containing one D-galactofuranose residue, two residues of L-rhamnopyranose, one of which is O-acetylated, and two D-GlcNAc residues:



The OPS of *P. fontium* 2740 was found to have a linear tetrasaccharide O-unit, containing two residues of L-rhamnopyranose and one residue each of D-GlcNAc and 2,3-diacetamido-2,3-dideoxy-D-manuronic acid (D-ManNAc3NAcA):



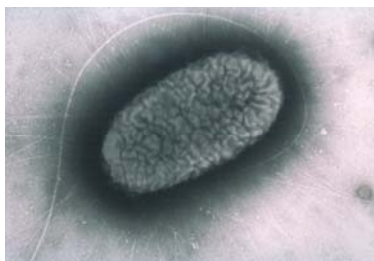
The OPS of *B. aquatica* 97U124 possesses a poly(glycerol phosphate) main chain decorated with D-glucose residues:



This and similar structures are characteristic of teichoic acids of Gram-positive bacteria but occur rarely in Gram-negative bacteria.

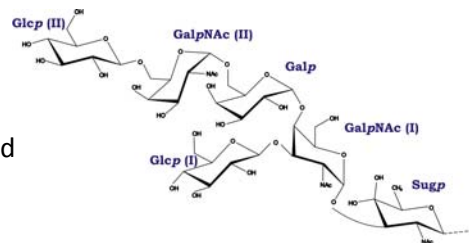
The structural data of the OPSs from the new enterobacterial species correlate well with data of serological investigations of the lipopolysaccharides with polyclonal antibodies against heat-killed cells (O-antisera).

This work was supported by the Russian Foundation for Basic Research (Project 09-04-90446) and the State Foundation for Basic Research of Ukraine (Project F28/223-2009).



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 18

#### The structure of the O-specific polysaccharide from the lipopolysaccharide of *Pectobacterium atrosepticum* SCRI1039

ZBIGNIEW KACZYŃSKI<sup>1</sup>, MAŁGORZATA CZERWICKA<sup>1</sup>, ANNA BYCHOWSKA<sup>1</sup>, HALINA  
DZIADZIUSZKO<sup>2</sup>, PIOTR STEPNOWSKI<sup>1</sup>

<sup>1</sup>Faculty of Chemistry, University of Gdańsk, Sobieskiego 18/19, 80-952 Gdańsk, Poland

<sup>2</sup>Department of Molecular Microbiology and Serology, National Salmonella Centre, Medical University of Gdańsk,  
Do Studzienki 38, 80-227 Gdańsk, Poland

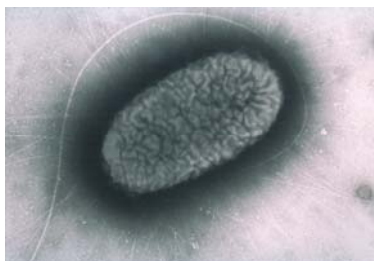
*Pectobacterium atrosepticum* is a Gram-negative enterobacterial pathogen. Previously it was known as *Erwinia carotovora* subsp. *atroseptica*. Several species of *Erwinia* were reclassified to the genus *Pectobacterium* at the end of the last century. Three of them (*P. atrosepticum*, *P. betavascularum* and *P. wasabiae*) were elevated to the genus level in 2003 [1]. Bacteria *Pectobacterium atrosepticum* cause soft rot and black leg effects in vegetables especially in potatoes.

Lipopolysaccharide (LPS), the major constituent of the cell surface of Gram-negative bacteria, acts as activator of the immune response. In general, complete (S-form) LPS consist of three regions: the O-specific polysaccharide (OPS), the core region and the lipid A, which in endotoxic active LPS represents the toxic moiety. For diagnostic and serological classification a structural determination of key parts of the LPS is required.

The structure of OPS isolated from LPS of *Pectobacterium atrosepticum* SCRI 1039 strain (serotype I) was investigated. Bacterial cells were lyophilized and extracted utilizing the hot phenol/water method. LPS was precipitated after removal of nucleic acids. The carbohydrate portion obtained by mild acid hydrolysis of the LPS was fractionated by gel-permeation chromatography and polymeric fraction was used to further structural analysis.

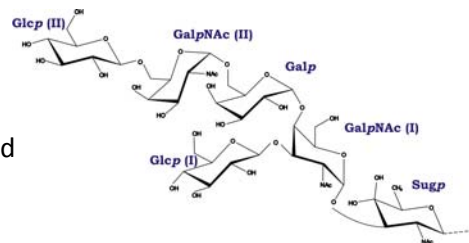
The compositional analyses (sugar analysis, methylation analysis, and absolute configuration assignment) revealed presence of D-GlcNAc, D-Glc, L-Rha, and L-Fuc in the repeating unit. The complete structure of OPS was done using one-dimensional (<sup>1</sup>H and <sup>13</sup>C), two-dimensional homonuclear (<sup>1</sup>H, <sup>1</sup>H COSY, TOCSY, and ROESY), as well as two-dimensional heteronuclear (<sup>1</sup>H, <sup>13</sup>C HMQC, HMQC-TOCSY and HMBC) NMR spectroscopy.

[1] Gardan L.; Gouy C.; Christen R.; Samson R., *Int. J. Syst. Evol. Microbiol.* **2003**, 53, 381-391.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT - **POSTER 19**

#### A new LC-MS Method for the Analysis of *O*-deacylated Lipopolysaccharides

BRIGITTE TWELKMEYER<sup>1</sup>, JACEK STUPAK<sup>2</sup>, ELKE K.H. SCHWEDA<sup>1</sup> JIANJUN LI<sup>2</sup>

<sup>1</sup> Karolinska Institutet, Novum, Huddinge, S-141-86, Sweden

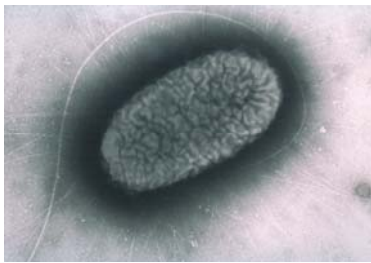
<sup>2</sup> Institute for Biological Sciences, National Research Council of Canada, Ottawa, K1A 0R6, Canada

Lipopolysaccharide (LPS) is the major outer-membrane component of gram-negative bacteria and plays an important role in their pathogenic and commensal behavior. Nontypeable *Haemophilus influenzae* (NTHi) is a major human pathogen causing otitis media and respiratory tract infections. For elucidating the role of its LPS and modifications thereof, structural analysis of LPS commonly implies either mild acid hydrolysis or mild *O*-deacylation using hydrazine.

Here we present a new liquid chromatography electrospray ionisation mass spectrometry (LC-MS) method for the analysis of *O*-deacylated lipopolysaccharide (LPS-OH) using LPS-OH derived from NTHi strain 1003 [1]. The choice of LC-MS provided the advantages of a robust instrument combination, high sensitivity and higher prevalence in many other laboratories than the established capillary electrophoresis MS. We demonstrated the potential for structural analysis in both positive and negative mode and applied the LC-MS method with advanced MS experiments as precursor ion scan and tandem MS analysis. Further, the adequacy on other rough type LPS expressed by Gram-negative bacteria from NTHi strain 1200, NTHi strain 375*lic1A*, *Haemophilus parainfluenzae* strain 22, *Pasteurella multocida* strain AL 857 and *Neisseria meningitidis* IL 4*galE* was demonstrated.

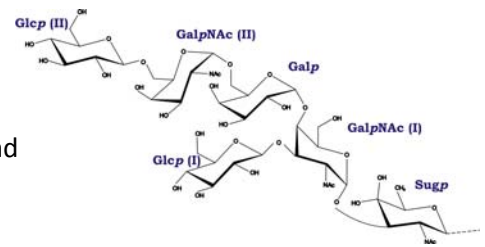
[1] M. Månsson, D.W.Hood, J. Li, J.C. Richards, E.R. Moxon and E.K.H. Schweda, Eur. J. Biochem., 269, 808-818 (2002)





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT - POSTER 20

#### Glycan diversity in *O*-linked protein glycosylation of *Neisseria* species

BENTE BØRUD<sup>1,2</sup>, RAIMONDA VIBURIENE<sup>1,2</sup>, FINN ERIK AAS<sup>1,2</sup>, ÅSHILD VIK<sup>1,2</sup>, WOLFGANG EGGE-JACOBSEN<sup>1,2,3</sup> AND MICHAEL KOOMEY<sup>1,2</sup>

<sup>1</sup> Centre for Molecular Biology and Neuroscience, University of Oslo, 0316 Oslo, Norway

<sup>2</sup> Department of Molecular Biosciences, University of Oslo, 0316 Oslo, Norway

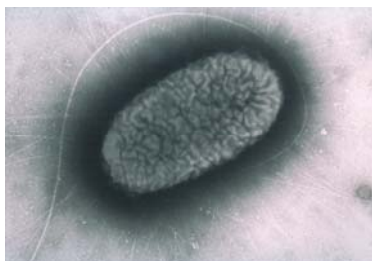
<sup>3</sup> Glyconor Mass Spectrometry & Proteomics Unit, University of Oslo, 0316 Oslo, Norway

Bacterial protein glycosylation systems are frequently being reported in pathogenic and symbiotic species. However, the functions associated with protein glycosylation in bacteria remain poorly understood. *O*-linked protein glycosylation in *Neisseria gonorrhoeae*, the etiologic agent of the human disease gonorrhea, is one of the better understood bacterial systems. We recently showed that in addition to *N. gonorrhoeae* strain N400<sup>1</sup>, one other gonococcal strain as well as isolates of *N. meningitidis* and *N. lactamica* expresses broad spectrum *O*-linked protein glycosylation<sup>2</sup>. All glycoproteins identified in *N. gonorrhoeae* strain N400 are predicted to be lipoproteins or transmembrane proteins localized in the periplasm or cell surface<sup>2</sup>. In order to characterize the level of protein glycan diversity in *Neisseria* species as well as to elucidate its genetic basis, we manipulate protein glycosylation (*pgl*) gene content and assess glycan structure by mass spectrometry and reactivity with monoclonal antibodies. We demonstrate that minimally nine distinct glycoforms can be expressed by the on-off (phase variable) expression of glycosyltransferases and *O*-acetylases<sup>2</sup>. Interestingly, we have identified a novel glycosyltransferase responsible for yet additional glycoforms. Together, our findings document a remarkably high degree of protein glycan diversification at both the interstrain and interspecies levels that may be driven by the adaptive immune response of the host.

#### References

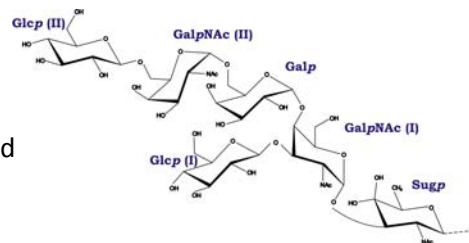
1. Å..Vik et al., *PNAS*, 2009, **106(11)**:4447-52
2. B. Børud et al., *J Bacteriol*, 2010, **192(11)**:2816-29





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT - POSTER 21

#### Characteristics of Lipopolysaccharides of *pssB-pssA* and *rosR* Mutants of *Rhizobium leguminosarum* bv. *trifolii*

TERESA URBANIK-SYPNIEWSKA, MONIKA JANCZAREK, JOLANTA KUTKOWSKA  
and MAŁGORZATA GEÇA

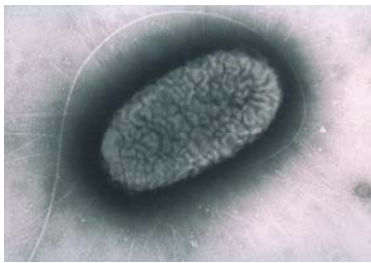
Department of Genetics and Microbiology, Maria-Curie Skłodowska University, Akademicka 19, 20-033 Lublin, Poland

*Rhizobium leguminosarum* bv. *trifolii* is a symbiotic nitrogen-fixing bacterium that elicits nodules on the roots of host plant *Trifolium* spp. For the emergence of an indeterminate type of nodule a proper quantities and structural features of the surface polysaccharides and lipopolysaccharides are required. In our previous studies mutation localized in the intergenic region *pssB-pssA* of RtTA1 affected exopolysaccharide (EPS) biosynthesis and qualitative alterations of the composition of polysaccharide part of LPS together with its electrophoretic pattern.

