ABSTRACT BOOK

INTERNATIONAL CONFERENCE ON TYPE IV SECRETION SYSTEM

17 - 20 February 2025
Nancy, France

T4SS

Structure

Function

Impact

SUMMARY

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ORAL COMMUNICATIONS

Detailed Program of Oral Communications

Session 1:

T4SS structure and function:
Structure and dynamics - Assembly mechanisms

S1.1	Monday 17th February – 14:45-16:40 Amphitheater K03 - Building K Chair: Elisabeth Grohmann	
14:45	KEYNOTE 45 min	Progress in the structural and molecular biology of bacterial conjugation in Gram-negative bacteria Gabriel WAKSMAN *Institute of Structural and Molecular Biology, Birkbeck, London, UK* *Institute of Structural and Molecular Biology, University College London, London, UK*
15:30	Short talk 20 min	Elucidating assembly and function of VirB8 cell wall subunits refines the DNA translocation model in Gram-positive Type IV secretion system Robine MAFFO-WOULEFACK *Université de Lorraine, INRAE, DynAMic, Nancy, France*
15:50	Short talk 20 min	Uncovering the molecular architecture of a Gram-positive Type IV secretion system Kieran DEANE-ALDER *Department of Medical Biochemistry & Biophysics, Umeå University, Sweden*
16:10	Main talk 30 min	Structural and functional analysis of the Legionella pneumophila Dot/Icm Type IV secretion system Melanie OHI *Life Sciences Institute, University of Michigan, Ann Arbor, USA* *Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, USA*

S1.2	Monday 17th February – 17:10-18:20 Amphitheater K03 - Building K Chair: Elisabeth Grohmann	
17:10	Main talk 30 min	Functional and Structural Characterization of essential and regulatory components of a Gram-positive Type IV secretion system Walter KELLER *Institute of Molecular Biosciences, University of Graz, Austria* *BioTechMed Graz, Graz, Austria*
17:40	Short talk 20 min	The VirB type IV secretion system is localized at the growth pole in *Brucella* abortus Charline FOCANT *Research Unit in Biology of Microorganisms (URBM), University of Namur* *Research Institute for Life Sciences (NARILIS), University of Namur*
18:00	Short talk 20 min	Molecular interactions required for secretion of Helicobacter pylori CagA Chiamaka OKOYE *Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN, USA*

Session 2:

Host-Pathogen Interactions:
Effectors diversity and function

S2	Tuesday 18th February – 9:00-11:15 Amphitheater K03 - Building K Chair: Abdelrahim Zoued	
9:00	KEYNOTE 45 min	The Ins and Outs of *Brucella*: deciphering the role of effectors in pathogenesis Suzana SALCEDO Department of Pathobiological Sciences, University of Wisconsin–Madison, USA
9:45	Short talk 20 min	Characterization of a new nucleomodulin of *Legionella pneumophila* Monica ROLANDO *Institut Pasteur, Université Paris Cité, Biologie des Bactéries Intracellulaires, Paris, France*
10:05	Short talk 20 min	The multifunction Coxiella effector Vice stimulates macropinocytosis and interferes with the ESCRT machinery Matteo BONAZZI *Institut de Recherche en Infectiologie de Montpellier (IRIM), CNRS, Université de Montpellier, France*
10:25	Short talk 20 min	Disruption of the nucleoli and translation by a *Legionella* Dot/Icm T4SS effector Gunnar SCHROEDER *Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK*
10:45	Main talk 30 min	Composition and function of the Helicobacter pylori cag pathogenicity island encoded type IV secretion system Steffen BACKERT *Friedrich Alexander University Erlangen-Nuremberg, Department of Biology, Division of Microbiology, Erlangen, Germany*

Session 3:

T4SS Structure and Function:
Conjugative systems - DNA import/export systems

S3	Tuesday 18th February – 16:30-18:45 Amphitheater K03 - Building K Chair: Kevin Macé	
16:30	KEYNOTE 45 min	Structural and Functional Plasticity of the T4SSs Exemplified Through Studies of the F system Peter CHRISTIE *Microbiology and Molecular Genetics, McGovern Medical School, UTHealth Houston, Texas, USA*
17:15	Short talk 20 min	Visualizing the structure and dynamics of the horizontal gene transfer during bacterial conjugation Shubha UDUPA *Department of Biochemistry and Pharmacology, University of Melbourne, Melbourne, VIC, Australia*
17:35	Short talk 20 min	Exploration of DNA processing mediated by MOBT relaxases during bacterial conjugation Haifa LAROUSSI *Université de Lorraine, INRAE, DynAMic, Nancy, France*
17:55	Short talk 20 min	Cryo-EM structure of the F plasmid relaxosome provides a molecular basis for DNA recruitment and processing in bacterial conjugation Sunanda WILLIAMS *Institute of Structural and Molecular Biology, School of Natural Sciences, Birkbeck College, London, UK*
18:15	Main talk 30 min	Molecular Mechanism For Loading Two Relaxases During Conjugation Initiation Aravindan ILANGOVAN *School of Biological and Behavioural Sciences, Queen Mary University of London, UK*

Session 4:

Bioinformatics, Genomics and Evolution:

Detection and classification, Diversity and Evolution, Bioinformatic Tools

S4	Wednesday 19th February – 8:45-11:00 Amphitheater K03 - Building K Chair: Virginie Libante	
8:45	KEYNOTE 45 min	Unveilling Integrative Conjugative Elements: detection and annotation in *Bacillota genomes* Hélène CHIAPELLO[1] and Nathalie LEBLOND-BOURGET[2] *1 - MaIAGE, Université Paris-Saclay, INRAE, Jouy-en-Josas, France* *2 - Université de Lorraine, INRAE, DynAMic, Nancy, France*
9:30	Short talk 20 min	PO-CBR for delimiting integrated mobile elements transferred by conjugation Toufik HAMADOUCHE *DynAMic, Université de Lorraine, INRAE, Nancy, France* *LORIA, Université de Lorraine, CNRS, Inria, Nancy, France*
9:50	Short talk 20 min	Prevalence and diversity of Integrative and conjugative or mobilizable elements carrying antimicrobial resistance genes in *Streptococcus suis* Sophie PAYOT *DynAMic, Université de Lorraine, INRAE, Nancy, France*
10:10	Short talk 20 min	Conjugative sabotage: new fertility inhibitors against broad-host-range plasmids Daniel GARCIA-LOPEZ *Instituto de Biomedicina y Biotencología de Cantabria, UC-CSIC, Santander, Spain*
10:30	Main talk 30 min	Evolution of plasmid mobility: origin and fate of non-conjugative plasmids Charles COLUZZI *Institut Pasteur, Université de Paris Cité, CNRS, UMR3525, Microbial Evolutionary Genomics, Paris, France*

Session 5:

Host-Pathogen Interactions:
Non-Canonical Systems, Interbacterial Systems

S5	Wednesday 19th February – 11:20-12:30 Amphitheater K03 - Building K Chair: Ronnie Bertsson	
11:20	Main talk 30 min	Substrate selection and fratricide avoidance by the bactericidal Type IV secretion system of *Xanthomonadaceae* Chuck FARAH *Departamento de Bioquímica, Universidade de São Paulo, São Paulo, Brazil*
11:50	Short talk 20 min	Type IV secretion system drives interbacterial competition in the plant pathogen *Xanthomonas* Chloé PEDUZZI *Earth & Life Institute, applied microbiology-plant health UCLouvain, Louvain-la-Neuve, Belgium*
12:10	Short talk 20 min	The non-canonical TraB-dependent Actinomycete ICEs (AICEs) mediate large-scale chromosomal transfer in *Streptomyces* Pauline GASCHT *Université de Lorraine, INRAe, DynAMic, Nancy, France*

Session 6:

Host-Pathogen Interactions:
Targeting eucaryotic signaling pathways

S6.1	Wednesday 19th February – 14:00-15:25 Amphitheater K03 - Building K Chair: Steffen Backert	
14:00	KEYNOTE 45 min	Effectors of *Legionella pneumophila* and their interaction with the host ubiquitin system Sagar BHOGARAJU *EMBL Grenoble, Auvergne-Rhône-Alpes, France*
14:45	Short talk 20 min	Revisiting effector functions: involvement of a putative T4SS effector in the envelope integrity of *Brucella* Maren KETTERER *Biozentrum, University of Basel, Basel, Switzerland*
15:05	Short talk 20 min	Exploring novel protein-protein interactions and functions of selected Helicobacter pylori Cag Type 4 secretion system (CagT4SS) outer proteins Christine JOSENHANS *Max von Pettenkofer-Institut, Chair for Medical Bacteriology and Hygiene, Ludwig Maximilian University Munich, München, Germany*

S6.2	Wednesday 19th February – 16:30-17:20 Amphitheater K03 - Building K Chair: Steffen Backert	
16:30	Main talk 30 min	Translocation of YopJ family effector proteins through the VirB/VirD4 T4SS of *Bartonella* Christoph DEHIO *Biozentrum, University of Basel, Basel, Switzerland*
17:00	Short talk 20 min	*Legionella pneumophila*: Assessment of effector secretion kinetics in real time in Acanthamoeba Carmen BUCHRIESER *Institut Pasteur, Université Paris Cité, Biologie des Bactéries Intracellulaires,Paris, France*

Session 7:

T4SS Structure and Function :
T4SS effectors recruitment

S7	Thursday 20th February – 8:45-10:05 Amphitheater K03 - Building K Chair: Christine Josenhans	
8:45	Main talk 30 min	The T4bSS of *Legionella* features a two-step secretion pathway with an inner membrane intermediate for secretion of transmembrane effectors Samuel WAGNER *Interfaculty Institute of Microbiology and Infection Medicine, Cellular and Molecular Microbiology, University of Tübingen, Tübingen, Germany Excellence Cluster 'Controlling Microbes to Fight Infections', University of Tübingen, Tübingen, Germany German Center for Infection Research, partner site Tübingen, Tübingen, Germany*
9:15	Short talk 20 min	Exploring the structure and function of *Brucella* abortus virulence factor VirJ in T4SS effectors translocation Chloé DUGELAY *CNRS-Université Lyon UMR5086, Molecular Microbiology and Structural Biochemistry, Lyon, France*
9:35	Main talk 30 min	Architectural asymmetry enables DNA transport through the Helicobacter pylori cag Type IV secretion system Carrie SHAFFER *Department of Microbiology, Immunology, and Molecular Genetics, University of Kentucky, Lexington, USA* *Department of Veterinary Science, University of Kentucky, Lexington, USA* *Markey Cancer Center, University of Kentucky, Lexington, USA*