Interestingly, very similar path of changes were observed in mutant Rt12A which is inactivated in *pssB* gene encoding inositol monophosphatase. The mutant in the *pssB-pssA* intergenic region lacked 3-N-methyl-3,6-dideoxyhexose, L-fucose, and 2-O-methyl-6-deoxyhexose, the sugars constituting the wild type O chain. Instead, two other 6-deoxyhexoses, i.e., 6-deoxy-L-talose and L-rhamnose were found. The oligosaccharides obtained by mild acid hydrolysis of the degraded polysaccharides were studied by methylation analyses, <sup>1</sup>H NMR and MALDI-ToF-mass spectroscopy. The oligosaccharides constituting the O-antigen chain of the mutant in the intergenic region were composed mainly of D-galacturonic acid, L-rhamnose, and 6-deoxy-L-talose, whereas that of RtTA1 contained D-galacturonic acid, L-fucose and 3-N-methyl-3,6-dideoxyhexose. A complex signal pattern in terms of peak intensities in the anomeric region ( $\delta$  4.7-5.6) indicated complex O-acetylation of sugar residues of O-polysaccharides from the mutant and the wild strain.

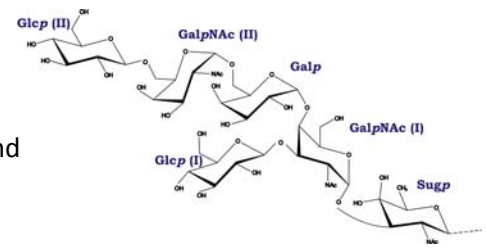
The mutants of wild strain Rt24.2 carrying the insertions in the *rosR* regulon encoding RosR protein - a general transcription regulator exhibited the pleiotropic phenotypes. They produced less EPS and elicited the formation of nodules that were unable to fix nitrogen. Additionally, the mutants produced cell-surface bound glucose-rich capsular polysaccharide extractable into water by the hot water-phenol method. These changes influenced the surface properties of the cells observed by adhesion to hexadecane. Mutation in *rosR* also resulted in qualitative alterations in polysaccharide part and electrophoretic mobility of LPS. The changes influenced the sugars constituting the O-polysaccharide and the linker part (3-deoxy-*lyxo*-2-heptulosaric acid, 6-deoxy-L-talose and L-rhamnose, and 2-N-acetamido-2,6-dideoxyglucose) of Rt24.2 lipopolysaccharide. Thus the novel regulatory pathway affecting mainly the biosynthesis of 6-deoxysugars could be influenced by the products of genes controlling the exopolysaccharide synthesis.

This report was supported by the Polish Ministry of Science and Higher Education (grant no. N303092234).



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 22

#### *Yersinia pseudotuberculosis* O:9 lipopolysaccharide reactivity with phage $\phi$ R1-37 and anti-YeO3-c-R1 antiserum

AGNIESZKA BECZAŁA<sup>1,2</sup>, JOANNA RADZIEJEWSKA-LEBRECHT<sup>2</sup>, OTTO HOLST<sup>1</sup>, MIKAEL SKURNIK<sup>3</sup>

<sup>1</sup> Division of Structural Biochemistry, Research Center Borstel, Borstel, Germany,

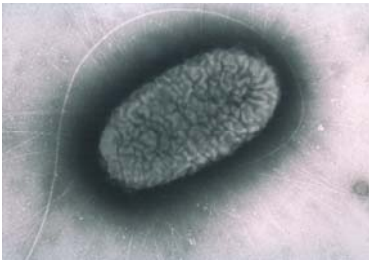
<sup>2</sup> Department of Microbiology, University of Silesia, Katowice, Poland,

<sup>3</sup> The Haartman Institute, University of Helsinki, Helsinki, Finland.

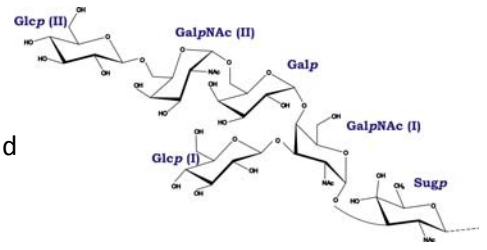
A rough mutant of *Yersinia enterocolitica* O:3 (YeO3-c-R1) serves as bacteriophage  $\phi$ R1-37 host strain with the receptor localized in the lipopolysaccharide (LPS) outer core which is furnished by Galp, GalpNac and 2-acetamido-2,6-dideoxy-D-xylo-hex-4-ulopyranose [1,2,3]. Phage  $\phi$ R1-37 also infects smooth, but not rough serotype O:9 strains of *Yersinia pseudotuberculosis* (*Y. pstb* O:9) suggesting the presence of a common structure in the LPSs of YeO3-c-R1 and *Y. pstb* O:9 [4]. The aim of the present studies was to elucidate the structure of the  $\phi$ R1-37 receptor in the O-specific polysaccharide (OPS) of *Y. pstb*. O:9 LPS [4]. The *Y. pstb*. O:9 OPS is composed of GlcpNacA3OAc, FucpAm, GlcpNac and Galp, thus Galp is the only common residue shared with the YeO3-c-R1 outer core. Phage inhibition test with *Y. pstb*. O:9 LPS deprived of Galp established that this sugar was required for phage recognition. In immunoblotting anti-YeO3-c-R1 antiserum reacted with *Y. pstb*. O:9 LPS indicating the existence of a common epitope(s) between the LPSs of these two strains. The epitopes were present in the LPS of low and medium molecular mass. There was also an evident difference between the reactivity of *Y. pstb*. O:9 LPS isolated from the water and phenol phases, with the latter reacting more intensively in immunoblotting. Experiments on the *Y. pstb*. O:9 LPS reactivity with the monoclonal antibody (mAb) 2B5 that is specific for the YeO3-c-R1 outer core gave negative results.

As Galp was the only identical sugar residue of both, *Y. pstb*. O:9 OPS and YeO3-c-R1 outer core region, the structure acting as the  $\phi$ R1-37 receptor could not be proposed by a simple comparison of the chemical composition. We therefore suggest that the outer core region and OPS share common conformational properties and that in *Y. pstb*. O:9 the Galp residue seems to be part of the phage receptor. The cross-reactivity of the anti-YeO3-c-R1 antiserum with *Y. pstb*. O:9 LPS indicates the presence of a common epitopes. The stronger reactivity of the LPS derived from the phenol phase could be due to higher content of hydrophobic molecules, *i.e.* rough type LPS or LPS with shorter OPS, where the epitopes would be more accessible to antibodies. Obviously, the epitopes recognized by the antiserum were different from the mAb 2B5. 2B5 is specific for YeO3-c-R1 outer core region and requires the presence of both Glcp I and Glcp II [2].

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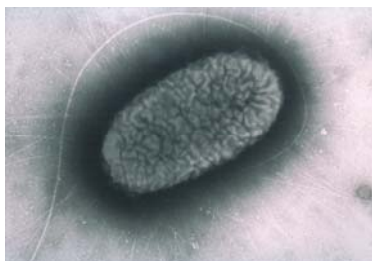
**4<sup>th</sup> Baltic meeting on  
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Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



# POSTER SESSION 2

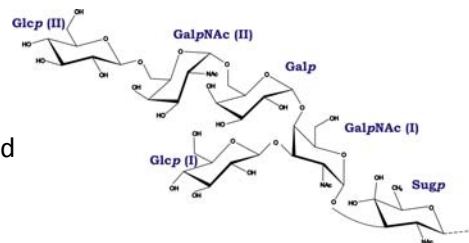
## ABSTRACTS 23-44

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## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 23

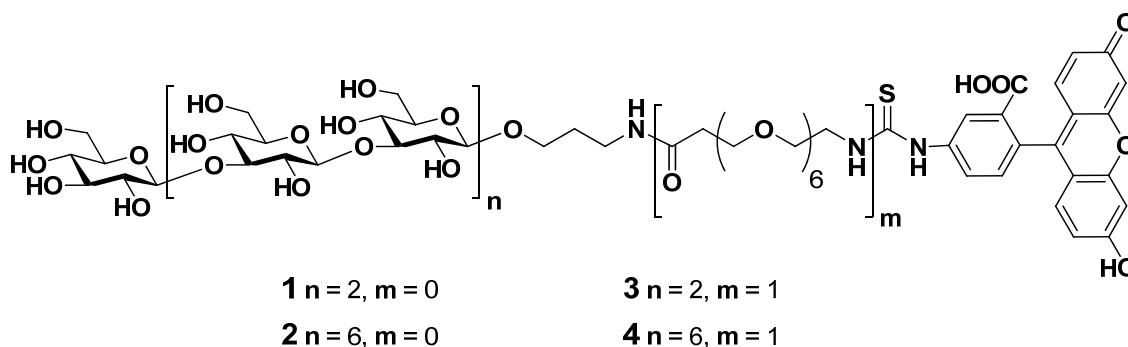
#### Binding of fluorescein labeled synthetic $\beta(1\rightarrow3)$ -glucans to human dendritic cells

MARINA L. GENING,<sup>1</sup> NATALIA YU. ANISIMOVA,<sup>2</sup> DMITRY V. YASHUNSKY,<sup>1</sup> YURY E. TSVETKOV,<sup>1</sup>  
MIKHAIL V. KISELEVSKY,<sup>2</sup> NIKOLAY E. NIFANTIEV<sup>1</sup>

<sup>1</sup> N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky prospect 47, 119991 Moscow, Russia

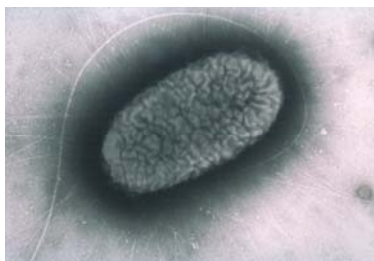
<sup>2</sup> N.N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences., Kashirskoe shosse 24, 115478 Moscow, Russia

$\beta$ -Glucans, especially  $\beta(1\rightarrow3)$ -glucans, are the main components of fungal cell walls. Dectin-1, the major receptor for  $\beta$ -glucans, is a C-type lectin that is highly expressed on dendritic cells (DCs). Binding of  $\beta$ -glucans to Dectin-1 is considered to be a key step in fungus recognition by host immune system. To date there is no information about precise structure of lectin binding domain of this receptor and the structure of  $\beta$ -glucan involved in this interaction. To reveal the effect of the length of glucan fragment on the efficiency of  $\beta$ -glucan-Dectin-1 interaction fluorescein labeled glucans were synthesized. Penta- and trideca- $\beta(1\rightarrow3)$ -glucans were conjugated with FITC either directly (compounds **1** and **2**, respectively) or through a bifunctional hexaethyleneglycol linker (compounds **3** and **4**).

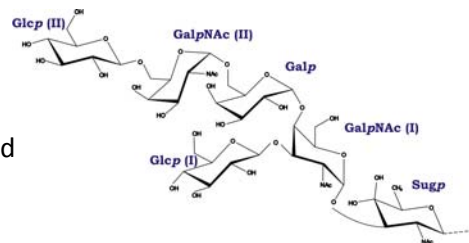


Binding of labeled probes **1–4** with DCs isolated from a serum of healthy human individual was studied by flow cytometry and electronic microscopy. According to cytometrical data conjugates **1** and **2** had a strong tendency to self-aggregation in solution and therefore no reliable results could be obtained using these probes. In contrast, probes **3** and **4** incorporating a flexible hydrophilic linker were much less prone to aggregation and are applicable for such experiments in combination with FITC-labeled polyethyleneglycol derivative (Peg-FITC) as a control of non-specific binding. It was found that both penta- and tridecasaccharide conjugates (**3** and **4**) but not Peg-FITC can stain DCs but quantitative data indicated that pentasaccharide probe **3** binds more efficiently than tridecasaccharide **4**.

Supported by RFBR grant 09-03-12240-ofi-m.



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**ABSTRACT - POSTER 24**

**Synthesis of new analogs of vancomycin with potential bactericidal properties**

MONIKA SZULC, JUSTYNA SAMASZKO, JANUSZ MADAJ

Chair of Sugar Chemistry, Faculty of Chemistry, University of Gdańsk, Sobieskiego 18/19, PL-80-952 Gdańsk, POLAND. monikas@chem.univ.gda.pl

Vancomycin was the first glycopeptide antibiotic to be discovered and was isolated in the 1950s during the screening of natural products from a soil sample [1]. The mechanism of action of vancomycin is to halt cell-wall biosynthesis of Gram-positive bacteria by binding to the terminal D-Ala-D-Ala sequence of the peptidoglycan cell-wall precursors [2]. In the common strains of vancomycin-resistant enterococci, VanA and VanB, the terminal residues are reprogrammed to the depsipeptide D-Ala-D-Lac [3]. Over the past decade the global emergence of vancomycin-resistant enterococci (VRE) and vancomycin-resistant *Staphylococcus aureus* (VRSA) reveals the need for more potent antibiotics. Consequently, the search for vancomycin analogs with improved activity against VRE and VRSA is an apposite and active area of research [4]. We want to present the results of our study on the synthesis of new analogs of vancomycin with potential bactericidal properties.

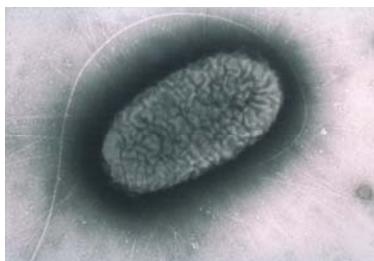
At first we want to define an influence for antibiotic activity amino group of vancosamine through protection of this group of casings, which we often use in peptide chemistry (acetyl, 9-fluorenylmethoxycarbonyl, benzyloxycarbonyl and benzoyl). We also intend to compare their activity and vancomycin. In addition we wish to determine an influence on modification of free carboxyl group of cyclic heptapeptide (modified of peptide fragments) for biological activity of vancomycin, respectively. Additionally we will be planning to define a part of the disaccharide fragment which is present in antibiotic, that is a treatment of last resort in therapeutics against Gram-positive bacteria. Modified this way analogs of vancomycin will be tested on VRSA strains.

**Acknowledgements:** "Publication part-financed by the European Union within the European Regional Development Fund".

**References:**

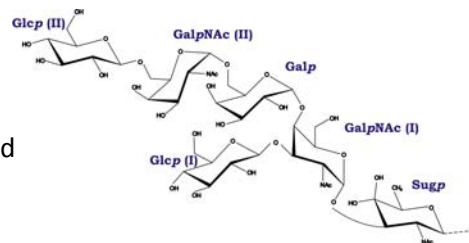
- [1] M.H. McCormick; W.M. Stark; *Antibiot. Annu.* 1956, 3, 606 – 611.
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### ABSTRACT - POSTER 25

#### Chemical synthesis of vancomycin derivatives modified with sugar and peptide fragments

JUSTYNA SAMASZKO, MONIKA SZULC, AGNIESZKA JOWSA, RAFAŁ ŚLUSARZ, JANUSZ MADAJ

Chair of Sugar Chemistry, Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, PL-80-952 Gdańsk, POLAND

New generation of drugs introduced into therapy against *Staphylococcus aureus* and other Gram-positive bacteria were glycopeptide antibiotics. The most widespread and most commonly used antibiotics were vancomycin and teicoplanin. They were discovered respectively in 1956 and 1978. For many years only these antibiotics were effective in the treatment of infections caused by MRSA strains. This opens a new chapter in the development of medicine and the fight against MRSA. For years, no vancomycin-intermediate resistant *S. aureus* (VISA) or vancomycin-resistant *S. aureus* (VRSA) strains were isolated. The first strains carrying full resistance against vancomycin appeared at the turn of the century.

Understanding the mechanisms of glycopeptide antibiotic action took many years. Pioneers in this area were Perkins and Nieto. In 1969 they discovered that mechanism of action of this antibiotic is leaning on selective binding of vancomycin with peptidoglycan precursor fragment UDP-MurNac-L-Ala-D-iGlu-L-Lys-D-Ala-D-Ala. Vancomycin creates a stable complex with the terminal fragment of murein (exactly with C-terminal dipeptide fragment D-Ala-D-Ala). Vancomycin inhibits the biosynthesis of peptidoglycan by accumulation of UDP-muramyl-peptide precursors in the cytoplasm.

The replacement of the last D-Ala residue in precursor of peptidoglycan fragment (UDP-MurNac-L-Ala-D-iGlu-L-Lys-D-Ala-D-Ala) to D-Lac or D-Ser decreases the vancomycin activity about 1000-fold.<sup>1,2,3)</sup> The frequency of resistance to glycopeptide antibiotics has increased significantly over the past decade. Considerable efforts have been made to obtain new semi-synthetic glycopeptides with improved pharmacological properties and activity against resistant strains. The design of new drugs and methods for their study is based on knowledge of the mechanism of action of existing antibiotics.<sup>4)</sup>

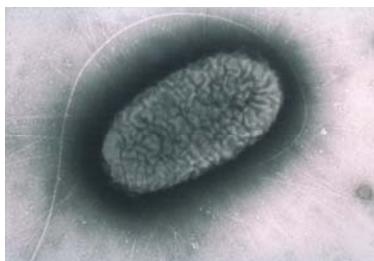
There are already many different types of vancomycin modifications aimed at improving its activity. In our work we try to modify the amino group of fragment heptapeptide of vancomycin by joining its sugar and peptide fragments. The introduction of its unit structure characteristic of the bacteria can make that it will interact strongly with parts necessary for the growth inhibition of peptidoglycan.

**Acknowledgements:** Publication part-financed by the European Union within the European Regional Development Fund

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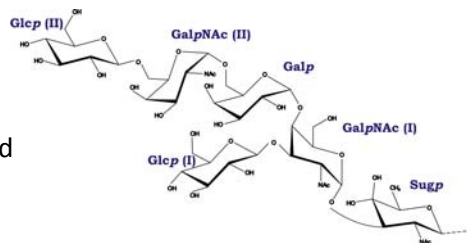
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### ABSTRACT - POSTER 26

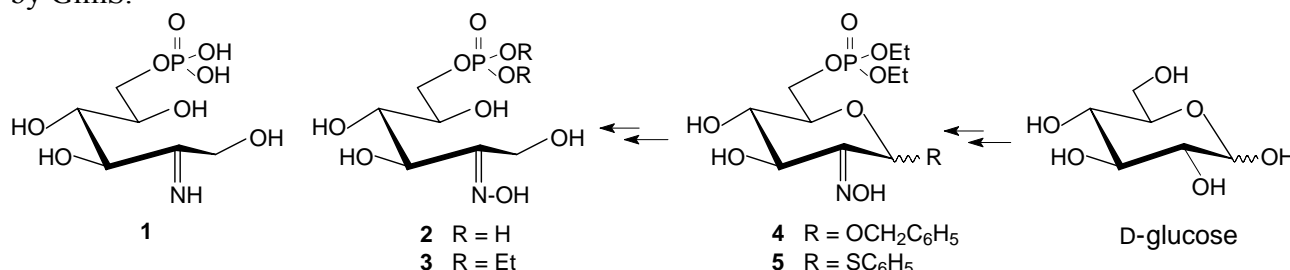
#### In search of new antifungal agents. Synthesis of benzyl and thiophenyl 2-deoxy-6-O-diethylphosphonato-2-hydroxyimino-D-hexopyranosides

MAGDALENA CYMAN, RAFAŁ ŚLUSARZ, BEATA LIBEREK

Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, PL-80-952 Gdańsk, Poland

Emerging challenge of systemic fungal infections, especially in immunocompromised patients, and a limited repertoire of effective antifungals stimulate a search for novel targets and drug candidates. Enzymes involved in biosynthesis of the fungal cell wall components are of a special interest in this respect. One of them is GlcN-6P synthase (GlmS), enzyme catalyzing the first committed step in chitin biosynthesis pathway, that is transformation of D-fructose-6-phosphate (Fru-6P) to D-glucosamine-6-phosphate (GlcN-6P).<sup>1</sup> Another one is phosphomannose isomerase (PMI), that catalyses the reversible isomerization of D-mannose-6-phosphate (Man-6P) and D-fructose-6-phosphate (Fru-6P). PMI is reported to play a crucial role in the biosynthesis of many mannosylated structures, including the cell wall components of fungi.<sup>2</sup> Both enzymes are proposed as the targets for antifungal chemotherapy and a search for their selective inhibitors has been continued.

The reaction performed by GlmS is believed to proceed through the formation of intermediate **1**, a Schiff base created between the keto group of the sugar and the ammonia generated from the glutamine amide function.<sup>3</sup> Mechanism of the reaction catalyzed by PMI is similar to that catalyzed by GlmS.<sup>4</sup>

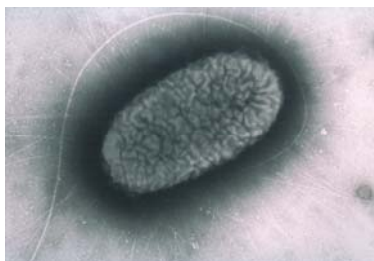


In the course of search of antifungal agents, we plan to synthesize 2-deoxy-2-hydroxyimino-6-O-phosphono-D-glucitol (**2**) and its diethyl ester (**3**), and explore their antifungal activity. Ethyl residues are incorporated into **2** to increase a lipophilicity of the molecule, which is supposed to be advantageous for better penetration of the derivative through the cytoplasmic cell membrane. Probably, diethyl ester **3** will be metabolized to **2** inside a cell.

Here, the first steps of our synthesis are presented. These involve the transformation of D-glucose into benzyl (**4**) and thiophenyl 2-deoxy-6-O-diethylphosphonato-2-hydroxyimino-D-hexopyranosides (**5**).

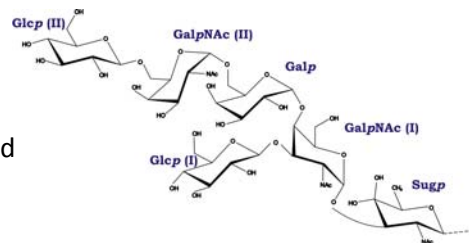
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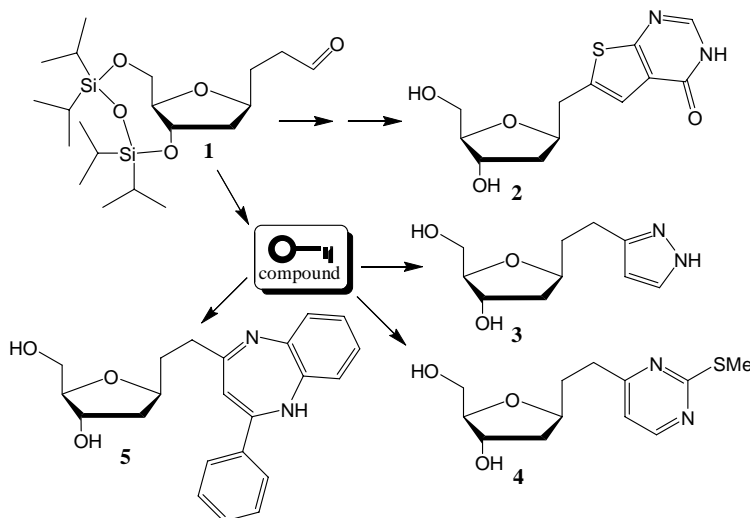
### ABSTRACT - POSTER 27

#### Efficient synthesis of $\beta$ -allyl C-glycosides of D-ribofuranose and 2-deoxy-D-ribofuranose and their use for the preparation of potential antimicrobial agents

CHRISTIAN VOGEL, DILVER PEÑA FUENTES, HEIKE OTERO MARTINEZ

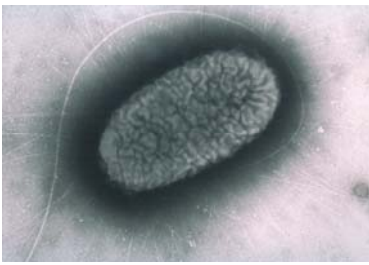
Department of Organic Chemistry, Institute of Chemistry, University of Rostock, Albert-Einstein Str. 3a, D-18059 Rostock, Germany [christian.vogel@uni-rostock.de](mailto:christian.vogel@uni-rostock.de)...

In order to synthesize new spacer-C-nucleoside analogues with potential biological activity a safe route for the synthesis of  $\beta$ -allyl C-glycosides of D-ribo- and 2-deoxy-D-ribofuranose became established.[1] Successful hydroboration-oxidation and stepwise oxidation provided a set of versatile intermediates which allow the synthesis of heterocycles belonging to quite different classes. For example, the tetraisopropylidisiloxan-propanal **1** was treated with 2-cyanoacetamide in the presence of aluminium oxide to yield a pentene acid intermediate. Cyclization with sulphur and triethylamine was performed to provide a thiophene derivative which was then treated with triethyl orthoformate to obtain after deprotection the thienopyrimidine nucleoside **2**.



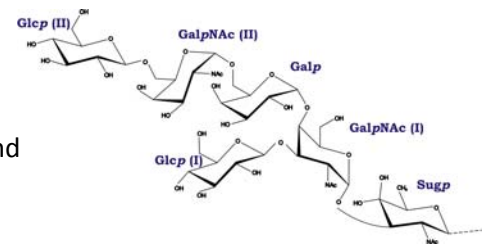
Reaction of propanal **1** with ethynylmagnesium bromide or lithium phenylacetylide in THF followed by oxidation, afforded pentinone intermediates. Treatment of these compounds with hydrazine, *S*-methylthiuronium sulfate and *o*-phenyldiamine provided the corresponding pyrazole, pyrimidine or benzodiazepine derivatives in good to excellent yields. Deprotection was performed by using tetrabutylammonium fluoride to furnish compounds **3**, **4** and **5** suitable for biological and pharmacological investigations.