Session 8:

Impact of Conjugative Transfer:
Dissemination in the environment, Consequence on bacterial fitness, Mobilization

S8	Thursday 20th February – 10:25-13:00 Amphitheater K03 - Building K Chair: Xavier Bellanger	
10:25	KEYNOTE 45 min	Insights into the T4SS-mediated spread of plasmids and antimicrobial resistance Uli KLÜMPER *TU Dresden, Dresden, Germany*
11:10	Short talk 20 min	Bacterial conjugation – based tools for manipulating and exploring long-range horizontal gene transfer Matxalen LLOSA *IBBTEC, University of Cantabria, Santander, Spain*
11:30	Short talk 20 min	Structural insights into a ParB-like protein of the F-plasmid that reprograms the gene expression profile of the recipient cell Laurent TERRADOT *Molecular Microbiology and Structural Biochemistry (MMSB), Université Lyon 1, CNRS, Inserm, UMR5086, Lyon, France*
11:50	Short talk 20 min	Competition between conjugative plasmids by fertility inhibition protein Sarah BIGOT *Laboratory of Molecular Microbiology and Structural Biochemistry (MMSB), CNRS-University Lyon,France*
12:10	Short talk 20 min	Uncovering the role of IMEs in antimicrobial resistance and horizontal gene transfer Virginie LIBANTE *DynAMic, Université de Lorraine, INRAE, Nancy, France*
12:30	Main talk 30 min	Entry and surface exclusion of IncC plasmids and SGI1: an asymmetric interplay gene transfer Vincent BURRUS *Department de biologie, Université de Sherbrooke, Sherbrooke, Canada*

Summary

Abstracts of Oral Communications

Session 1:

T4SS structure and function:
Structure and dynamics - Assembly mechanisms

Elucidating assembly and function of VirB8 cell wall subunits refines the DNA translocation model in Gram-positive T4SSs

Robine MAFFO-WOULEFACK[1], Abbas MOHAMAD ALI[1], Haifa LAROUSSI[1], Julien CAPPÈLE[2], Felipe ROMERO-SAAVEDRA[3], Nancy RAMIA[1], Emilie ROBERT[1#], Sandrine MATHIOT[2], Nicolas SOLER[1], Yvonne ROUSSEL[1], Rémi FRONZES[4], Johannes HUEBNER[3], Claude DIDIERJEAN[2], Frédérique FAVIER[2*], Nathalie LEBLOND-BOURGET[1*]and Badreddine DOUZI[1]

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ABSTRACT

Conjugative Type IV secretion systems (T4SSs) are membrane-spanning multiprotein complexes specialized in the transfer of mobiles genetic element between bacteria. They are considered as main drivers for the spread of antibiotic resistance genes. While T4SSs are extensively studied in Gram-negative bacteria, their architecture and mode of function in Gram-positive bacteria remain poorly understood[1]. According to the envelope structure in Gram-positive bacteria, it has been proposed that T4SSs are made by two functional complexes, the cell wall complex and the translocon[2]. To bring new insights into the biology of these bacterial nanomachines, we focused on this study on characterizing VirB8-like proteins. Our funding revealed that the VirB8-like are cell wall components where they act as trimeric subunits, adopting a conserved assembly pattern, distinct from their counterparts in Gram-negative bacteria. Additionally, we identified the interaction of VirB8-like proteins with T4SS components, in particular the predicted translocon components, highlighting the central role of VirB8-like proteins in T4SS assembly. Notably, we revealed a direct interaction between VirB8-like proteins and DNA, indicating their direct involvement in DNA transfer during conjugation. Altogether, our data allowed us to propose a model of Gram-positive T4SSs, where VirB8-like proteins, assemble a channel-like structure in the cell wall, a model distinct from what it was described in Gram-negative bacteria[3].

KEY WORDS: Conjugation, T4SS, VirB8-like, Gram-positive bacteria.

REFERENCES

[1] Bhatty, M., Laverde Gomez, J.A., Christie, P.J., 2013. The expanding bacterial type IV secretion lexicon. Res Microbiol 164, 620–639. https://doi.org/10.1016/j.resmic.2013.03.012.

[2] Grohmann, E., Christie, P.J., Waksman, G., Backert, S., 2018. Type IV secretion in Gram-negative and Gram-positive bacteria. Mol Microbiol 107, 455–471. https://doi.org/10.1111/mmi.13896

[3] Maffo-woulefack, R., Mohamad Ali, A., Laroussi, H., CAPPELE, J., Romero Saavedra, F., Ramia, N., Robbert, E., Mathiot, S., Soler, N., Roussel, Y., Fronzes, R., HUEBNER, J., Didierjean, C., Favier, F., Leblond-Bourget. N., Douzi, B. 2024. Elucidating assembly and function of VirB8 cell wall subunits refines the DNA translocation model in Gram-positive T4SSs. *In revision*.

Uncovering the molecular architecture of a Gram-positive type 4 secretion system

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ABSTRACT

Gram-positive (G+) bacteria are major contributors to the burden of antimicrobial resistance. The spread of resistance is facilitated by the transfer of resistance genes through conjugal type 4 secretion systems (T4SSs), which transport single-stranded DNA from host to donor cells. Recent advances in cryogenic electron microscopy have enabled a high-resolution structure of the T4SS from the Gram-negative (G-) *E. coli* R388 plasmid (1). However, no overall structure is available for any T4SS of G+ origin, though we have characterized some components of the T4SS encoded by the pCF10 plasmid in the G+ organism *Enterococcus faecalis* (2). Here we present three ongoing approaches to elucidate a more complete structure.

Firstly, we used recent developments in bioinformatics tools to build an *in silico* structure model of the core channel. As the membrane-associated subunits of the T4SS from pCF10 have little sequence identity to any proteins from G- systems, we employed AlphaFold3 (AF3) and Foldseek (3) in a 'divide and conquer' approach. These results indicate that the central channel might be more structurally conserved than previously predicted. Combining the AF3 predictions, experimental data, and homology to G- systems, we constructed a model that can be used as a starting point for future experiments.

Secondly, we have endogenously expressed affinity-tagged pCF10 T4SS and purified the assembled native channel. Using negative stain EM, we obtained the first low-resolution 2D images of a G+ T4SS. Pleasingly, the channel is intact and similar to the assembly predicted by AF3. Efforts to obtain a high-resolution structure using cryo-EM are ongoing.

Thirdly, we present the basic groundwork towards using cryogenic electron tomography (cryo-ET) to study the full pCF10-encoded T4SS *in situ*. Together, these approaches allow us to better understand the structure and function of the pCF10 T4SS, and G+ T4SS in general.

KEYWORDS: *cryo-EM, cryo-ET, AlphaFold*

REFERENCES

[1] Macé K, Vadakkepat AK, Redzej A, Lukoyanova N, Oomen C, Braun N, et al. (2022) Cryo-EM structure of a type IV secretion system. *Nature*. 607(7917):191–6.

[2] Sun WS, Torrens G, Ter Beek J, Cava F, Berntsson RPA. (2024) Breaking barriers: pCF10 type 4 secretion system relies on a self-regulating muramidase to modulate the cell wall. *mBio*. 15(8):e00488-24.

[3] van Kempen M, Kim SS, Tumescheit C, Mirdita M, Lee J, Gilchrist CLM, et al. (2024) Fast and accurate protein structure search with Foldseek. *Nat Biotechnol*. 42(2):243–6.

Structural and functional analysis of the *Legionella pneumophila Dot/Icm* Type IV secretion system

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ABSTRACT
Legionella pneumophila is a pathogenic gram-negative bacterium that causes Legionnaires' disease. The main virulence factor of *L. pneumophila* is its Dot/Icm Type IV Secretion System (T4SS). The T4SS injects effector proteins into the cytoplasm of the host cell, allowing the bacterium to establish a replicative niche. The Dot/Icm T4SS machinery localized between the bacterial inner and outer membranes is the called the outer membrane core complex (OMCC) and can be divided into subcomplexes termed the dome, an outer membrane cap (OMC), a periplasmic ring (PR), and a stalk. We have determined high-resolution structures of the Dot/Icm T4SS OMCC using cryo-electron microscopy (cryo-EM)[1,2]. This structural analysis showed multiple symmetry mismatches between the dome, OMC, and PR and identified previously uncharacterized components of the Dot/Icm T4SS (Dis1, Dis2, and Dis3)[1,2]. In this study we have determined a structure of the Dot/Icm T4SS OMCC that includes the stalk and a "plug", allowing us to identify additional symmetry mismatches across the complex. We also characterize how Dis2 and Dis3 contribute to the structure of the T4SS and pathogenicity of *L. pneumophila*. We found that OMCCs purified from a *Δdis2* strain are only missing the density for Dis2, while OMCCs purified from the *Δdis3* strain lack densities for Dis3 and DotF. Despite missing proteins, the mutant OMCCs purified from each strain remain structurally stable. Both the *Δdis2* and *Δdis3* strains efficiently replicate in human macrophages; however, they do have different rates of translocation for four tested substrates. In summary, our structural and functional analysis of the Dot/Icm T4SS characterizes the structure of the stalk and plug, identifies new symmetry mismatches across the complex, and explores the importance of Dis2 and Dis3 for T4SS translocation.

KEYWORDS: *Legionella pneumophila, cryo-EM, biochemistry*.

REFERENCES

[1] Durie, C. L.; Sheedlo, M. J.; Chung, J. M.; Byrne, B. G.; Su, M.; Knight, T.; Swanson, M.; Lacy, D. B.; Ohi, M. D. Structural Analysis of the *Legionella pneumophila* Dot/Icm Type IV Secretion System Core Complex. *eLife* **2020**, *9*, e59530. https://doi.org/10.7554/eLife.59530.

[2] Sheedlo, M. J.; Durie, C. L.; Chung, J. M.; Chang, L.; Roberts, J.; Swanson, M.; Lacy, D. B.; Ohi, M. D. Cryo-EM Reveals New Species-Specific Proteins and Symmetry Elements in the *Legionella pneumophila* Dot/Icm T4SS. *eLife* **2021**, *10*, e70427. https://doi.org/10.7554/eLife.70427.

Functional and Structural Characterization of essential and regulatory components of a Gram-positive Type IV Secretion System (T4SS).