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### ABSTRACT - POSTER 28

#### Identification and Characterization of a *Helicobacter* Sialyltransferase from GT-42

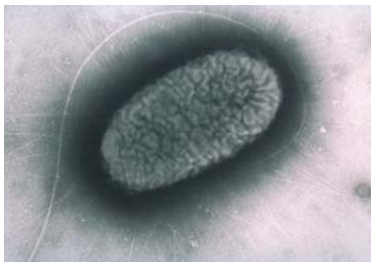
MIRKO ROSSI<sup>1</sup>, BRIGITTE TWELKMEYER<sup>2</sup>, MELISSA J. SCHUR<sup>3</sup>, PRADEEP KONDADI<sup>1</sup>, J.LI<sup>3</sup>, ELKE SCHWEDA<sup>2</sup>, MARJA-LIISA HÄNNINEN<sup>1</sup> AND WARREN WAKARCHUK<sup>3</sup>

<sup>1</sup>Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, P.O. Box 66, Agnes Sjöbergin katu 2, FI-00014 Helsinki, Finland;

<sup>2</sup>Södertörn University & Karolinska Institutet, Clinical Research Center, Huddinge Hospital, NOVUM, S-141 86 Huddinge, Sweden;

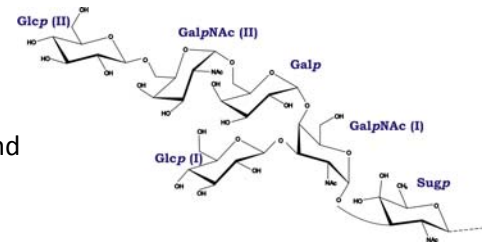
<sup>3</sup>National Research Council of Canada, Institute for Biological Sciences, 100 Sussex Drive, Ottawa, ON, K1A 0R6, Canada.

The canine Gram-negative *Helicobacter bizzozeronii* is one out of five species in the group of “*H. heilmannii*” type 2 that are detected in 0.17-2.3% in gastric biopsies of human patients with gastritis. At the present, *H. bizzozeronii* is the only non-pylori gastric *Helicobacter* sp. isolated from human, representing a good alternative model of human helicobacter disease. In contrast to *H. pylori*, little is known about the structural features and the biological proprieties of *H. bizzozeronii* Lipopolysaccharide (LPS). We used a functional genomics approach to characterize genes to be involved in *H. bizzozeronii* LPS biosynthesis. Starting from the shotgun genome sequences of the type strain and the human strain CIII-1, we identified potential glycosyltransferases using BLAST. One of these candidates, which appears to encode GT-42 family sialyltransferases and co-transcribed with 3 genes involved in the biosynthesis of N-acetylneuraminic acid, was expressed in *Escherichia coli*. This gene (*Helicobacter Bizzozeronii* Sialyltransferase-2, HBS-2) is indeed a functional sialyltransferase different from Cst-II, showing a preference for LacNAc substrates and the ability to use Lewis-X as an acceptor. We sequenced this operon in 7 canine *H. bizzozeronii* strains and the human strain R53 and we found that it is likely phase-variable. The LPS from all the strains were shown to contain sialic acid by SDS-PAGE after neuraminidase treatment and confirmed by HPAEC-PAD. Dot blot analysis showed that *H. bizzozeronii* cells bind Cholera Toxin (CT) and anti-sialyl-Lewis-X antibody, however this is not a common characteristic of all the strains. Structural information of the sialylated LPS was obtained on LPS-OH from the human strain CIII-1, which showed strong reaction with CT, employing CE-ESI-MS and CE-ESI-MS/MS. The sialylation site of *H. bizzozeronii* strain CIII-1 was determined to be Nana-Hex-HexNAc, suggesting sialyl-lactoseamine. Terminal sialic acid is commonly found in LPS from several mucosal pathogens and known to be an important virulence factor. Although it has been shown that also *H. pylori* LPS could express sialyl-Lewis-X Ag, the presence of sialyl-LPS is rarely observed in this species and the sialyltransferase involved has not been characterized. Here we reported the characterization of a *Helicobacter* Sialyltransferase which showed a preference for LacNAc and which seems involved in the biosynthesis of sialyl-LPS in *H. bizzozeronii* strains. More studies are needed in order to understand the role of sialyl-LPS in the pathogenesis of *Helicobacter* infections.



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### ABSTRACT - POSTER 29

#### Immunological studies of *Salmonella* Telaviv somatic antigen epitopes

HALINA DZIADZIUSZKO<sup>1</sup>, JOLANTA KUMIRSKA<sup>2</sup>, SYLWIA MUŻA<sup>1</sup>, DANUTA KUNIKOWSKA<sup>1</sup>, PIOTR STEPNOWSKI<sup>1</sup>

<sup>1</sup>Department of Molecular Microbiology and Serology; National Salmonella Centre, Medical University of Gdańsk, Do Studzienki 38, 80-227 Gdańsk, Poland

<sup>2</sup>Department of Environmental Analysis, Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk, Poland

The serotype of *Salmonella* is based on the immunoreactivity of three different types of antigens: somatic O, flagellar H and capsular Vi. The serological O-specificity of *Salmonella* is defined by the structure of the O-antigen (O-polysaccharide, OPS), being a part of the lipopolysaccharide (LPS) which is one of the major components of the outer surface of smooth-type Gram-negative bacteria. Now, more than 2500 serologically distinct types of *Salmonella* are listed in the Kauffmann-White scheme, later modified by Popoff & Le Minor [1]. The bacterium *Salmonella* Telaviv belongs to the serogroup O:28 (formerly M) containing 101 serovars. Strains from this serogroup contain only the O28 factor, which was divided into three subfactors - O28<sub>1</sub>, O28<sub>2</sub>, and O28<sub>3</sub>, - without structural differences being described [2]. *Salmonella* Dakar has subfactors O28<sub>1</sub> and O28<sub>3</sub>, whereas *Salmonella* Telaviv - O28<sub>1</sub> and O28<sub>2</sub>. So far, only the structure of the *S. Dakar* O-polysaccharide (OPS) has been published [3] and the structure of *S. Telaviv* OPS was determined, but it has not been published yet [4]. The establishment of the structure of *S. Telaviv* OPS and *S. Dakar* OPS was crucial for the determination of the O28 epitope size and the explanation of the molecular basis for subdividing it into subfactors.

The aim of this study was to determine immunological properties of the native LPS and the native and chemically modified OPS isolated from *Salmonella* Telaviv [28:y:enz<sub>15</sub>] and comparative analyses with and the native LPS, and the native and chemically modified OPS obtained from *Salmonella* Dakar [28:a:1,6]. The immunological studies were performed with application of ELISA test and immunological assay. In the experiments rabbit serum and monoclonal antibodies directed against O28<sub>1</sub> epitope were used. In the results, the saccharide units responsible for serological specificity of O28<sub>1</sub> epitope were established.

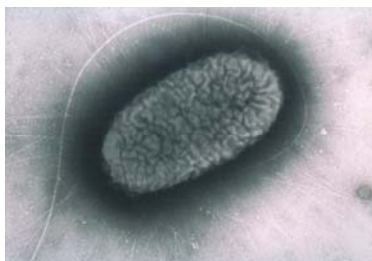
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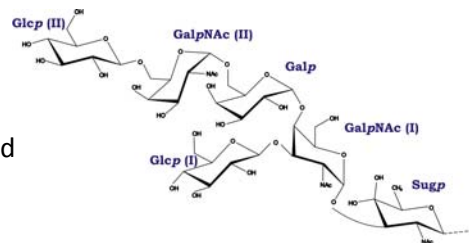
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Acknowledgement: Financial support was provided by the Polish Ministry of Research and Higher Education under grants BW/8200-5-0457-0 and BW173.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT - POSTER 30

#### Flagellar glycosylation in the bio-threat agent *Burkholderia pseudomallei*

ANDREW E. SCOTT<sup>1</sup>, SUSAN M. TWINE<sup>2</sup>, KELLY M. FULTON<sup>2</sup>, RICHARD W. TITBALL<sup>3</sup>, ANGELA E. ESSEX-LOPRESTI<sup>1</sup>, TIMOTHY P. ATKINS<sup>1</sup>, JOANN L. PRIOR<sup>1</sup>

<sup>1</sup> Defence Science and Technology Laboratory, Porton Down, Salisbury, UK.

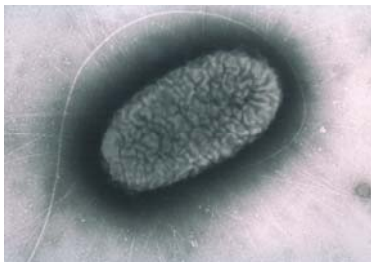
<sup>2</sup> NRC Institute for Biological Sciences, Ottawa, Canada.

<sup>3</sup> School of Biosciences, University of Exeter, Exeter, UK.

Glycosylation of proteins is known to impart novel physical properties and biological roles to proteins from both eukaryotes and prokaryotes. In this study, gel based glycoproteomics were used to identify glycoproteins of the potential bio-threat agent *Burkholderia pseudomallei* and the closely related but non-pathogenic *B. thailandensis*. Top-down and bottom-up MS analysis identified that the flagellin proteins of both species were post-translationally modified by novel glycans. Analysis of protein from two strains of each species demonstrated that *B. pseudomallei* flagellin proteins were modified with a glycan of mass 291 Da whilst *B. thailandensis* flagellin protein was modified with related glycans of mass 300 or 342 Da. Structural characterization of the *B. thailandensis* carbohydrate moiety suggest that it is a tetra-acetylated hexuronic acid. In addition, we have identified through mutagenesis a gene from the LPS O-antigen biosynthetic cluster which is involved in flagella glycosylation, inactivation of which eliminates flagella glycosylation in *B. pseudomallei*. This is the first report to conclusively demonstrate the presence of a carbohydrate covalently linked to a protein in *B. pseudomallei* and *B. thailandensis* and suggests new avenues to explore to examine the marked differences in virulence between these two species.

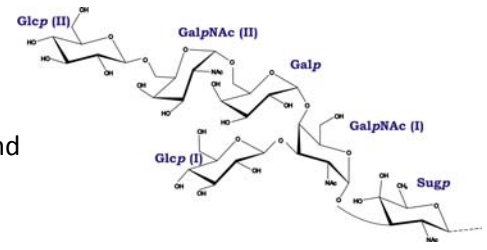
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## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT - POSTER 31

#### Mannan-binding lectin (MBL) interaction with lipopolysaccharides (LPS) from rough mutants of *Yersinia enterocolitica* O:3 serotype of various chemotypes

KATARZYNA KASPERKIEWICZ<sup>1</sup>, ANNA ST ŚWIERZKO<sup>2</sup>, MACIEJ CEDZYŃSKI<sup>2</sup>, MIKAEL SKURNIK<sup>3</sup>, JOANNA RADZIEJEWSKA-LEBRECHT<sup>1</sup>

<sup>1</sup> Dept of Microbiology, Faculty of Biology and Environmental Protection, University of Silesia, Katowice, Poland

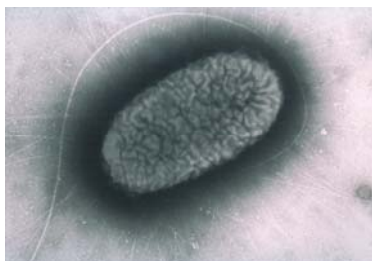
<sup>2</sup> Laboratory of Immunobiology of Infections, Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland

<sup>3</sup> Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland

Mannan-binding lectin (MBL) is a serum defence collectin. It interacts with D-Man, D-GlcNAc and L-Fuc residues being components of microbial cell surface structures, including lipopolysaccharide (LPS). It contributes to the clearance of pathogens via lectin pathway activation of complement. Moreover, it opsonizes microbial cells thus enhancing the phagocytosis by immune cells. MBL insufficiency is believed to be the most common human immunodeficiency, having association with enhanced susceptibility to various infections or influencing the disease course. *Yersinia enterocolitica* is a Gram-negative pathogen transmitted through contaminated food or water, leading to human and animal enteric infections. It was also detected in synovial samples from reactive arthritis patients. The aim of this study was to evaluate the reactivity of MBL with cells and isolated LPS from *Y. enterocolitica* O:3 wild-type strain and its mutants synthesizing LPS of various chemotypes. Bacteria were cultivated at a temperature 22°C or 37°C. LPS was isolated with the help of phenol/water or PCP procedures. LPS samples were separated in SDS-PAGE and their interaction with MBL was evaluated by immunoblotting employing human serum as a source of the collectin, murine anti-human MBL monoclonal antibody and peroxidase-conjugated secondary antibody. The interaction of MBL with LPS was further investigated in ELISA, employing the same source of the collectin as well as antibodies. Moreover, the MBL-dependent lectin pathway activation of complement by *Yersinia* lipopolysaccharides was tested. The binding of MBL to formalin-inactivated bacterial cells was evaluated with the help of flow cytometry.

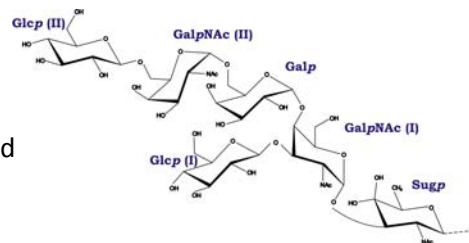
All LPS samples separated in SDS-PAGE and blotted to nitrocellulose bound human MBL. The strongest reactivity was observed for low molecular mass fractions of LPS. On the other hand, in ELISA, practically no binding was detected in the case of S- and Ra-type of LPS. The LPS having truncated or lacking the outer core oligosaccharide (isolated from bacteria cultivated at 22°C) bound MBL and in addition activated complement via the lectin pathway. However, when corresponding LPS from strains grown at 37°C were tested, a very weak (or no) reactivity was noted. In contrast, MBL recognized inactivated microbial cells more efficiently when bacteria were cultivated at a higher temperature. These results suggest that the target structure for human MBL is located in the inner core oligosaccharide region of *Y. enterocolitica* O:3 LPS. The binding properties depend not only on LPS chemotype but also on bacterial growth conditions as well as on the arrangement of LPS molecules.





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### ABSTRACT - POSTER 32

#### The correlation of anti-LPS *Proteus mirabilis* antibodies level with *tlr4* (Thr399Ile) gene polymorphism in rheumatoid arthritis

MICHAŁ ARABSKI<sup>1</sup>, ANNA KOZA<sup>1</sup>, WOJCIECH GARBACZ<sup>1</sup>, ŁUKASZ MADEJ<sup>1</sup>, EWELINA NOWAK<sup>1</sup>,  
AGNIESZKA MATUSIK<sup>1</sup>, WIESŁAW KACA<sup>1</sup>

<sup>1</sup> Department of Microbiology, Jan Kochanowski University, Świętokrzyska 15, 25-406, Kielce, Poland

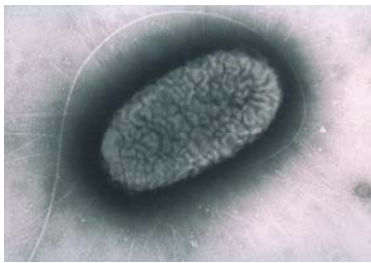
Rheumatoid arthritis is a crippling joint disease with environmental and genetic components. The cause of RA is most probably linked to the triad of microbial trigger, genetic predisposition and autoimmunity. Bacteria of the genus *Proteus* are facultative human pathogens responsible for a number of infections as wounds or burns and are one of the most important urinary tract infection pathogens. A role of *Proteus* surface antigens (like LPS) in the pathogenesis of rheumatoid arthritis has also been suggested. The presence of enterobacterial LPSs lead to the high and non-specific activation of the human immunological systems in patients with rheumatoid arthritis. One of the most important are the pathogen-recognition mechanisms, which include TLR4 receptor.

In our investigation we determined the level of anti-LPSs *P. mirabilis* O3, O23, O10, O9, O40 and O49 strains antibodies in serum of 50 (37 females and 13 males) from 33 to 86 years (median age 59) with rheumatoid arthritis by ELISA. We did not observe the correlation between the levels of all tested anti-LPSs *P. mirabilis* antibodies and amounts of C-reactive protein, RF factor, Waaler-Rose reaction, *tlr4* (Thr399Ile) gene polymorphism and standing of disease. However, we observed statistically higher level ( $p < 0.006$ ) of anti-O3, O23, O49 and O40 *P. mirabilis* antibodies in rheumatoid arthritis patients sera in comparison to 50 healthy blood donors. It is worth to taking into consideration that in 66% of rheumatoid arthritis patients sera the individual level of anti-O9 LPSs *P. mirabilis* antibodies have lower level of anti-O9 antibodies than in control sera.

We do not observed the correlation between anti-*P. mirabilis* antibodies and *tlr4* gene polymorphism in rheumatoid arthritis patients. One can postulated that lower level of peculiar anti-O *P. mirabilis* antibodies in rheumatoid arthritis patients sera i.e. O9 LPS might be due to its not-branched, linear structure of O-polysaccharide antigen. The differences of specificity and level of anti-*Proteus* sp. LPSs antibodies might have an diagnostic potential in autoimmunity diseases.

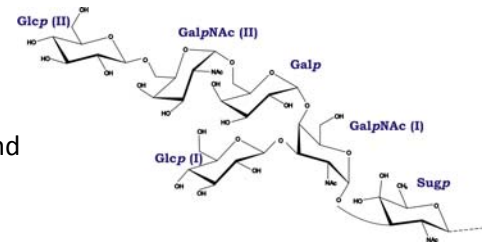
#### Acknowledgements

This work was supported by grand from Jan Kochanowski University, Kielce, Poland (BW/2010 for M. Arabski).



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 33

#### The effect of VLDL-associated LPS on macrophages in periodontitis patients

K.A. ELISA KALLIO<sup>1</sup>, KATI HYVÄRINEN<sup>1</sup>, PETRI T. KOVANEN<sup>2</sup>, MATTI JAUHAINEN<sup>3</sup>, PIRKKO J. PUSSINEN<sup>1</sup>

<sup>1</sup> Institute of Dentistry, University of Helsinki, Helsinki, Finland

<sup>2</sup> Wihuri Research Institute, Helsinki, Finland

<sup>3</sup> National Institute for Health and Welfare, Biomedicum, Helsinki, Finland

**Objectives.** Periodontitis, a chronic oral infection caused mainly by Gram-negative bacteria, induces endotoxemia and has been associated with an increased risk of atherosclerosis. We investigated the proatherogenic properties of very low density lipoprotein (VLDL) isolated from periodontitis patients before and after periodontal treatment.

**Methods.** The study included 30 systemically healthy patients (16 males, 14 females, aged 50±7 years). VLDL was isolated and characterized before and three months after periodontal treatment. VLDL-derived cholesterol uptake and gene expression levels of TNF- $\alpha$  and MCP-1 were investigated in human THP-1 macrophages.

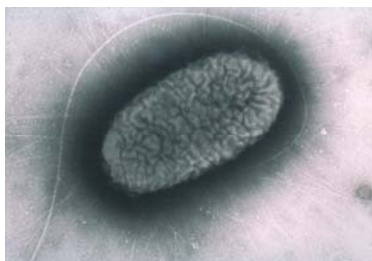
**Results.** Cholesterol uptake was nearly 2-fold in the presence of VLDL and correlated positively with the VLDL-associated lipopolysaccharide (LPS) activity ( $r=0.436$ ,  $p=0.016$ ). Before the treatment, VLDL of patients with more severe periodontitis

- i) contained more LPS ( $3.7\pm 2.1$  vs.  $2.2\pm 1.6$  EU/ml,  $p=0.017$ )
- ii) caused 18 % higher cholesterol uptake ( $p=0.014$ )
- iii) induced higher mRNA expression of TNF- $\alpha$  ( $p=0.009$ ) and MCP-1 ( $p=0.041$ )

than VLDL of patients with moderate periodontitis. Although clinically successful, periodontal treatment did not affect VLDL composition or its potential to induce cholesterol uptake or gene expression by the macrophages.

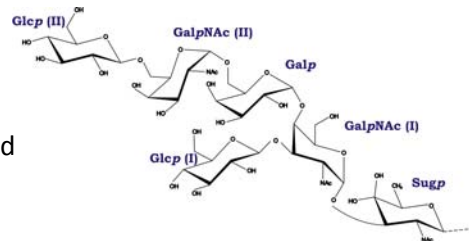
**Conclusion.** Periodontitis-induced inflammation and endotoxemia may increase VLDL-derived macrophage activation and foam cell formation, and thereby atherogenesis. Such systemic effect of periodontitis seems to be refractory to periodontal treatment.

**Funding.** This research was supported by The Academy of Finland, The Finnish Dental Society Apollonia, Helsinki Biomedical Graduate School, and Jenny and Antti Wihuri Foundation.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 34

#### Structural and cross-serological analyses of the O-, C- and H-antigens of *Azospirillum brasilense* type strain Sp7

YULIA P. FEDONENKO<sup>1</sup>, OLGA N. SMOL'KINA<sup>1</sup>, ALEKSEY YE. BELYAKOV<sup>1</sup>, GENNADIY L. BURYGIN<sup>1</sup>,  
SERGEY YU. SHCHEGOLEV<sup>1</sup>, SVETLANA A. KONNOVA<sup>1,2</sup>, VLADIMIR V. IGNATOV<sup>1,2</sup>

<sup>1</sup> Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia

<sup>2</sup> Saratov State University, Saratov, Russia Russian Federation

Bacteria of the genus *Azospirillum* are gram-negative nitrogen-fixing plant-growth-promoting rhizobacteria. The bacterial attachment to plant roots is known to occur in two stages, namely, adsorption and anchoring, where the important role is played by cell surface proteins, such as the polar flagellin (PF) and major outer membrane protein, and bacterial cell surface polysaccharides (PS), such as capsular polysaccharides (CPS), and lipopolysaccharide (LPS).

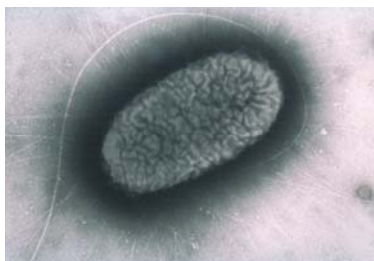
It is common knowledge that glycosylation of bacterial proteins (including PF) can carry by either independent way or by way connected with biosynthesis LPS. In this paper we report the results of the comparative research of the carbohydrate components from PF, LPS, and CPS of *A. brasilense* Sp7.

We have obtained rabbit antibodies to LPS isolated from outer membrane of *A. brasilense* wild-type strain Sp7. The rabbit polyclonal anti-LPS antibodies were characterized by their reactivity in immunodiffusion assay with non-purified and deproteinized LPS, CPS and PF and by their ability to bind high-molecular-mass fraction of the molecules in Western immunoblotting.

Mild acid degradations of the LPS, CPS and PF from *A. brasilense* Sp7 resulted in the obtainment of high-molecular-mass polysaccharides (PS1, PS2, PS3 respectively), which were separated by GPC on Sephadex G-50. Test for sugars made by GLC of the alditol acetates after full acid hydrolysis of each polysaccharide showed the presence of rhamnose, fucose, xylose, galactose and glucosamine in the ratio of 5:5:1:4:3 and 3:3:1:2:1 for PS1 and PS3 respectively. The PS2 differed from them in the absence of xylose, the low glucosamine content, and the presence of glucuronic acid in its structure. The GLC determination of the absolute configurations of the acetylated glycosides with a chiral alcohol indicated that rhamnose and fucose had the L configuration whereas galactose and glucosamine had the D configuration.

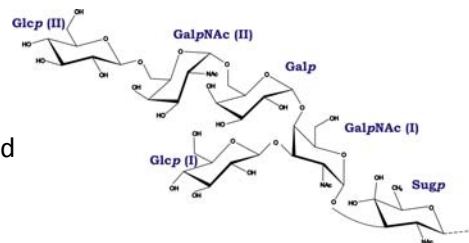
Thereby, the polysaccharides from LPS, CPS, and PF of *A. brasilense* Sp7 have similar monosaccharide compositions and probably common structural fragments resulting in the revealed serological cross-reactivity of the polysaccharides with the anti-LPS antibodies.

This work was funded in part by RFBR (project 08-04-00669-a).



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 35

#### Structural investigation of the O-polysaccharide of *Azorhizobium caulinodans* HAMBI 216 consisting of rhamnose, 2-O-methylrhamnose and 3-C-methylrhamnose

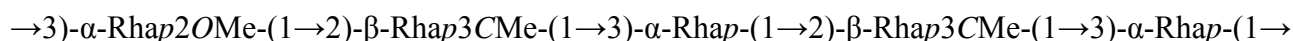
OLGA A. VALUEVA<sup>1</sup>, EVELINA L. ZDOROVENKO<sup>1</sup>, VADIM V. KACHALA<sup>1</sup>, ALEXANDER S. SHASHKOV<sup>1</sup>, YURIY A. KNIREL<sup>1</sup>, IWONA KOMANIECKA<sup>2</sup>, ADAM CHOMA<sup>2</sup>

<sup>1</sup>N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991, Moscow, Russia

<sup>2</sup>Department of Genetics and Microbiology, Maria Curie-Skłodowska University, 20-033, Lublin, Poland

Rhizobacteria are unique in their ability to interact with roots of legumes and to form nitrogen-fixing nodules. An important role in establishing the plant-bacterium symbiosis is played by bacterial surface polysaccharides, including lipopolysaccharides and their most exposed O-polysaccharide chains. Knowing their structure is crucial for understanding of the mechanism of the nodule development. In this work, we report the structure of the O-polysaccharide of *Azorhizobium caulinodans* HAMBI 216 (ORS 571), a representative of rhizobacteria from the family *Xanthobacteraceae*. *A. caulinodans* induces nitrogen-fixing nodules on roots and stem of water-tolerant tropical legume *Sesbania rostrata*.