Kirill KUHLMANN[1], Tamara BERGER[1], Claudia MICHAELIS[2], Anna REISENBICHLER[1], Tea PAVKOV-KELLER[1,4], Bernd GESSLBAUER[3,4], Elisabeth GROHMANN[2] and **Walter KELLER[1,4]**

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ABSTRACT

The occurrence of multi-resistant strains among pathogenic bacteria, many of which are causing severe nosocomial infections, is one of the most pressing problems of our health system. Bacterial conjugation is one of the important mechanisms of DNA transfer between bacteria and mediates the rapid spread of bacterial resistances within bacterial communities. Type IV secretion systems (T4SS) are the molecular machineries enabling the efficient transport of nicked, single-stranded plasmid DNA across the cell walls of the donor as well as the recipient cell [1]. The T4SS from the antibiotic resistance plasmid pIP501, occurring in *Enterococci* and related Gram-positive bacteria, is encoded within a single operon comprised of 15 putative transfer factors.

Here we present our investigations on essential factors of the pIP501 encoded T4SS: the relaxase TraA and its DNA-binding behavior, the putative recruitment factor TraB [2] and the essential component of the core complex TraF. We also present the mutational study of the DNA binding protein TraN [3], which is an effective repressor of transcription and plays a regulatory role in the formation of the T4SS complex.

KEYWORDS: *relaxase, TraA, repressor, DNA-binding, recruitment factor, pIP501, Enterococcus faecalis*

REFERENCES

1. Alvarez-Martinez CE, and Christie PJ (2009) Biological diversity of prokaryotic type IV secretion systems. *Microbiol Mol Biol Rev* **73**, 775-808.
2. Berger TMI, Michaelis C, Probst I, Sagmeister T, Petrowitsch L, Puchner S, Pavkov-Keller T, Gesslbauer B, Grohmann E, Keller W. (2022) *Front Mol Biosci. 9:867136*. doi: 10.3389/ fmolb.2022.867136.
3. Michaelis C, Berger TMI, Kuhlmann K, Ghulam R, Petrowitsch L, Besora Vecino M, Gesslbauer B, Pavkov-Keller T, Keller W, Grohmann E. (2024) Effect of TraN key residues involved in DNA binding on pIP501 transfer rates in Enterococcus faecalis. *Front Mol Biosci. 11:1268647.* doi: 10.3389/fmolb.2024.1268647.

The VirB type IV secretion system is localized at the growth pole in *Brucella abortus*

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ABSTRACT

Brucella abortus is an intracellular pathogenic alpha-proteobacterium, causing bovine brucellosis. It resides in three types of vacuoles (endosomal, replicative and autophagic, respectively eBCV, rBCV and aBCV where BCV stands for *Brucella*-containing vacuole) [1]. To reach its replication niche (rBCV), *Brucella* requires a type IV secretion system (T4SS) named VirB. VirB, formed by a complex of ten multimeric proteins, injects effector proteins into the host cell [2]. Our team previously demonstrated that unipolar growth of *B. abortus* begins in eBCVs [3], where T4SS expression is induced by starvation and acidic pH. We hypothesized that envelope generation at the growth pole could be accompanied by VirB incorporation into the envelope. To follow the biogenesis of the T4SS, translational fusions for different VirB subunits with mNeonGreen were constructed. The residual virulence during infection was tested to determine if the fusions preserve VirB function, which was the case for most of the C-terminal fusions since these strains were able to replicate intracellularly. Strains with a functional T4SS were analyzed by fluorescence microscopy during macrophage infection to localize the fused VirB subunits. We were able to localize the two ATPases of the system, VirB4 and VirB11, and also VirB3 from the Inner Membrane Complex, strictly to the growth pole. VirB3 and VirB4 show a circular subpolar pattern, reminiscent of the RgsE/GPR localization. VirB11, the last subunit recruited to the T4SS, shows a focalized localization at the growth pole. These patterns of localization are independent of the presence of a O-chain in the lipopolysaccharide. A vast majority (90 to 96%) of the bacteria detected in macrophages display of polarly localized VirB, arguing against population heterogeneity based on T4SS expression. These data suggest VirB is recruited to the growth pole, opening new avenues to study connections between envelope growth and virulence mechanisms in *B. abortus*.

KEYWORDS*: Brucella, VirB, pole, growth, infection, BCV*

REFERENCES

[1] Celli, J. The intracellular life cycle of *Brucella* spp. *Microbiol. Spectr.* 7, 1–11 (2019).

[2] O'Callaghan, D., *et al.* A homologue of the *Agrobacterium tumefaciens* VirB and *Bordetella pertussis* Ptl type IV secretion systems is essential for intracellular survival of *Brucella suis*. *Molecular microbiology*, *33*(6), 1210-1220. (1999).

[3] Deghelt, M., *et al*. G1-arrested newborn cells are the predominant infectious form of the pathogen *Brucella abortus*. *Nat. Commun.* 5, 1–12 (2014).

Molecular interactions required for secretion of *Helicobacter pylori* CagA

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ABSTRACT

Gastric colonization with *Helicobacter pylori* strains that produce CagA and the Cag Type IV Secretion System (T4SS) confers an increased risk of stomach cancer. The Cag T4SS delivers CagA, a bacterial oncoprotein, into gastric cells. Our previous studies showed that CagF, a putative chaperone for CagA, can serve as a bait for isolating CagA and the Cag T4SS outer membrane core complex (OMCC), but the molecular interactions involved in this process remain elusive. Single-particle cryo-EM analyses of the OMCC revealed that it contains multiple copies of 5 proteins (CagY, CagX, CagT, CagM, and Cag3). To elucidate molecular interactions required for the binding of CagA to CagF and binding of the CagA-CagF complex to the Cag T4SS OMCC, we used a crosslinking mass spectrometry approach. We purified CagF and CagA from a T4SS-assembly deficient strain of *H. pylori* (Δ*cagX*), and we purified partially assembled OMCC complexes, consisting of only CagX and CagY, from a Δ*cagM* mutant. We then treated the complexes with the amine-reactive crosslinker BS3. Samples were analyzed by tandem mass spectrometry to identify sites of monolinks, intraprotein crosslinks, and interprotein crosslinks. Our analysis of the CagA-CagF complex revealed 83 interprotein crosslinks, distributed in multiple domains of CagA. Our analysis of samples from the Δ*cagM* mutant revealed 41 CagX-CagY crosslinks, 12 CagF-CagA crosslinks, and 2 CagF-CagX crosslinks. Within the cryo-EM structure of the Cag T4SS OMCC, the calculated distances between multiple crosslinked CagX/CagY lysine residues were within 30 Å. Structural models of a CagF-CagA complex were generated using AlphaFold 3; the calculated distances between multiple crosslinked CagF/CagA lysine residues in the model were within 30 Å. These results provide new insights into the molecular interactions required for secretion of CagA and suggest that CagF has important chaperone and adaptor functions in the recruitment of CagA to the Cag T4SS.

KEYWORDS: *Helicobacter pylori, Cag T4SS, gastric cancer.*

REFERENCES

[1] Frick-Cheng, A. E., Pyburn, T. M., Voss, B. J., McDonald, W. H., Ohi, M. D., & Cover, T. L. (2016). Molecular and structural analysis of the *Helicobacter pylori* cag type IV secretion system core complex. *mBio*, 7(1), 10-1128.

[2] Sheedlo, M. J., Chung, J. M., Sawhney, N., Durie, C. L., Cover, T. L., Ohi, M. D., & Lacy, D. B. (2020). Cryo-EM reveals species-specific components within the *Helicobacter pylori* Cag type IV secretion system core complex. *eLife*, *9*, e59495.

[3] Roberts, J. R., Tran, S. C., Frick-Cheng, A. E., Bryant, K. N., Okoye, C. D., McDonald, W. H., Cover, T.L., & Ohi, M. D. (2024). Subdomains of the *Helicobacter pylori* Cag T4SS outer membrane core complex exhibit structural independence. *Life Science Alliance*, *7*(6).

Session 2:

Host-Pathogen Interactions:
Effectors diversity and function

Legionella pneumophila LpDot1 interferes with host nuclear functions by altering paraspeckles

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ABSTRACT

Understanding how pathogens manipulate the epigenetic regulation of the host to proliferate and survive may help finding new strategies to fight infectious diseases (1). One of these pathogens is *Legionella pneumophila*, a bacterium that replicates naturally in aquatic amoeba, but can also infect human cells and cause a severe pneumonia in humans, called Legionnaires' disease. Uniquely, *L. pneumophila* encodes a large repertoire of proteins encoding eukaryotic-like motifs acquired from its hosts and translocated via a specialized T4SS called Dot/Icm. Some of them target the host cell nucleus and reprogram the cellular response to the bacterial advantage (2). Here we identified and characterized a *L. pneumophila* protein, predicted to encode a lysine methyltransferase similar to human Dot1L (disruptor of telomeric silencing-1), therefore named *Lp*Dot1. In humans, Dot1L is known to catalyze the methylation of Lys79 on histone H3 (H3K79). Interestingly, in the genome of its protozoan host *Acanthamoeba castellanii*, we identified three Dot1-like proteins: two that are more similar to the human one, and a shorter version that is similar to the bacterial LpDot1, suggesting that the *lpDot1* gene was acquired through horizontal gene transfer from its protozoan host. Here we hypothesized that LpDot1 mimics the functions of its homologous proteins in eukaryotes. We solved the crystal structure of LpDot1 and determined its 3D structure at >2.4Å resolution. By using a MS/MS approach on the nuclear cell proteome, we identified specific LpDot1 targets and could show that LpDot1 is not a histone methyltransferase like in humans, but it has methyltransferase activity on nuclear non-histone proteins, predominantly RNA binding proteins that play multiple regulatory roles in the nucleus, such as subnuclear body formation, transcriptional regulation, genome stability and alternative splicing. For one of them we identified the unique site of methylation, that occurs in a structural motif important for its activities. Furthermore, we observed that *L. pneumophila* infection causes its degradation in a *Lp*Dot1-dependent manner, altering subnuclear body formation with resulting in a slight influence of LpDot1 on protein synthesis and a dramatic effect on alternative splicing on a gene involved in cell death. Altogether, these results suggest that *L. pneumophila* hijacks host nuclear functions through a unique secreted methyltransferases that modifies a RNA binding protein in the host cell to help its intracellular survival.

KEYWORDS: *Legionella pneumophila*, epigenetics, nuclear effectors

REFERENCES

[1] Bierne H, Pourpre R. 2020. Bacterial Factors Targeting the Nucleus: The Growing Family of Nucleomodulins. Toxins (Basel) 12:220–19.