The O-polysaccharide was studied by sugar and methylation analyses by GLC-MS along with 1D and 2D NMR spectroscopy, including <sup>1</sup>H,<sup>1</sup>H ROESY and <sup>1</sup>H,<sup>13</sup>C HMBC experiments, and found to contain rhamnose, 2-O-methylrhamnose (Rha2OMe) and 3-C-methylrhamnose (Rha3CMe, evalose). Analysis of glycosylation effects in the <sup>13</sup>C NMR spectrum of the polysaccharide showed that all constituent monosaccharides have the same absolute configuration, whose determination is in progress. The following linear structure of the polysaccharide was established, which is unique among the known bacterial polysaccharide structures:

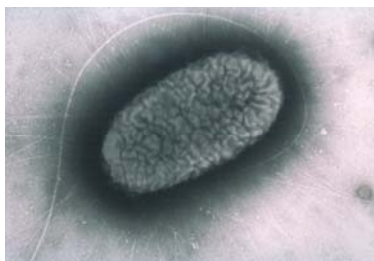


Evalose is a rare monosaccharide in nature. Earlier, this sugar of unknown absolute configuration has been reported as a constituent of the O-polysaccharide of *Nitrobacter* X<sub>14</sub> [1]. D-Evalose is a component of the natural antibiotic everninomicin B [2]. Both enantiomers of 2-O-methylrhamnose have been found in a number of bacterial carbohydrates and antibiotics; in bacterial polysaccharides, they often occur in non-stoichiometric amounts.

A highly lipophilic character of the O-polysaccharide of *A. caulinodans* HAMBI 216 may play a role in the interaction of the bacterium with the host plant.

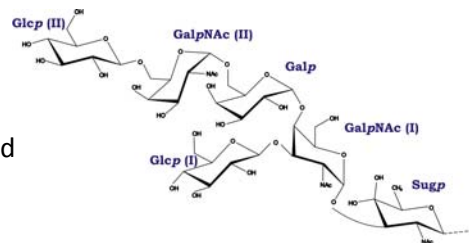
[1] Mayer H., Bock E., Weckesser J. *FEMS Microbiol. Lett.* **1983**, *17*, 93-96.

[2] Ganguly A.K., Saksena A.K. *J. Chem. Soc. Chem. Commun.* **1973**, 531-532.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 36

#### Bacterial Carbohydrate Structure Database 3: novel version

PHILIP V TOUKACH

*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russia*

Carbohydrates are one of the major constituents of the bacterial cell wall important for immunological properties of microorganisms, signal transduction and cell-cell recognition. The role of the provision of data on bacterial carbohydrates to the scientific community in biomedical and immunological research can hardly be overestimated. However, in contrast to other disciplines studying molecular basis of life, glycomics is lacking information-technology-based advantages. Universal integration standards and computer-assisted tools in glycomics are still in the making. Existing carbohydrate databases are focused on particular properties, utilize proprietary formats, do not provide complete coverage and, accordingly to our dedicated study, are full of errors.

Bacterial Carbohydrate Structure Database (BCSDB) aims at closing this gap its by curated content and cross-database integration, thus bringing of glycomics to the same level of integrity as exists in genomics and proteomics. BCSDB has been continuously developed and updated since 2005 to provide the data on bacterial carbohydrates with known primary structure. Currently it is the only free database with primary data on carbohydrate structures published up to 2009. Now we present version 3, the result of the database architecture rearrangement done in 2009.

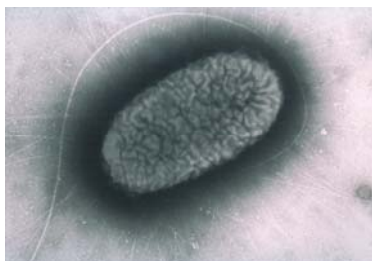
Two key features of this project are coverage and data consistency. The database contains structural, taxonomical, bibliographical, assigned NMR spectroscopic and other (elucidation methods, conformational, biochemical, and genetic data etc.) information on ~8000 bacterial carbohydrates and glycoconjugates, including glycoproteins and glycolipids. The coverage approaches nearly all structures published within this class before 2009. This means that even negative answer to the search request to BCSDB still remains a valuable scientific information.

The source of data were “CarbBank” (manually curated carbohydrate database ceased in 1996), manual and semi-automated retrospective processing of publications. All data have been checked for consistency by experts in carbohydrate biochemistry prior to the upload and corrected when necessary, which makes BCSDB the only glycoinformatic project with fully-moderated content.

The BCSDB interface includes the web-based user part, administrator part and gateways for automated data interchange with other databases. Users can search the database using fragments of structure, bibliography, taxonomical annotations, fragments of NMR spectra. The integration with other projects in glycomics (“GlycomeDB”, meta-database from Deutsches Krebsforschungszentrum, bibliographical (PubMed) and taxonomical databases from National Center for Biotechnology Information) has been achieved on the level of programming interface. The unambiguous but nevertheless human-readable carbohydrate structure description language has been developed for this project and translation tools to and from other known glycan representations are provided.

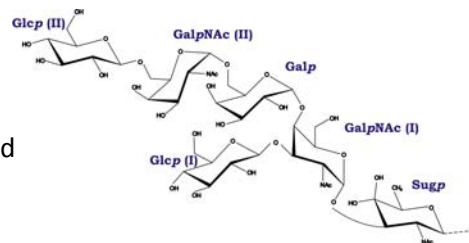
BCSDB is available on the Internet for free usage and validated user data submission (<http://www.glyco.ac.ru/bcsdb3/>).





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### ABSTRACT - POSTER 37

#### Diversity of exopolysaccharide structure isolated from *Lactobacillus* species (colitis “+”) present in murine intestinal microflora

S GÓRSKA-FRĄCZEK,<sup>1</sup> C SANDSTROM,<sup>2</sup> M STRUS,<sup>3</sup> P HECZKO,<sup>3</sup> L KENNE<sup>2</sup> AND A GAMIAN<sup>1</sup>

<sup>1</sup>*L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland*

<sup>2</sup>*Department of Chemistry, Swedish University of Agricultural Sciences, P. O. Box 7015, SE-750 07, Uppsala, Sweden*

<sup>3</sup>*Chair of Microbiology, Jagiellonian University Medical College, Czysła 18, 31-121 Kraków, Poland;*

Studies on the intestinal microbiota revealed a dysbiosis, in the context of inflammatory bowel disease (IBD). The inflammatory response interferes with the state of tolerance to the intestinal bacteria and leads to characteristic changes in the biostructure of the faecal microbiota. This dysbiosis is accompanied by a loss of bacteria with anti-inflammatory properties, which may explain the installation of chronic inflammation in IBD. However very little is known on the bacterial effector molecules involved in bacteria-host crosstalk, and the role of bacterial surface antigens. The aim of our work was to assess structure of exopolysaccharide isolated from mouse with experimentally induced IBD (colitis “+”) and from healthy mouse.

Exopolysaccharides (EPS) are high-molecular-mass sugar biopolymers forming a bacterial envelope called capsule. Bacterial cell, like *Lactobacillus* secrete EPS to the surrounding environment. Owing to the wide diversity in composition, exopolysaccharides have found multifarious applications in various food and pharmaceutical industries. Some evidences for the health properties of these biopolymers have already been found [1,2]. These cell surface antigens are also thought to play an essential role in the adhesion phenomenon. It has been shown that EPS from lactic acid bacteria can modify the adhesion of pathogenic bacteria to intestinal mucus [3]. During the investigation concerning the immunological activity of various exopolysaccharides from bacterial strains, the information about the molecular structure of the polysaccharide is essential.

Here we present exopolysaccharide structures isolated from *Lactobacillus animalis/murinus* 116 and 148 isolated from mouse with IBD. Structures were analyzed by standard chemical methods and NMR.

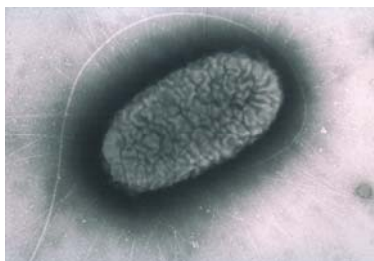
Strains of *Lactobacillus animalis/murinus* 116 and 148 produced neutral and charged EPS with specific various motifs and were different compared to those isolated from healthy mouse.

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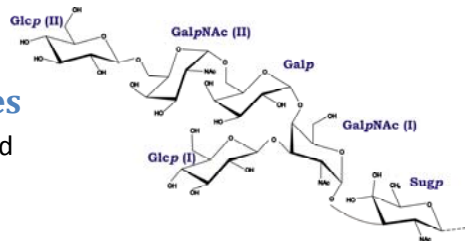
[3] Vesterlund S.; Karp, M.; Salminen, S.; Ouwehand, A. C. *Microbiol.-Sgm*, **2006**, 152, 1819–1826





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 38

#### Chemical structure of the polysaccharidic O-antigen of *Cronobacter turicensis*

ANNA BYCHOWSKA<sup>1</sup>, STEPHEN FORSYTHE<sup>2</sup>, MAŁGORZATA CZERWICKA<sup>1</sup>, KINGA MARSZEWSKA<sup>1</sup>,  
ALICJA MAZGAJCZYK<sup>1</sup>, HALINA DZIADZIUSZKO<sup>3</sup>, PIOTR STEPNOWSKI<sup>1</sup>, ZBIGNIEW KACZYŃSKI<sup>1</sup>

<sup>1</sup> Faculty of Chemistry, University of Gdańsk, Sobieskiego 18/19, 80-952 Gdańsk, Poland

<sup>2</sup> School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, United Kingdom

<sup>3</sup> Department of Molecular Microbiology and Serology; National Salmonella Centre, Medical University of Gdańsk, Do Studzienki 38, 80-227 Gdańsk, Poland

*Cronobacter* spp., formerly named *Enterobacter sakazakii*, is a Gram-negative opportunistic pathogen known as important cause of necrotizing enterocolitis, meningitis, and septicemia with a high mortality rate in neonates [1]. Those pathogens are widely distributed in the environment and in foods especially in dairy products such as cheese, milk powder and ultrahigh-temperature pasteurized milk. *Cronobacter* spp. has been also found in the hospital environment, including air, formula-mixing utensils, and enteral-feeding tubes [2]. Although *Cronobacter* virulence factors have not been extensively studied, it is known that lipopolysaccharide (LPS) can induce the translocation of those bacteria across the intestinal wall [3].

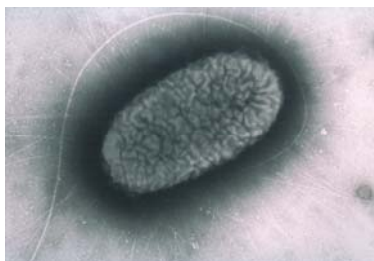
The main aim of this study was to isolated and assigned the chemical structure of the O-polysaccharide (O-PS) from *Cronobacter turicensis* strain NTU 57. The lipopolysaccharide was obtained according to the classical hot phenol–water extraction of the dried bacterial cells [4]. The aqueous phase was dialyzed against distilled water and the LPS was precipitated with ethanol and centrifugation. The LPS pellet was dissolved in water, dialyzed, freeze-dried and then hydrolyzed with acetic acid. Finally gel permeation chromatography (GPC) on Bio-Gel P-100 column with water as eluent was used for purifying poly- and oligosaccharides. Whole purification process was monitored with a refractometer detector. Sample for structural analysis was selected on the basis of results of nuclear magnetic resonance spectroscopy (<sup>1</sup>H-NMR). To determine a complete chemical structure of the isolated polysaccharides the following procedures were used: sugar analysis, methylation analysis, D, L configuration assignment. All obtained derivatives were analyzed by chromatographic methods combined with mass spectrometry.

[1] Baldwin, A.; Loughlin, M.; Caubilla-Barron, J.; Kucerova, E.; Manning, G.; Dowson, C.; Forsythe, S. BMC Microbiol. 2009, 9, 223.

[2] Hurrell, E.; Kucerova, E.; Loughlin, M.; Caubilla-Barron, J.; Hilton, A.; Armstrong, R.; Smith, C.; Grant, J.; Shoo, S.; Forsythe, S. BMC Inf. Dis. 2009, 9, 146.

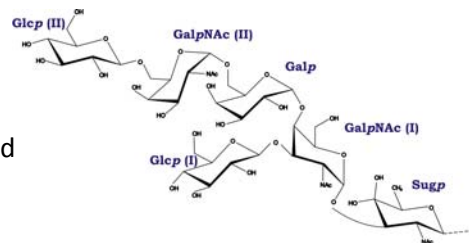
[3] Townsend, S.; Caubilla Barron, J.; Loc-Carrillo, C.; Forsythe, S. J. Food Microbiol. 2007, 24, 67–74.

[4] Westphal, O.; Jann, K. Methods Carbohydr. 1965, 5, 83–91.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT - POSTER 39

#### Structure of the O-polysaccharide isolated from *Cronobacter sakazakii* 767

MAŁGORZATA CZERWICKA<sup>1</sup>, STEPHEN FORSYTHE<sup>2</sup>, ANNA BYCHOWSKA<sup>1</sup>, HALINA DZIADZIUSZKO<sup>3</sup>,  
DANUTA KUNIKOWSKA<sup>3</sup>, PIOTR STEPNOWSKI<sup>1</sup>, ZBIGNIEW KACZYŃSKI<sup>1</sup>

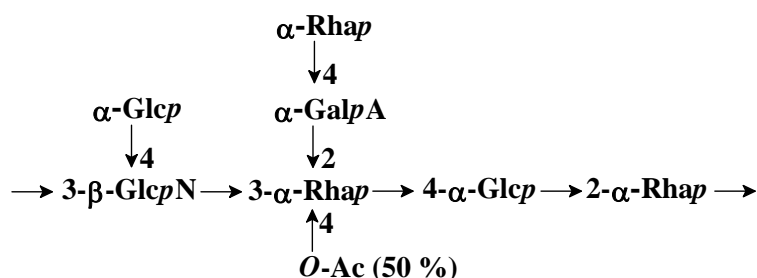
<sup>1</sup> Faculty of Chemistry, University of Gdańsk, Sobieskiego 18/19, 80-952 Gdańsk, Poland

<sup>2</sup> School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, United Kingdom

<sup>3</sup> Department of Molecular Microbiology and Serology; National Salmonella Centre, Medical University of Gdańsk, Do Studzienki 38, 80-227 Gdańsk, Poland

The *Cronobacter* spp. (previously *Enterobacter sakazakii*) are opportunistic Gram-negative bacterial pathogens that can cause infections in all age groups [1]. However neonates, particularly those of low-birth weight have the highest risk of infection and can cause necrotizing enterocolitis, meningitis and septicaemia, with a high mortality rate [2]. Characterization of LPS structure and consequently O-polysaccharide structure can be important in developing identification schemes for *Cronobacter* spp. based on serotyping.

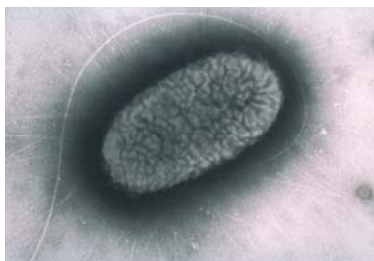
The major goal of this study was to determine the chemical structure of the O-polysaccharide (O-PS) of *C. sakazakii* 767. This strain was isolated from neonate who died from meningitis at a NICU in 1994 [3]. The lipopolysaccharide was separated from the water phase after the phenol-water extraction of dry bacterial cells. Mild hydrolysis of LPS followed by lipid A centrifugation and purification by gel permeation chromatography provided the O-PS. Part of the obtained sample was de-*O*-acetylated using hydrazine. For compositional determination the classical procedure including sugar analysis, methylation analysis, and L, D configuration assignment was used. Moreover, the native and de-*O*-acetylated polysaccharides were studied by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy, including two-dimensional DQF-COSY, TOCSY, ROESY, HMQC, and HMBC experiments. In summary, the data identified the structure of the repeating unit of O-specific polysaccharide from *Cronobacter sakazakii* 767 as



[1] Iversen, C.; Mullane, N.; McCardell, B.; Tall, B. D.; Lehner, A.; Fanning, S.; Stephan, R.; Joosten, H. *Intl. J. System. Evol. Microbiol.* 2008, 58, 1442-1447.

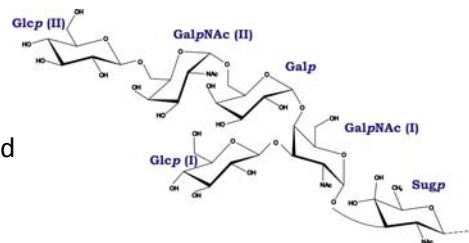
[2] Forsythe, S. J. *Maternal Child Nutr.* 2005, 1, 44-50.

[3] Caubilla-Barron, J.; Hurrell, E.; Townsend, S.; Loc-Carrillo, C.; Cheetham, P.; Fayet, O.; Prère M-F.; Forsythe, S. J. *J. Clin. Microbiol.* 2007, 45, 3979-3985.



## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 40

#### Cell wall teichuronic and teichulosonic acids of gram-positive bacteria

ELENA M. TUL'SKAYA<sup>1</sup>, ALEXANDER S. SHASHKOV<sup>2</sup>, GALINA M. STRESHINSKAYA<sup>1</sup>, SOF'YA N. SENCHENKOVA<sup>2</sup>, NATALIA V. POTEKHINA<sup>1</sup>, YULIA I. KOZLOVA<sup>1</sup>, LUDMILA I. EVTUSHENKO<sup>3</sup>

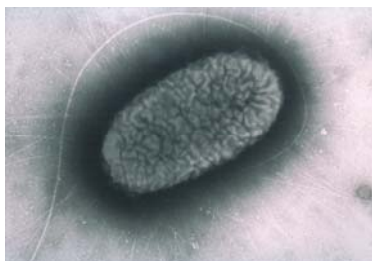
<sup>1</sup> School of Biology, M. V. Lomonosov Moscow State University, Moscow 119991, Russian Federation;

<sup>2</sup> N. D. Zelinsky Institute of Organic Chemistry, RAS, Moscow 119991, Russian Federation;

<sup>3</sup> All-Russian Collection of Microorganisms (VKM), G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, RAS, Pushchino, Moscow Region, 142290, Russian Federation

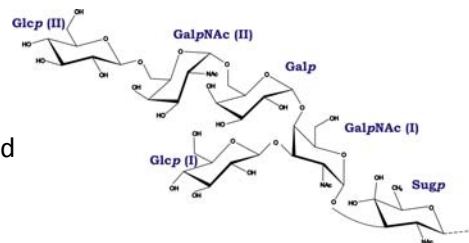
Teichuronic and teichulosonic acids represent cell wall glycopolymers of gram-positive bacteria. Unlike other anionic polymers (teichoic acids, sugar phosphate polymers), these compounds impart a negative charge to the cell wall due to the presence of carboxyl groups rather than phosphoric acid residues. Teichuronic acids are natural biopolymers known for about 50 years. Their biosynthesis often occurs under conditions of phosphate limitation in the medium. We demonstrated the simultaneous presence of both teichuronic acids and other glycopolymers in the cell walls of representatives of the order *Actinomycetales*. The wide structural diversity of teichuronic acids is accounted for by i) the nature of uronic acids (glucuronic, mannuronic as well as their monoamino- and diamino-derivatives) and monosaccharide residues (glucose and its monoamino- and diamino-derivatives) as the polymer repeating unit constituents; ii) the amount of monomers in the repeating unit and the number of repeating units in the chain; iii) the presence of different *N*-acyl substituents in amino sugars and iv) the type of glycosidic linkages in the repeating unit. Natural biopolymers that may be called teichulosonic acids were discovered about a decade ago, they comprise ald-2-ulosonic acids as essential constituents. These polymers have been found in cell walls of actinomycetes including sporogenous phytopathogenic and thermophilic streptomycetes, sporangial as well as asporogenous (nocardio- and coryneform) actinomycetes. The teichulosonic acids studied to date are represented by two types of polymers. The backbone of polymers and oligomers of one of them is built of 3-deoxy-D-glycero-D-galacto-non-2-ulosonic acid (Kdn). The Kdn homopolymer chains comprise from twenty to forty  $\beta$ -(2 $\rightarrow$ 4)-linked residues. All of the Kdn polymers examined so far bear lateral 8- and/or 9-linked Glc, Gal, 3-*O*-Me-Gal and GlcN residues.

The second type of polymers contain, in the basic chain, 5,7-diacylamido-3,5,7,9-tetra-deoxy-L-glycero-L-manno-nonulosonic acid (pseudaminic acid, Pse). In all cases, the *N*-acyl substituent at C-7 is 4-hydroxy- or 3,4-dihydroxybutyric acid. A characteristic feature of all the teichulosonic acids is that the monosaccharide residues are glycosidically linked with the C-4 hydroxyl group of hydroxybutyric acid(s). The Galp, 3-*O*-Me-Galp, 2,3-*O*-Me-Galp or Rhap residues were found as the side substituents at C-4 of Pse in the majority of the polymers studied. This type of teichulosonic acids contains 30-40 of sugar residues. Addressing teichuronic acids and teichulosonic acids is related to the demonstration of the natural diversity of biopolymers, this can help understanding of the mechanisms of the interaction of bacteria within the microbial community and with environment, higher organisms in particular.



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### ABSTRACT - POSTER 41

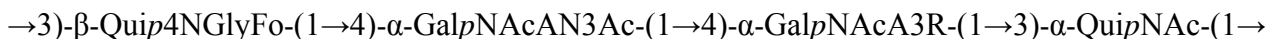
#### Structures of the O-polysaccharides of *Photorhabdus asymbiotica* subsp. *asymbiotica* and subsp. *australis* resembling that of *Francisella tularensis*

ANNA N. KONDAKOVA<sup>1</sup>, NADEZHDA A. KIRSHEVA<sup>2</sup>, ALEXANDER S. SHAHSHKOV,<sup>1</sup> RIMA Z. SHAIKHUTDINOVA<sup>2</sup>, SERGEY A. IVANOV<sup>2</sup>, ANDREY P. ANISIMOV<sup>2</sup>, YURIY A. KNIREL<sup>1</sup>

<sup>1</sup> N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia

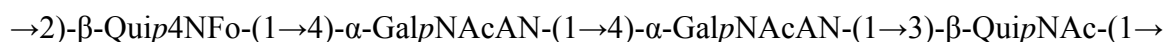
<sup>2</sup> State Research Center for Applied Microbiology and Biotechnology, Obolensk, Moscow Region, Russia

The genus *Photorhabdus* from the family *Enterobacteriaceae* includes three entomopathogenic species associated with nematodes of the genera *Heterorhabditis*, one of which, *Photorhabdus asymbiotica*, is an emerging human pathogen [1]. No data on the lipopolysaccharide structure of these bacteria have been reported. In this work, we studied the O-polysaccharides from the lipopolysaccharides of two *P. asymbiotica* strains of subsp. *asymbiotica* (US-86) and subsp. *australis* (AU46). The following structures differing only in the pattern of O-acetylation were established using chemical methods and one- and two-dimensional <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy:



where GalNacA stands for 2-acetamido-2-deoxygalacturonic acid, GalNacAN for amide of GalNacA, QuiNac for 2-acetamido-2,6-dideoxyglucose, Qui4NGlyFo for 4,6-dideoxy-4-(*N*-formylglycyl)aminoglucose; R = Ac in strain US-86 or H in strain AU46.

Remarkably, the structures of the O-polysaccharides studied resemble that of a taxonomically remote bacterium *Francisella tularensis*, which differs in i) the *N*-acyl substituent on Qui4N, ii) the mode of the linkage between the repeating units ( $\beta$ -1 $\rightarrow$ 2 *versus*  $\alpha$ -1 $\rightarrow$ 3), iii) amidation of both GalNacA residues, and iv) the lack of O-acetylation [2]:



To the best of our knowledge, *N*-formylglycyl has not hitherto been found on Qui4N or another amino sugar in bacterial polysaccharides. Earlier, the presence of the formyl group on Qui4N has been demonstrated to be crucial for the synthesis of the long-chain lipopolysaccharide in a vaccine strain *F. tularensis* 15, which is important for bacterial virulence [3].

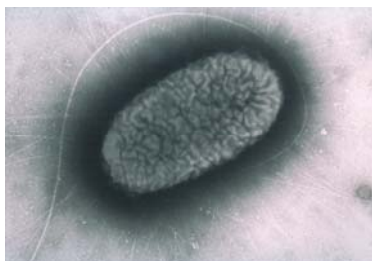
This work was supported by the Russian Foundation for Basic Research (Project 09-04-00501).

[1] Costa S.C., Chavez C.V., Jubelin G., Givaudan A., Escoubas J.M., Brehélin M., Zumbihl R. *Microbes Infect.* **2010**, *12*, 182-189.