[2] Schator D, Mondino S, Berthelet J, Silvestre CD, Assaya MB, Rusniok C, Rodrigues-Lima F, Wehenkel A, Buchrieser C, Rolando M. 2023. Legionella para-effectors target chromatin and promote bacterial replication. Nat Commun 14:2154.

The multifunction *Coxiella* effector Vice stimulates macropinocytosis and interferes with the ESCRT machinery

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ABSTRACT

Coxiella burnetii, the causal agent of the Q fever, is an obligate intracellular pathogen that replicates within host cells. This lifestyle relies on the formation of *Coxiella*-containing vacuoles (CCVs), specialized vacuoles that provide a shielded environment for bacterial replication, enabling *C. burnetii* to evade host immune defences and establish persistent infections[1]. CCV development hinges on bacterial effector proteins translocated into host cells through a Type 4 Secretion System (T4SS), which interact with host proteins to subvert membrane trafficking. Expanding on the earlier identification of CvpB as a *C. burnetii* Lipid-Interacting Effector (LIE) that manipulates PI(3)P metabolism[2], we have conducted further investigations into the lipid composition of CCVs. Use of lipid binding probes revealed that PI(3)P, PI(4)P and LBPA were actively enriched at the CCV by T4SS effectors. We have identified the effector Vice as an additional LIE responsible for LPBA enrichment at the CCV. Vice interacts with phosphoinositides and late endosomal membranes, enriched in phosphatidylserine (PS) and lysobisphosphatidic acid (LBPA)[3]. CCVs generated by the *vice*::Tn mutants are negative for LBPA, showed a strong defect in size and an altered morphology as compared to wt CCVs. Strikingly, ectopic expression of Vice in eukaryotic cells leads to the formation of compartments reminiscent of early CCVs in both morphology, protein and lipid composition. The biogenesis of these Vice-induced compartments (VICs) is driven by an intriguing dual role of Vice. Initially, the effector localizes at the plasma membrane of eukaryotic cells, triggering the internalization of large vacuoles through the induction of macropinocytosis. Subsequently, Vice stabilizes VICs by disrupting the ESCRT machinery, impeding the formation of intraluminal vesicles (ILVs), with consequences on the release of extracellular vesicles (EVs). Taken together, our findings highlight Vice as a pivotal *C. burnetii* effector capable of exploiting two major cellular pathways to shape the bacterial replicative environment[3].

KEYWORDS: *Host-pathogen interactions, effector proteins, Coxiella burnetii, intracellular traffic*

REFERENCES

1. Burette, M. & Bonazzi, M. From neglected to dissected: How technological advances are leading the way to the study of Coxiella burnetii pathogenesis. *Cell Microbiol* **22**, e13180 (2020).

2. Martinez, E. *et al.* Coxiella burnetii effector CvpB modulates phosphoinositide metabolism for optimal vacuole development. *Proc National Acad Sci* **113**, E3260–E3269 (2016).

3. Bienvenu, A. *et al.* The multifunction Coxiella effector Vice stimulates macropinocytosis and interferes with the ESCRT machinery. *Proc. Natl. Acad. Sci.* **121**, e2315481121 (2024).

Disruption of the nucleoli and translation by a *Legionella* Dot/Icm T4SS effector

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ABSTRACT

Upon host cell contact, the facultative intracellular bacterial pathogen *Legionella pneumophila* translocates more than 300 effector proteins through the Dot/Icm type IV secretion system. These effector proteins manipulate numerous cellular processes to the benefit of the bacteria, enabling the evasion of phagolysosomal degradation and the generation of the replication-permissive *Legionella*-containing vacuole.

Using bioinformatics we identified a family of peptidase C58-like T4SS effectors encoded in a wide variety of *Legionella* species. Functional analysis of the most conserved member of the family revealed that it is a protease that cleaves itself and, upon ectopic expression, has profound effects on the physiology of eukaryotic cells, inducing changes to ER structure, Golgi apparatus and nuclear morphology, without causing cell membrane permeability or lysis.

Substrate trapping experiments with the inactive enzyme in combination with mass spectrometry yielded a large number of interactors involved in ribosome biogenesis and function. Subsequent *in vitro* and *in cellulo* analysis showed that the active protease compromised translation directly but in cells in addition triggered a complete loss of the nucleoli and accumulation of nucleolar proteins in the cytoplasm.

During infection, the effector did not induce as profound effects on host cell physiology as upon ectopic expression; likely because *L. pneumophila* 130b seems to control its expression tightly and delivers only small amounts of the effector. Nevertheless, reduced replication of the *L. pneumophila* strain lacking the effector in macrophages and mice showed that it has a significant, non-redundant role for the manipulation of host cells by the bacteria.

KEYWORDS: *Legionella, Dot/Icm effector, functional characterization, protease, translation, nucleoli*

REFERENCES

[1] Lockwood, Amin, Costa and Schroeder (2022). The *Legionella pneumophila* Dot/Icm type IV secretion system and its effectors. Microbiology; vol. 168, issue 5 DOI 10.1099/mic.0.001187

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Composition and function of the Helicobacter pylori cag pathogenicity island encoded type IV secretion system

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ABSTRACT

Many Gram-negative pathogens harbour type IV secretion systems (T4SSs) that translocate bacterial virulence factors into host cells to hijack cellular processes for multiple purposes. Helicobacter pylori is a human-specific bacterium that causes persistent infections in the stomach associated with pathologies ranging from chronic gastritis, peptic ulceration to gastric cancer. The virulence of H. pylori strongly depends on the serine protease HtrA, the T4SS encoded by the cytotoxin-associated genes (cag) pathogenicity island and the injected effector protein CagA.

This T4SS forms a needle-like pilus, which is induced upon host cell contact, and its assembly is accomplished by multiple protein-protein interactions. We discovered two gastric cancer-associated single nucleotide polymorphisms (SNPs) in the bacteria, which control effective T4SS functions upon infection of polarized gastric epithelial cells. Those include an alanine-to-threonine polymorphism in the tyrosine phosphorylation motif EPIYA-B of CagA, which affects gastric cancer risk by modifying intracellular signal transduction events involved in inflammation and cell division. The second SNP causes a serine-to-leucine change in HtrA that is associated with (i) elevated trimer formation and proteolytic activity, (ii) boosted cleavage of epithelial junction proteins occludin, claudin-8 and E-cadherin, (iii) induction of paracellular H. pylori transmigration, (iv) pronounced T4SS-pilus formation and injection of CagA, (v) promoted gastric inflammation and β-catenin-mediated cell proliferation as well as (vi) enhanced introduction of DNA double-strand breaks (DSBs) in the host chromosome and micronuclei formation upon infection. These activities highlight the importance of the cag T4SS and SNPs in bacterial virulence, which co-operatively elicit malignant alterations in the gastric epithelium that are discussed.

Session 3:

T4SS Structure and Function:
Conjugative systems - DNA import/export systems

Visualizing the structure and dynamics of the horizontal gene transfer during bacterial conjugation

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ABSTRACT
Bacterial conjugation is a sophisticated process that facilitates horizontal gene transfer between bacterial cells and is also responsible for the proliferation of antibiotic resistance genes among the bacterial population[1]. Conjugation is a process of unidirectional transfer of single-stranded DNA from donor cells to recipient cells via a contact-dependent (tight transfer) manner or through the pilus lumen in physically distant cells[2,3]. The conjugational transfer of DNA is mediated by the Type IV Secretion System (T4SS), one of the most versatile and elaborate secretion systems in bacterial cell envelopes[1]. T4SS assembles highly dynamic filamentous structures (T4SS pilus) that undergo cycles of extension and retraction. The T4SS pilus plays a major role in bringing the donor and recipient cells together to form a stable mating junction, and it is also shown to serve as a conduit for DNA transfer in physically distant cells[3]. The biochemical, molecular, and structural framework of conjugation remains poorly understood. Here, we use genetics, fluorescence microscopy, and cryo-electron tomography (cryo-ET) to investigate the molecular and structural basis of conjugation. Our study revealed the molecular anatomy of conjugating cells. This study provides key insights into the T4SS-dependent conjugation process, thus advancing our understanding of bacterial horizontal gene transfer and its role in spreading antibiotic resistance.

KEYWORDS : *Bacterial conjugation, Cryo-ET, T4SS.*

REFERENCE:

1. Costa, T.R.D., et al. Structural and functional diversity of type IV secretion systems. Nat Rev Microbiol 22, 170–185 (2024).
2. Couturier, A. et al. Real-time visualisation of the intracellular dynamics of conjugative plasmid transfer. Nat Commun 14, 294 (2023).
3. Goldlust, K. et al. The F pilus serves as a conduit for the DNA during conjugation between physically distant bacteria. PNAS,120(47) (2023).

Exploration of DNA processing mediated by MOB_T relaxases during bacterial conjugation

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ABSTRACT

Integrated and Conjugative Elements (ICEs) are major players mediating bacterial conjugation, and are thus responsible for the spread of antibiotic resistance and virulence genes. During the initiation of conjugation, the DNA of the mobile element is processed by the relaxase, a transesterase that recognizes its origin of transfer (*oriT*) [1]. ICE*St3*/Tn*916* conjugative elements encode relaxases belonging to a unique family called MOB_T that is distantly related to *Rep-trans* proteins. Whereas the *nic* site of *oriT* is conserved with the *Rep-trans* proteins[2], the DNA binding site recognized by these MOB_T relaxases was still unknown. The aim of this study was to determine the sequence recognized by the relaxase of ICE*St3* (RelSt3) within the *oriT* region and to characterize the enzymatic features of MOB_T relaxases. We also studied two proteins encoded by ICE*St3*, OrfL and OrfM, which could be involved in the relaxosome complex.

To perform this work, we used multidisciplinary approaches combining *in vivo* (mating assays, bacterial two-hybrid) and *in vitro* (EMSA, SEC-MALS, ITC) techniques.

We identified a *bind* site of RelSt3 unexpectedly distant from the *nic* site. We revealed that the binding of RelSt3 on its *bind* site is required for efficient nicking activity[3]. We also characterized OrfL and OrfM as OB-fold proteins involved in ICE*St3* relaxosome. We demonstrated an interaction network between these accessory proteins and several DNA processing enzymes, especially the RelSt3 and the PcrA helicase.

In this study, we deciphered the role of a MOB_T relaxase in the initial and final stages of ICE conjugation. We also characterized OrfL and OrfM proteins as the first example of OB-fold accessory proteins involved in DNA processing during the first steps of conjugation.

Keywords: *DNA processing, bacterial conjugation, ICE, relaxase, OB-fold*

REFERENCES

[1]: De La Cruz, F., Frost, L.S., Meyer, R.J., and Zechner, E.L. (2010). Conjugative DNA metabolism in Gram-negative bacteria. FEMS Microbiol Rev 34, 18–40. https://doi.org/10.1111/j.1574-6976.2009.00195.x.

[2]: Soler N, Robert E, Chauvot De Beauchêne I, *et al*. Characterization of a relaxase belonging to the MOB_T family, a widespread family in Firmicutes mediating the transfer of ICEs. Mob DNA. 2019;10(1):18. doi:10.1186/s13100-019-0160-9

[3]: Laroussi H, Aoudache Y, Robert E, *et al.* Exploration of DNA processing features unravels novel properties of ICE conjugation in Gram-positive bacteria. Nucleic Acids Res. 2022;50(14):8127-8142. doi:10.1093/nar/gkac607

Cryo-EM structure of the F plasmid relaxosome provides a molecular basis for DNA recruitment and processing in bacterial conjugation

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ABSTRACT

Bacterial conjugation is the unidirectional transport of genetic materials from a donor to a recipient cell. It is mediated in the donor cell by three major complexes: a DNA-processing machinery called the relaxosome, a double-membrane spanning transfer machinery termed type 4 secretion system (T4SS), and an extracellular appendage termed pilus. While the structures of the T4SS and pilus are known, that of the relaxosome is not. Here, we describe the cryo-EM structure of the fully assembled relaxosome encoded by the F plasmid in two different states corresponding to various functional steps along the DNA processing reaction. Altogether, the structural and molecular biology results we will present shed unprecedented light on a complex essential in the spread of antibiotic resistance genes among bacterial populations.

KEYWORDS: *Conjugation, Structural Biology, Relaxosome, Protein-protein interactions, Protein-DNA interactions*

Molecular Mechanism For Loading Two Relaxases During Conjugation Initiation

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ABSTRACT

Conjugation is the primary mode of horizontal gene transfer responsible for spreading antibiotic resistance genes in bacteria. This process is mediated by the Type-IV secretion system, the conjugative pilus, and a nucleoprotein complex called the relaxosome. At the core of the relaxosome is a protein called the relaxase, which plays a crucial role in DNA transfer, likely piloting the single-stranded DNA (ssDNA) during conjugation.

The archetypical relaxase from the F/R1 plasmid family is a bi-functional enzyme with two distinct activities: a transesterase or ssDNA nicking activity and a DNA unwinding activity. Our previous studies have shown that this protein can adopt a structured, closed state corresponding to its helicase activity and an open, unstructured state, potentially aligned with the translocation-compatible mode (1). These functional modes are determined by the specific DNA sequences engaged with the relaxase's domains. Importantly, these activities are mutually exclusive, meaning that two relaxase molecules are required to perform these tasks independently (2).

In our latest work, we have captured this relaxase dimer on a near-native DNA template and obtained the cryo-EM structure of the TraI dimer. Additionally, we have also captured TraI helicase in its unwinding state, bound to a forked DNA substrate. These structures expand our understanding of TraI's function as a helicase and how the interplay of its domains enables DNA processing at the origin of transfer (*oriT*).

Building on the cryo-EM structures and through biochemical analysis using both native DNA templates and assembled oligonucleotide templates, we can now elucidate key mechanistic steps involved in the loading of two relaxase molecules prior to the initiation of conjugation. Crucially, our results suggest a possible structural regulatory mechanism governing the loading of these relaxases, with potential implications for the initiation of conjugative DNA transfer itself.

KEYWORDS: *Relaxase, TraI, Cryo-EM.*

REFERENCES

[1] Ilangovan, A., Kay, C.W., Roier, S., El Mkami, H., Salvadori, E., Zechner, E.L., Zanetti, G. and Waksman, G., 2017. Cryo-EM structure of a relaxase reveals the molecular basis of DNA unwinding during bacterial conjugation. *Cell*, *169*(4), pp.708-721.

[2] Dostál L, Shao S, Schildbach JF. Tracking F plasmid TraI relaxase processing reactions provides insight into F plasmid transfer. *Nucleic Acids Res*. 2011 Apr;39(7):2658-70.

Structural and Functional Plasticity of the T4SSs Exemplified Through Studies of the F System

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ABSTRACT

A long-term goal of this laboratory is to decipher the structural bases underlying the functional versatility of the T4SSs. In the F plasmid transfer system, two structural studies established the foundation for our more recent work. First, by *in situ* cryoelectron tomography, we determined that the F system elaborates distinct structures at the *E. coli* cell surface, including the translocation channel without or with the associated F pilus as well as two novel F-pilus-associated basal platforms. Second, by single particle cryoelectron microscopy, we showed that OMCC subassembly of the F T4SS ($T4SS_F$) consists of a 13-fold symmetrical outer ring complex (ORC) built from 26 copies of VirB9-like TraK and VirB7-like TraV C-terminal domains, and a 17-fold symmetrical central cone (CC) composed of 17 copies of VirB10-like TraB β-barrels. To further interrogate Tra subunit or domain requirements for assembly of functional $T4SS_F$s and F pili, we phenotypically characterized mutant machines deleted of individual Tra subunits or domains, or chimeric machines built by swapping of Tra subunits or domains between the classical F plasmid and a distantly related IncFV plasmid, pED208. Analyses of the deletion mutants identified contributions of F-specific subunits to $T4SS_F$ channel function, the extension of F pili, or the deposition of F pili on alternative platforms. Corresponding studies of the chimeric F systems showed that swaps of OMCC subunits, certain F-specific subunits, or TraA pilins support DNA transfer but completely block pilus production. Our findings indicate that the requirements for elaboration of dynamic F pili are considerably more constrained than of functional translocation channels. Mechanistically, the ability to genetically "uncouple" pathways for channel vs F pilus assembly sets the stage for further definition of structural features and Tra partner interactions required for one but not the second of these dynamic processes.

KEYWORDS: *DNA conjugation, fluorescence microscopy, chimeric systems*

REFERENCES

[1] Kishida, K., Bosserman, R.E., Harb, L., Khara, P., Song, L., Hu, B., Zeng, L. and Christie, P.J. (2022) Contributions of F-specific subunits to the F plasmid-encoded type IV secretion system and F pilus. *Mol. Microbiol.* 117:1275-1290. doi: 10.1111/mmi.14908. PMCID: PMC9359479

[2] Kishida, K., Li, Y.G., Ogawa-Kishida, N., Khara, P., Al Mamun, A.A.M., Bosserman, R.E. and Christie, P.J. (2024) Chimeric systems composed of swapped Tra subunits between distantly-related F plasmids reveal striking structural and functional plasticity among type IV secretion machines. *PLOS Genetics* 20(3):e1011088. doi.org/10.1371/journal.pgen.1011088. PMCID: PMC10939261

[3] Costa, T.R.D., Patkowski, J.B., Mace, K., Christie, P. J., Waksman, G. 2024. Structural and functional diversity of type IV secretion systems. *Nat. Rev. Microbiol.* 22:170-185. doi: 10.1038/s41579-023-00974-3.

Session 4:

Bioinformatics, Genomics and Evolution:
Detection and classification, Diversity and Evolution, Bioinformatic Tools

PO-CBR for Delimiting Integrated Mobile Elements transferred by Conjugation

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ABSTRACT

Integrative Conjugative Elements (ICEs) and Integrative Mobilizable Elements (IMEs) are crucial drivers of bacterial plasticity. These mobile genetic elements integrate into bacterial genomes and disseminate adaptive traits, such as antibiotic resistance and virulence factors, via the T4SS conjugation machinery. To understand the role of ICEs and IMEs in bacterial evolution and adaptability, their delineation is essential. This study aims to develop an automated tool for the accurate detection of ICE and IME boundaries.

Our approach is based on case-based reasoning (CBR), an artificial intelligence approach, where a case is the representation of a problem-solving episode. CBR is often used to represent an expert's experience through a set of cases [1]. More specifically, our approach is based on process-oriented CBR, i.e., CBR in which cases represent processes. In this context, a case involves detailed procedures used to delineate a specific mobile element within the bacterial genome. Indeed, different case processes have been implemented to delimit numerous mobile elements, depending on the enzyme catalyzing integration and the targeted insertion gene. For example, our process case, CASE-1, employs data and integration characteristics from a known reference mobile element to define the boundaries of similar elements by determining their exact locations [2]. This approach enables precise modeling of delimitation processes and provides the flexibility to continually update our case base with new cases. PO-CBR also ensures transparent decision-making, essential for scientific validation and understanding the effectiveness of our approach.

Our findings confirm the effectiveness of our approach in delineating ICEs and IMEs in streptococcal genomes, revealing new genomic elements. The alignment of our delineation work with those reported in existing publications [3, 4] establishes the reliability of our method. These promising outcomes highlight its potential and encourage its application to more complex delineation tasks in other genera of the Bacillota phylum.

KEYWORDS: *Integrative Conjugative Elements, Integrative Mobilizable Elements, PO-CBR, Cases, mobile element delineation, Automated delineation.*

REFERENCES

[1] Lieber J, Bresson B. Case-Based Reasoning for Breast Cancer Treatment Decision Helping. In: Blanzieri E, Portinale L, editors. Advances in Case-Based Reasoning. Berlin, Heidelberg: Springer; 2000. p. 173–85.

[2] Lao J, Lacroix T, Guédon G, Coluzzi C, Payot S, Leblond-Bourget N, et al. ICEscreen: a tool to detect Firmicute ICEs and IMEs, isolated or enclosed in composite structures. NAR Genomics and Bioinformatics [Internet]. 2022 Dec 1 [cited 2024 Jun 26];4(4):lqac079. Available from: https://doi.org/10.1093/nargab/lqac079

[3] Ambroset C, Coluzzi C, Guédon G, Devignes MD, Loux V, Lacroix T, et al. New Insights into the Classification and Integration Specificity of Streptococcus Integrative Conjugative Elements through Extensive Genome Exploration. Front Microbiol. 2015;6:1483.

[4] Coluzzi C, Guédon G, Devignes MD, Ambroset C, Loux V, Lacroix T, et al. A Glimpse into the World of Integrative and Mobilizable Elements in Streptococci Reveals an Unexpected Diversity and Novel Families of Mobilization Proteins. Front Microbiol [Internet]. 2017 Mar 20 [cited 2024 Oct 1];8.