[2] Vinogradov E.V., Shashkov A.S., Knirel Y.A., Kochetkov N.K., Tochtamysheva N.V., Averin S.P., Goncharova O.V., Khlebnikov V.S. *Carbohydr. Res.* **1991**, *214*, 289-297.

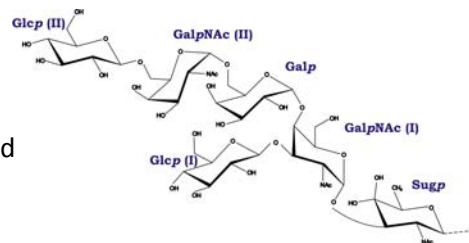
[3] Mokrieovich A.N., Kondakova A.N., Valade E. et al. *Biochemistry (Moscow)* **2010**, *75*, 443-451.





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

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### ABSTRACT - POSTER 42

#### The completed *Salmonella enterica* O-antigen structure elucidation

SOFIYA SENCHENKOVA,<sup>1</sup> ANDREI V. PEREPELOV,<sup>1</sup> SERGEI D. SHEVELEV,<sup>1</sup>  
ALEXANDER S. SHASHKOV,<sup>1</sup> YURIY A. KNIREL,<sup>1</sup> BIN LIU,<sup>2</sup> LU FENG,<sup>2</sup> LEI WANG<sup>2</sup>

<sup>1</sup>*N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991, Moscow, Russia*

<sup>2</sup>*TEDA School of Biological Sciences and Biotechnology, Nankai University, Tianjin, People's Republic of China*

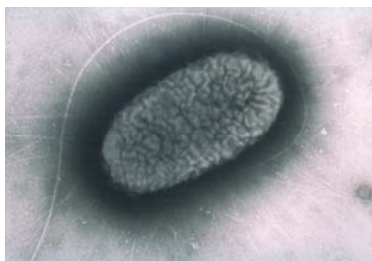
*Salmonella enterica* is recognized as an important pathogen of humans and animals, which in many countries is the leading cause of outbreaks of food-borne infections. The O-antigen (O-polysaccharide chain of the lipopolysaccharide) appears to be the major target of the host immune system and bacteriophages, and its specificity is important for bacterial virulence and niche adaptation. The O-antigen diversity is mainly due to genetic variations in the O-antigen gene clusters and is the basis for bacterial serotyping. Currently, 46 *S. enterica* O-serogroups are described in the Kauffmann-White serotyping scheme.

The unique O-antigens of *S. enterica* A, B, D and E (serogroups O2, O4, O9 and O3,10) are historically the first O-polysaccharides whose structures have been elucidated in detail. The O-antigens of five O-serogroups (O6,14, O30, O35, O48 and O50) have been found to be shared by *Escherichia coli* O-serogroups. However, until recently, O-antigen structures in many other *S. enterica* O-serogroups remained unknown. Aiming to fill the gap, we elucidated new unique O-antigen structures of seven O-serogroups (O21, O41, O44, O53, O56, O59 and O60) and found that in 13 more O-serogroups (O13, O16, O17, O38, O42, O47, O51, O52, O55, O57, O58, O65 and O66) the O-antigens are structurally identical or closely related to those of various *E. coli* O-serogroups.

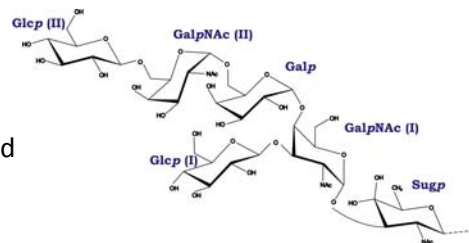
The O-polysaccharide structures were established by sugar analysis along with one- and two-dimensional <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy; when necessary, methylation analysis and Smith degradation were employed. The O-polysaccharides were found to have linear or branched tetra- to hexa-saccharide O-units containing at least one residue of GlcNac or GalNac, which is evidently the first monosaccharide of the O-unit whose transfer to a lipid carrier initiates the O-antigen biosynthesis. In addition to these and a number of other components already reported in the O-antigens of *S. enterica*, we identified 3-formamido-3,6-dideoxy-D-galactose (serogroup O60), *N*-acetyl-L-seryl (O56) and *N*-[(*S*)-3-hydroxybutanoyl]-D-alanyl (O58) derivatives of 4-amino-4,6-dideoxy-D-glucose and D-ribitol 5-phosphate (O47). Some O-polysaccharides are modified by non-stoichiometric O-acetylation (O16, O17, O53 and O66) or glucosylation (O16).

O-antigen gene clusters of all *S. enterica* strains studied were sequenced. The functions were tentatively assigned to the genes by a comparison with sequences in the available databases and were found to be in full agreement with the O-polysaccharide structures established. The structural relatedness of the O-antigens in the newly discovered pairs of the related *S. enterica* and *E. coli* O-serogroups was demonstrated to be due to the identity or close similarity of their O-antigen gene clusters. These findings may be useful for understanding serological relationships between the O-antigens in the family *Enterobacteriaceae* and for learning the evolutionary history of their diversification.

This work was supported by the Russian Foundation for Basic Research (Projects 08-04-01205 and 08-04-92225-NNSF).



4<sup>th</sup> Baltic meeting on  
Microbial Carbohydrates  
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**ABSTRACT - POSTER 43**

**Structural characterization of the lipid A from *Bradyrhizobium yuanmingense***

ADAM CHOMA<sup>1</sup>, IWONA KOMANIECKA<sup>1</sup>, ANNA TURSKA-SZEWCZUK<sup>1</sup>, BUKO LINDNER<sup>2</sup>, OTTO HOLST<sup>3</sup>

<sup>1</sup> Department of Genetics and Microbiology, Maria Curie-Skłodowska University, Akademicka 19, PL-20-033 Lublin (Poland)

Divisions of: <sup>2</sup> Immunochemistry and <sup>3</sup> Structural Biochemistry, Research Center Borstel, Parkallee 10 and 4a, Leibniz-Center for Medicine and Biosciences, D- 23845 Borstel (Germany)

At present, the *Bradyrhizobium* genus consists of eight species, i.e. *Bradyrhizobium canariense*, *B. elkanii*, *B. iriomotense*, *B. japonicum*, *B. jicamae*, *B. liaoningense*, *B. pachyrhizi*, and *B. yuanmingense*. The last species is a nitrogen-fixing microsymbiont of the legume genus *Lespedeza*.

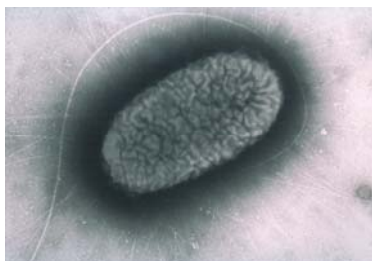
The chemical structure of the lipid A liberated by mild acid hydrolysis from the lipopolysaccharide of *Bradyrhizobium yuanmingense* type strain CCBAU 10071 was determined utilizing compositional, nuclear magnetic resonance spectroscopic, and mass spectrometric methods.

The lipid A was a mixture of species composed of an identical disaccharide acylated by 3-hydroxylauryl and 3-hydroxymyristyl primary fatty acids, which, in turn, were esterified with secondary very long chain ( $\omega$ -1)-hydroxylated fatty residues. Those substituents contained from 26 to 33 carbon atoms in the aliphatic chain. *B. yuanmingense* synthesized extremely hydrophobic lipid A species containing three or four acyloxyacyl residues. Moreover, one of these could be further acylated by 3-hydroxy butyric acid linked to the ( $\omega$ -1)-hydroxy group.

The disaccharide backbone was exclusively composed of 2,3-diamino-2,3-dideoxy-D-glucopyranose (Glc pN3N) which was substituted at both sides. The distal Glc pN3N was decorated with a Manp-(1→6)-Manp disaccharide, whereas the reducing hydroxyl of the proximal Glc pN3N was substituted by a galacturo lactone or with a galacturonic acid.

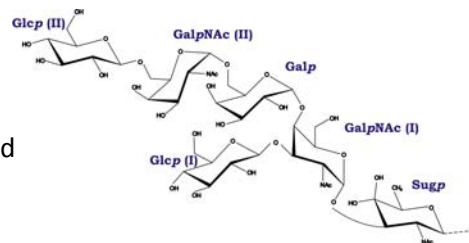
This work was partly supported by the Polish Ministry of Science and Higher Education (grant no. 303 109 32/3593).





## 4<sup>th</sup> Baltic meeting on Microbial Carbohydrates

Hyytiälä Forestry Field Station, Finland  
September 19-22, 2010



### ABSTRACT - **POSTER 44**

#### Structural characterization of the O-specific polysaccharide from the lipopolysaccharide of the fish pathogen *Aeromonas bestiarum* strain P1S

ANNA TURSKA-SZEWCZUK,<sup>1</sup> LESZEK GUZ,<sup>2</sup> HUBERT PIETRAS,<sup>1</sup> BUKO LINDNER,<sup>3</sup> IWONA KOMANIECKA,<sup>1</sup> ADAM CHOMA,<sup>1</sup> RYSZARD RUSSA<sup>1</sup> AND OTTO HOLST<sup>4</sup>

<sup>1</sup>Department of Genetics and Microbiology, M. Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland

<sup>2</sup>Department of Fish Diseases, Agriculture University, Akademicka 12, 20-033 Lublin, Poland

Divisions of <sup>3</sup>Biophysics and <sup>4</sup>Structural Biochemistry, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Parkallee 10 and 4a/c, 23845 Borstel, Germany

*Aeromonas* species are ubiquitous water-borne bacteria responsible for a wide spectrum of diseases among aquatic and terrestrial animals as well as being important pathogens of humans. *Aeromonas bestiarum* strain P1S was isolated from an outbreak of motile aeromonad septicaemia in a carp farm in Poland. The varied clinical picture of *Aeromonas* infections suggests a multifactorial model for the pathogenesis, in which the lipopolysaccharide plays a crucial role.

Sugar analysis of the *A. bestiarum* strain P1S O-specific polysaccharide (OPS) showed the presence of D-ribose (D-Rib), 3-amino-3,6-dideoxy-D-glucose (D-Qui3N), 2-amino-2,6-dideoxy-D-galactose (D-FucN) and D-galactose (D-Gal) residues at a molar ratio of ~1:1.4:1.2:2. GLC-MS analysis of the partially methylated monosaccharides as acetylated alditols-1-*d* resulted in the identification of 2-substituted Ribf, 4-substituted Galp, 2-substituted Qui3N, and 3-substituted FucpN. The spin systems for four monosaccharide residues  $\beta$ -Rib,  $\alpha$ -FucN,  $\beta$ -Qui3N, and  $\beta$ -Gal were identified on the basis of the <sup>1</sup>H and <sup>13</sup>C chemical shifts and the coupling constant values, and those of amino sugars were confirmed by correlations revealed by the <sup>1</sup>H, <sup>13</sup>C HSQC experiment. The <sup>13</sup>C NMR spectrum of the studied OPS contained, *inter alia*, signals for four anomeric carbons at  $\delta$  96.85, 104.78, 105.83 and 107.65, two nitrogen-bearing carbons (FucN C-2, and Qui3N C-3) at  $\delta$  48.42 and 56.35, two methyl groups of 6-deoxysugars (FucN and Qui3N) at  $\delta$  16.28 and 17.85, one *N*-acetyl group (CH<sub>3</sub> at  $\delta$  23.2 and CO group at 175.04), and one *N*-(3-hydroxybutyryl) group (CH<sub>3</sub> at  $\delta$  22.87 and CO group at  $\delta$  175.19, CH-OH and CH<sub>2</sub> at  $\delta$  65.60 and 45.94, respectively). The presence of the signal at  $\delta$  84.28 confirmed the occurrence of ribose in the furanose form. The site of attachment of the 3-hydroxybutyryl group at the amino group of Qui3N was confirmed by the cross-peak at  $\delta_C/\delta_H$  175.19/3.96 observed on the <sup>1</sup>H, <sup>13</sup>C HMBC spectrum. The charge-deconvoluted ESI FT-ICR MS spectrum (negative-ion mode) of the OPS showed mass peaks at 2867.2, 3579.5, 4291.8, 5004.1, 5716.4, and 6428.7 u, with the difference in the molecular mass of 712.3 Da that corresponded to the molecular mass of the repeating unit composed of RibGalFucNAcQui3Nacyl. The sequence of the sugar residues in the repeating unit determined by <sup>1</sup>H, <sup>1</sup>H NOESY and <sup>1</sup>H, <sup>13</sup>C HMBC experiments had the following structure:

→2)- $\beta$ -D-Ribf-(1→4)- $\beta$ -D-Galp-(1→3)- $\alpha$ -D-FucpNAc-(1→2)- $\beta$ -D-Quip3Nacyl-(1→