Prevalence and diversity of Integrative and Conjugative or Mobilizable Elements carrying antimicrobial resistance genes in *Streptococcus suis*

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ABSTRACT

Streptococcus suis, commensal of the upper respiratory tract of pigs, can lead to severe infections in post-weaning animals but also in humans after close contact with infected animals or through consumption of pork. Cases are usually sporadic but large outbreaks with fatal cases have been reported in Asia (1). This zoonotic pathogen frequently carries antibiotic resistance genes (ARGs), most of them being located on Mobile Genetic Elements (MGEs) in particular Integrative and Conjugative Elements (ICEs) and Integrative and Mobilizable Elements (IMEs) (2). The purpose of this work was to make an extensive study of the prevalence and diversity of MGEs carrying ARGs in *S. suis*. A total of 2588 genomes were clustered using dRep in order to select the best representative genomes based on assembly quality and genomic diversity. Genomes were then scaffolded using 21 reference *S. suis* genomes and annotated before searching ARGs – using ResFinder, CARD and homemade databases – and ICEs/ IMEs using ICEscreen (3). Clades were defined after alignment of core genes using PPanGGOLiN and phylogenetic tree construction. A dataset of 411 high-quality genomes (with associated metadata: country, year of isolation, host, isolation source, serotype) was created that covers the diversity of the *S. suis* species. A total of 1943 ARGs and 584 ICEs (7 families)/1069 IMEs (12 families) were detected in these genomes. Only 12 strains were devoid of ARGs. Multi-dimensional statistical analyses of the dataset revealed: (i) specific MGE co-occurrences, and (ii) statistical links between MGE and ARG contents and clade and isolation site (upper or lower respiratory tract or systemic). This study confirms the huge diversity of ICEs and IMEs carrying ARGs in *S. suis* and identifies a few avenues about the interplay between the various families of ICEs and IMEs that will be interesting to test experimentally.

KEYWORDS: *Streptococcus suis, antimicrobial resistance, Integrative Conjugative Elements, Integrative Mobilizable Elements*

REFERENCES

[1] Dutkiewicz, J., Sroka, J., Zając, V., Wasiński, B., Cisak, E., Sawczyn, A., Kloc, A. and Wójcik-Fatla, A.(2017) *Streptococcus suis*: a re-emerging pathogen associated with occupational exposure to pigs or pork products. Part I – Epidemiology, *Ann Agric Environ Med* 24(4):683-695, http://dx.doi.org/10.26444/aaem/79813

[2] Dechêne-Tempier, M., Marois-Créhan, C., Libante, V., Jouy, E., Leblond-Bourget, N. and Payot, S.(2021) Update on the Mechanisms of Antibiotic Resistance and the Mobile Resistome in the Emerging Zoonotic Pathogen *Streptococcus suis*, *Microorganisms* 9(8):1765. http://dx.doi.org/10.3390/microorganisms9081765

[3] Lao, J., Lacroix, T., Guédon, G., Coluzzi, C., Payot, S., Leblond-Bourget, N. and Chiapello, H. (2022) ICEscreen: a tool to detect Firmicute ICEs and IMEs, isolated or enclosed in composite structures. *NAR Genom Bioinform* 4(4):lqac079. http://dx.doi.org/10.1093/nargab/lqac079

Conjugative sabotage: new fertility inhibitors against broad-host-range plasmids

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ABSTRACT

Plasmids are key players in the acquisition and spread of antimicrobial resistance genes. Given the global concern over the rise of multidrug-resistant strains, it is essential to explore strategies that prevent their transmission without exerting selective pressure [1]. In nature, we can find such anticonjugative strategies and exploit them to our advantage. One of these strategies is the fertility inhibition, where a plasmid prevents the transmission of other unrelated co-resident plasmids [2]. Despite its potential, many questions remain unanswered regarding this phenomenon. In this work, using bioinformatic tools, we have expanded the catalog of fertility inhibition factors, previously described as isolated examples, providing a broader view of their distribution in plasmids and chromosomes. Using mating assays, we have validated the specificity of different factors against a set of conjugative plasmids. Besides, we selected those that inhibit plasmids from the Plasmid Taxonomic Units W (PTU-W) and P1 (PTU-P1) to clarify their mechanism of action. We studied whether they block the Type 4 Secretion System, or they target the components of the relaxosome. We also generated and analyzed a collection of mutant plasmids capable of escaping fertility inhibition, with the aim of unravelling the molecular basis of this phenomenon.

KEYWORDS: *Conjugation, Fertility inhibition, Plasmid interactions.*

REFERENCES

[1] Baquero, F., Coque, T. M. & Cantón, R. (2014) Counteracting antibiotic resistance: breaking barriers among antibacterial strategies. *Expert Opin. Ther. Targets* 18, 851–861. http://dx.doi.org/10.1517/14728222.2014.925881

[2] Getino, M. & De La Cruz, F. (2018) Natural and Artificial Strategies To Control the Conjugative Transmission of Plasmids. *Microbiol. Spectr*. 6, 6.1.03. https://doi.org/10.1128/ microbiolspec.mtbp-0015-2016

Evolution of plasmid mobility: origin and fate of non-conjugative plasmids

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ABSTRACT

Conjugation drives the horizontal transfer of adaptive traits across prokaryotes. A fourth of the plasmids can conjugate autonomously, the others being eventually mobilizable by conjugation. They transport functions such as antibiotic resistance, virulence factors or metabolism pathways, allowing bacterial communities to adapt rapidly. To understand the evolution of plasmid mobility, we studied plasmid size, gene repertoires, and conjugation-related genes of 11806 plasmids. We found that plasmid gene repertoires vary rapidly in relation to the evolutionary rate of relaxases. So much that plasmids with almost identical relaxases (>95% identity) have less than 50% of homologs. We identified 249 recent transitions of mobility type and we observed a clear tendency for plasmids to lose their autonomy to conjugate. These transitions are associated with even greater changes in gene repertoires and they seem to be mediated by transposable elements. At the micro-evolutionary scale of plasmid taxonomic units, transitions of mobility type are frequent. They are often associated with significant changes in plasmid gene content, and sometimes result in the creation of novel taxonomic units. Interestingly, most transitions from conjugative to mobilizable plasmids seem to be short-lived. This suggests a source-sink dynamic, where conjugative plasmids generate non-conjugative plasmids that tend to be poorly adapted. In conclusion, the evolution of plasmid mobility is frequent, it impacts the dynamics of gene repertoires, and the ecology of plasmids. Our results shed further light on the huge plasticity of plasmids, and quantify how changes in plasmid mobility affect their contribution to bacterial genomes.

Session 5:

T4SS Structure and Function:
Non-Canonical Systems, Interbacterial Systems

Substrate Selection and Fratricide Avoidance by the Bactericidal Type IV Secretion System of Xanthomonadaceae

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ABSTRACT

Many bacterial species from the orders Xanthomonadales, Burkholderales, and Neisseriales carry a type IV secretion system (T4SS) specialized in translocating effector proteins into other gram-negative species, leading to target cell death [1]. These effectors, known as X-Tfes, carry a carboxyl-terminal domain of ~120 residues, termed XVIPCD, characterized by several conserved motifs and a glutamine-rich tail. We show that the XVIPCD interacts with the central all-alpha domain of VirD4 and use NMR spectroscopy to map the interaction interfaces of the two proteins in the complex [2].

X-Tfes are encoded along with cognate immunity proteins, X-Tfis, that could in principle (i) protect against "friendly-fire" (*trans*-intoxication) from neighboring sister cells and/or (ii) protect against internal *cis*-intoxication (suicide). Here, we investigate these two mechanisms in the case of the bactericidal *Xanthomonas citri* X-T4SS. We show that the X-Tfi immunity proteins are dedicated to protecting cells against *cis*-intoxication [3] while *trans*-intoxication is avoided by a more general, previously undescribed mechanism.

KEYWORDS: *self/non-self discrimination; suicide avoidance, fratricide avoidance, Xanthomonas citri, bacterial warfare*, XVIPCD, VirD4

REFERENCES

[1] Souza, D.P. et al (2015) Bacterial killing via a type IV secretion system. *Nature Commun.* 6:6453 doi.org/10.1038/ncomms7453

[2] Oka, G.U. et al (2022) Structural basis for effector recognition by an antibacterial type IV secretion system *PNAS U.S.A.* 119 No. 1 e2112529119 doi.org/10.1073/pnas.2112529119

[3] Oka, G.U. et al (2024) *Xanthomonas* immunity proteins protect against the *cis*-toxic effects of their cognate T4SS effectors *EMBO Reports* doi.org/10.1038/s44319-024-00060-6

Type IV secretion system drives interbacterial competition in the plant pathogen *Xanthomonas*

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ABSTRACT

Bacteria have evolved diverse antibacterial strategies to eliminate competitors from their environment. Recently, a novel T4SS in the order *Xanthomonadales* (X-T4SS) was shown to have bacterial killing activity by injecting toxic effectors into bacteria in a contact-dependent manner [1]. This machinery exhibits structural features that distinguish it from the canonical conjugative T4SS [2]. The genus *Xanthomonas* is a large group of Gram-negative plant-associated bacteria causing diseases in more than 350 host plants worldwide. Although X-T4SSs are widely distributed among xanthomonads, knowledge about X-T4SS evolution affecting microbial ecology is limited. Here we studied X-T4SS function and evolution in *Xanthomonas* including the cereal pathogen, *Xanthomonas translucens* (*Xt*). A genomics-informed screening for X-T4SS gene clusters was conducted among all xanthomonads. Interestingly, the heterogeneous distribution of X-T4SS was mutually exclusive with the distribution of another antibacterial system, the type VI secretion system (T6SS). Using a combination of genetic and fluorescence-based methods, we demonstrate that both X-T4SS and T6SS are crucial for interbacterial competition in *Xt*. Comparative genetic and phylogenetic analyses showed that X-T4SS was likely acquired ancestrally at an early stage in *Xanthomonas* evolution, then subject to loss events in some *Xanthomonas* lineages. In addition, competition assays demonstrated that the X-T4SS was required for fitness against a cereal bacterial consortium. This work provides a framework to define the mechanisms promoting fitness in cereal phytobiomes.

KEYWORDS: *antibacterial, ecology, evolution*

REFERENCES

[1] Souza, D. P., Oka, G. U., Alvarez-Martinez, C. E., Bisson-Filho, A. W., Dunger, G., Hobeika, L., ... and Farah, C. S. (2015). Bacterial killing via a type IV secretion system. Nature Communications, 6(1), 6453.

[2] Sgro, G. G., Oka, G. U., Souza, D. P., Cenens, W., Bayer-Santos, E., Matsuyama, B. Y., ... and Farah, C. S. (2019). Bacteria-killing type IV secretion systems. Frontiers in microbiology, 10, 1078.

The non-canonical TraB-dependent Actinomycete ICEs (AICEs) mediate large-scale chromosomal transfer in *Streptomyces*

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ABSTRACT

Streptomyces are spore-forming and mycelial-growing bacteria that are ubiquitous in soils and crucial for the production of metabolites used in medicine, agriculture and industry. Conjugation is the main mechanism of horizontal gene transfer in these bacteria. In addition to conjugative plasmids and integrative and conjugative elements (ICEs) using canonical T4SS, other conjugative elements that are often encountered are AICEs (*Actinomycete* ICEs). AICEs do not use T4SS to translocate their DNA, but a single necessary and sufficient DNA translocase called TraB, which is related to the FtsK/SpoIIIE system involved in chromosome segregation. During AICE transfer, after excision from the chromosome and circularisation of the element, it is transferred to the recipient strain as double-stranded DNA through a pore formed by a hexamer of the TraB protein. Here we show that at the scale of a *Streptomyces* population (conspecific strains co-isolated from soil grains), there is a large diversity of AICEs [1] and associated TraB proteins that are thought to contribute to massive gene transfer in soils [2]. We confirmed the efficiency of AICE transfer in the laboratory and showed that this process also involves the mobilisation of chromosomal DNA, either in *cis* and/or in *trans* [3]. This phenomenon, which we call '*Streptomyces* chromosome transfer' (SCT), can lead to a massive exchange of up to a third of the recipient chromosome, resulting in a mixture of parental chromosomes and a novel metabolic arsenal in the exconjugant offspring. The relationship between TraB and SCT is discussed.

KEYWORDS: *conjugation, Actinomycete ICEs, TraB, Streptomyces, chromosome blending*

REFERENCES

[1] Choufa C, Tidjani AR, Gauthier A, Harb M, Lao J, Leblond-Bourget N, Vos M, Leblond P, Bontemps C. Prevalence and mobility of integrative and conjugative elements within a *Streptomyces* natural population. Front Microbiol. 2022 Sep 13;13:970179. doi: 10.3389/fmicb.2022.970179. PMID: 36177458; PMCID: PMC9513070.

[2] Tidjani AR, Lorenzi JN, Toussaint M, van Dijk E, Naquin D, Lespinet O, Bontemps C, Leblond P. Massive Gene Flux Drives Genome Diversity between Sympatric *Streptomyces* Conspecifics. mBio. 2019 Sep 3;10(5):e01533-19. doi: 10.1128/mBio.01533-19. PMID: 31481382; PMCID: PMC6722414.

[3] Vos M, Buckling A, Kuijper B, Eyre-Walker A, Bontemps C, Leblond P, Dimitriu T. Why do mobile genetic elements transfer DNA of their hosts? Trends Genet. 2024 Sep 19:S0168-9525(24)00174-4. doi: 10.1016/j.tig.2024.07.008. Epub ahead of print. PMID: 39304387.

Session 6:

Host-Pathogen Interactions:
Targeting eucaryotic signaling pathways

Revisiting effector functions: Involvement of a putative T4SS effector in the envelope integrity of *Brucella*

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ABSTRACT

Intracellular pathogens encounter various stressful environments while trafficking towards their replicative niche inside the host cell. To successfully replicate, they have either to withstand the stress or manipulate the environment to their needs. We intended to understand the function of the putative Type 4 secretion system effector BspD of the zoonotic bacterium *Brucella* in infection. However, through *in silico* analyses, classical growth assays, morphological analyses, stress assays, as well as host cell infections we reveal that BspD is critical for envelope integrity of *Brucella*. We show that BspD is conserved in the Rhizobiales without evidence of co-evolution with the presence of a T4SS or a certain lifestyle. Further, we show that BspD is critical for *Brucella abortus* envelope integrity in the stationary phase and in the presence of the outer membrane destabilizing compound EDTA *in vitro*. Deletion of *bspD* leads to swelling, elongation, and plasmolysis. In infection the absence of BspD led to a survival defect, culminating in the formation of fewer and smaller microcolonies in a macrophage infection model. From our observations, we propose that BspD of *B. abortus* is critical for preserving the integrity of the bacterial envelope, particularly under stressful conditions, which may enhance *Brucella*'s ability to survive within host cells independently of its putative status as a Type 4 secretion system effector.

KEYWORDS: *Effector, function, Brucella, infection, envelope integrity*

Exploring novel protein-protein interactions and functions of selected *Helicobacter pylori* Cag Type 4 Secretion System (CagT4SS) outer proteins

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ABSTRACT

Background and Questions. The *Helicobacter pylori cag* pathogenicity island (*cag*PAI) is an important virulence factor of *H. pylori* and encodes a complex type IV secretion system (CagT4SS). Our earlier work has helped us to generate hypotheses concerning exposed, variable outer proteins that can interact with host factors. Recently, structural information on the CagT4SS has been substantially improved by cryo-EM. However, important details, in particular on protein interactions between the OMC and the surface "outer" complex of the CagT4SS, consisting of T4SS outer proteins, are missing. This also concerns functional details on putative active and inactive conformations of the export complex, and on the transport functions in general.

Methods and Results. Using bacterial two hybrid system (BACTH) and biochemical characterization methods, we have enhanced our knowledge on protein-protein interactions in the CagT4SS outer proteins. This includes interactions of outer membrane proteins, of the VirB2 homolog CagC and the outer protein CagN of yet unknown function. We have determined and quantitated homo-dimerization of CagC and CagN, elucidated novel interactions of all tested proteins by BACTH, and confirmed them using protein purification and interaction analysis, for instance by Octet biolayer interferometry analysis and high-resolution microscopy.

Conclusions. Novel interactions of *H. pylori* outer membrane proteins and CagT4SS outer proteins were found and further characterized using biochemical methods and microscopy. This will help to refine structural and functional details of the CagT4SS transport and translocation complex and machinery, also in contact with human cells.

KEYWORDS: *CagT4SS; protein interactions; Helicobacter pylori; outer membrane proteins; interactome*

Translocation of YopJ family effector proteins through the VirB/VirD4 T4SS of *Bartonella*

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ABSTRACT

The evolutionary conserved YopJ family comprises numerous type-III-secretion system (T3SS) effectors of diverse mammalian and plant pathogens that acetylate host proteins to dampen immune responses. Acetylation is mediated by a central acetyltransferase domain that is flanked by conserved regulatory sequences, while a nonconserved N-terminal extension encodes the T3SS-specific translocation signal. *Bartonella* spp. are facultative-intracellular pathogens causing intraerythrocytic bacteremia in their mammalian reservoirs and diverse disease manifestations in incidentally infected humans. Bartonellae do not encode a T3SS, but most species possess a type-IV-secretion system (T4SS) to translocate *Bartonella* effector proteins (Beps) into host cells. Here we report that the YopJ homologs present in Bartonellae species represent genuine T4SS effectors. Like YopJ family T3SS effectors of mammalian pathogens, the "*Bartonella* YopJ-like effector A" (ByeA) of *Bartonella taylorii* also targets MAP kinase signaling to dampen proinflammatory responses, however, translocation depends on a functional T4SS. A split NanoLuc luciferase-based translocation assay identified sequences required for T4SS-dependent translocation in conserved regulatory regions at the C-terminus and proximal to the N-terminus of ByeA. The T3SS effectors YopP from *Yersinia enterocolitica* and AvrA from *Salmonella* Typhimurium were also translocated via the *Bartonella* T4SS, while ByeA was not translocated via the *Yersinia* T3SS. Our data suggest that YopJ family T3SS effectors may have evolved from an ancestral T4SS effector, such as ByeA of *Bartonella*. In this evolutionary scenario, the signal for T4SS-dependent translocation encoded by N- and C-terminal sequences remained functional in the derived T3SS effectors due to the essential role these sequences coincidentally play in regulating acetyltransferase activity [1].

KEYWORDS: *Bartonella; YopJ; effectors; type III secretion system; type IV secretion system*

REFERENCES

[1] Fromm, K., Ortelli, M., Boegli, A, and Dehio, C. (1924) Translocation of YopJ family effector proteins through the VirB/VirD4 T4SS of *Bartonella*, Proc. Natl. Acad. Sci. U. S. A. 121:e2310348121. doi: 10.1073/pnas.2310348121.

Legionella pneumophila: Assessment of effector secretion kinetics in real time in *its* natural host

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ABSTRACT

Legionella pneumophila is an intracellular pathogen that can infect free-living amoebae but also lung macrophages, causing Legionnaires' disease, a severe form of pneumonia in humans. Due to its co-evolution with eukaryotic hosts, this bacterium encodes a large arsenal of eukaryotic-like effectors that are translocated via a specialized type IV secretion system (T4SS) called Dot/Icm into the host cell, promoting bacterial replication [1]. To elucidate the virulence mechanisms conferred by the secretion of these over 330 effectors is necessary to assess their secretion. Mainly two effector secretion systems have been developed and are widely used: the β-lactamase FRET based translocation assay and the calmodulin-dependent adenylate cyclase domain, derived from the cyclolysin (CyaA) toxin from *Bordetella pertussis*, both are used as translocation reporters. The first is an end-point assay limiting the study both are based on artificial expression of the proteins using IPTG, thus they do not allow to study the kinetics of effector secretion

We have developed a secretion assay which uses the small and bright NanoLuc luciferase to sensitively assess secretion of *Dot/Icm* effector proteins in real time. We fuse 8 amino acids (Hibit) to the N terminal of an effector and express the remaining part (LgBiT) in the host cell of interest (either Thp-1 or *Acanthamoeba castellanii*). Furthermore, we have set up this assay using either an IPTG inducible promoter or the endogenous promoter. The assay using the endogenous promoter allows to assess effector secretion in real time as the regulation of secretion by the bacteria is not disturbed. Thus, we report here, the first bacterial effector secretion assay in protozoa, the natural host of *Legionella* spp. and many other environmental bacteria in real time, which promises to open up the field of molecular effector biology.

KEYWORDS: *Legionella pneumophila, type 4 secretion, real time effector secretion assay, Split Luciferase assay*

REFERENCES

[1] Mondino, S., Schmidt, S., Rolando, M., Escoll, P., Gomez-Valero, L., Buchrieser, C., (2020). Legionnaires' disease:
State of the Art Knowledge of Pathogenesis mechanisms of Legionella sp. *Annu. Rev. Pathol. Mech. Dis.* 15, 439–466

Session 7:

T4SS Structure and Function:
T4SS effectors recruitment

The T4bSS of *Legionella* features a two-step secretion pathway with an inner membrane intermediate for secretion of transmembrane effectors

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ABSTRACT

To promote intracellular survival and infection, *Legionella spp.* translocate hundreds of effector proteins into eukaryotic host cells using a type IV b protein secretion system (T4bSS). T4bSS are well known to translocate soluble as well as transmembrane domain-containing effector proteins (TMD-effectors) but the mechanisms of secretion are still poorly understood. Herein we investigated the secretion of hydrophobic TMD-effectors, of which about 80 were previously reported to be encoded by *L. pneumophila*. A proteomic analysis of fractionated membranes revealed that TMD-effectors are targeted to and inserted into the bacterial inner membranes of *L. pneumophila* independent of the presence of a functional T4bSS. While the T4bSS chaperones IcmS and IcmW were critical for secretion of all tested TMD-effectors, they did not influence inner membrane targeting of these proteins. As for soluble effector proteins, translocation of all investigated TMD-effectors depended on a C-terminal secretion signal. A deeper analysis of the TMD-effector SidF showed that this signal needed to be presented towards the cytoplasmic side of the inner membrane and that a small periplasmic loop was required for efficient translocation. We propose that strongly hydrophobic TMD-effectors are secreted in a two-step secretion process: Initially, an inner membrane intermediate is formed, that is extracted towards the cytoplasmic side, possibly by the help of the type IV coupling protein complex and subsequently secreted into eukaryotic host cells by the T4bSS core complex. Overall, our study highlights the amazing versatility of T4bSS to secrete soluble and TMD-effectors from different subcellular locations of the bacterial cell.

KEYWORDS: *Type IV b secretion system, transmembrane effectors, Legionella, mechanism of secretion*

REFERENCES

[1] The T4bSS of *Legionella* features a two-step secretion pathway with an inner membrane intermediate for secretion of transmembrane effectors.
Silke Malmsheimer, Iwan Grin, Erwin Bohn, Mirita Franz-Wachtel, Boris Macek, Tobias Sahr, Fabian Smollich, David Chetrit, Amit Meir, Craig Roy, Carmen Buchrieser, Samuel Wagner bioRxiv 2024.03.14.584949; doi: https://doi.org/10.1101/2024.03.14.584949

Exploring the structure and function of *Brucella abortus* virulence factor VirJ in T4SS effectors translocation

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ABSTRACT

Type IV secretion systems (T4SSs) are bacterial nanomachines used to transport macromolecules across membranes. In the case of animal and human pathogen Brucella spp, the T4SS is required for intracellular replication. More that 15 effectors have been identified to be translocated by VirB, targeting various signaling and metoblic pathways (1). *Brucella* spp VirB is similar to *Agrobacterium tumefaciens* VirB/D system but does not encode the VirD4 coupling protein, raising questions about the mechanism of effector translocation. Both bacteria encode a 40 kDa periplasmic protein named VirJ (or AcvB in *A. tumefaciens*), associated with the T4SS function (2, 3). AcvB is required for membrane lipid homeostasis and T-DNA transfer in *A. tumefaciens* (2). VirJ is important for *Brucella* infection in mice and involved in translocating of some effectors (3). We present our biochemical, structural and functional analysis of VirJ from *B. abortus*. Multi-angle light scattering and Small angle X-ray scattering experiments demonstrated that purified VirJ is monomeric and globular in solution. Structural prediction found that VirJ contains two domains, D1 and D2, each with an α/β hydrolase fold and in agreement with SAXS data. The crystal structure of D1 was solved at 1.7 Å and confirmed the α/β hydrolase fold. Our analysis revealed a potential conserved catalytic triad, presents only in D2. Enzymatic activity assays showed that VirJ hydrolyzes Lysyl-Phosphatidyl glycerol (L-PG) into PG with activity carried by D2 domain. Finally, in vivo effector translocation experiments using wt and DeltaVirJ *B. abortus* strains confirmed that VirJ is essential for the translocation of a T4SS-dependant effector. Altogether, our data suggest that VirJ could couple lipid homeostasis to T4SS translocation for some effectors.

KEYWORDS: *Brucella, T4SS, VirJ, Hydrolase, X-ray crystallography*

REFERENCES

[1] Celli J. (2019) The Intracellular Life Cycle of *Brucella* spp., *Microbiol Spectr*, http://dx.doi.org/10.1128/microbiolspec.BAI-0006-2019

[2] Groenewold MK, Hebecker S, Fritz C, Czolkoss S, Wiesselmann M, Heinz DW, Jahn D, Narberhaus F, Aktas M, Moser J. (2018) Virulence of Agrobacterium tumefaciens requires lipid homeostasis mediated by the lysyl-phosphatidylglycerol hydrolase AcvB. Mol Microbiol, http://dx.doi.org/10.1111/mmi.14154

[3] Del Giudice MG, Döhmer PH, Spera JM, Laporte FT, Marchesini MI, Czibener C, Ugalde JE. (2016) Brucella Virulence Factor Involved in the Secretion of Type IV Secreted Substrates J Biol Chem http://dx.doi.org/10.1074/jbc.M116.730994.

Architectural asymmetry enables DNA transport through the *Helicobacter pylori cag* type IV secretion system

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ABSTRACT

Structural asymmetry within secretion system architecture is fundamentally important for apparatus diversification and biological function. However, the mechanism by which substructure symmetry mismatch contributes to nanomachine assembly and interkingdom effector translocation are undefined. Here, we show that architectural asymmetry orchestrates dynamic substrate selection and enables trans-kingdom DNA conjugation through the *Helicobacter pylori cag* type IV secretion system (*cag* T4SS). Structural analyses of asymmetric units within the *cag* T4SS periplasmic ring complex (PRC) revealed intermolecular π–π stacking interactions that coordinate DNA binding and license trans-kingdom conjugation without disrupting the translocation of protein and peptidoglycan effector molecules. Additionally, we identified the VirB2-like pilin ortholog CagC as a proximal translocation channel gating mechanism that regulates cargo loading and governs substrate transport across the outer membrane. We thus propose a model whereby the organization and geometry of architectural symmetry mismatch exposes π–π interfaces within the PRC to facilitate DNA transit through the *cag* T4SS translocation channel.

KEYWORDS: *Helicobacter pylori, cag T4SS, DNA, structure, architecture*.

Session 8:

Impact of Conjugative Transfer:
Dissemination in the environment, Consequence on bacterial fitness, Mobilization

Bacterial Conjugation – based tools for manipulating and exploring long-range horizontal gene transfer

Dolores Lucia GUZMÁN-HERRADOR[1], Andrea FERNÁNDEZ-GÓMEZ[1], Pablo GURIDI-FERNÁNDEZ[1], David BIKARD[2] and **Matxalen LLOSA[1]**

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ABSTRACT

Bacterial conjugation is a promiscuous process widely used to genetically transform distantly related bacteria, especially those which are recalcitrant to transformation, as happens often with wild-type strains.

We have focused on the fact that the conjugatively transferred substrate is a nucleoprotein complex in order to design new delivery tools which bypass the need to express the transferred DNA. The actual substrate of the conjugative Type IV Secretion Systems is the conjugative relaxase, which leads the covalently linked DNA strand into the recipient cell. Thus, any peptide fused to the relaxase could in principle be delivered to any recipient cell. We have applied this principle to send CRISPR-Cas systems into recipient bacteria [1]. We have shown that fusions of conjugative relaxases TrwC (R388) and MobA (RSF1010) to Cas nucleases or Base Editors can be translocated and the fusion proteins are active in the recipient cells. These fusions are translocated covalently attached to a DNA molecule encoding the gRNA or even the desired mutations, which is incorporated by DSB-induced homologous recombination. To note, protein substrates of close to 300 kDa in size were translocated and functional in transconjugants.

Our next goal is to extend these Relaxase-Cas tools to wild-type bacteria of commercial or biomedical interest, such as streptomycetes or lactic-acid bacteria, which can only be reached by conjugation. We have established conjugation protocols to introduce DNA into wild-type strains of lactobacilli [2], bifidobacterial or staphylococci. Optimization of these protocols will open the door to the modification of bacteria for which there are currently insufficient tools.

Another aspect of our research aims to analyse horizontal gene transfer in nature, especially among distantly related species. We are developing approaches to study microbe-host genetic communication, as well as tools to trace the movement of single plasmids in complex communities such as the microbiota.

KEYWORDS: *Bacterial conjugation, CRISPR-Cas, targeted genetic modification, recalcitrant Gram-positive bacteria, Horizontal Gene Transfer.*

REFERENCES

[1] Guzmán-Herrador, D.L., Fernández-Gómez, A., Depardieu, F., Bikard, D. and Llosa, M. (2024). In vivo delivery of functional Cas:DNA nucleoprotein complexes into recipient bacteria through a Type IV Secretion System. *Proc Natl Acad Sci USA, in press.*

[2] Samperio, S., Guzmán-Herrador, D.L., May-Cuz, R., Martín, M.C., Álvarez, M.A., Llosa, M. (2021) Conjugative DNA Transfer From *E. coli* to Transformation-Resistant Lactobacilli. Front Microbiol. 2021 Feb 11;12:606629. doi: 10.3389/fmicb.2021.606629. PMID: 33643236; PMCID: PMC7905204.

Structural insights into a ParB-like protein of the F-plasmid that reprograms the gene expression profile of the recipient cell

Chloé VIROLLE[1], Sybille FERRARIN[1], Agathe COUTURIER, Jérémy GUERIN[1], Gaël PANIS[2], Daouda A K TRAORE[3,4,5], Frédéric DELOLME[6], Adeline PAGE[6], Rania ZENATI[1], Annick BERNE-DEDIEU[1], Sarah BIGOT[1], Patrick VIOLLIER[2], **Laurent TERRADOT[1]** and Christian LESTERLIN[1]

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ABSTRACT
DNA conjugation is a contact-dependent horizontal gene transfer mechanism by which plasmids are transferred between bacterial cells, thus causing the spread of antibiotics resistance [1]. The F plasmid is composed of different genetic regions including the leading, the maintenance and the transfer regions, which are sequentially transferred into the recipient bacterium. Genes carried by the leading region are not expressed in the donor cell but only transiently upon entry of the DNA in the new host cell, thus suggesting a role in the early steps of plasmid establishment [2]. In this study we focus on the yfjB gene, which is conserved amongst a variety of conjugative plasmids where it is generally annotated parB2 due to its homology with ParB-like family proteins. Live-cell microscopy reveals that YfjB forms bright and dynamic foci associated with the nucleoid of the transconjugant cells. The structure of YfjB was then investigated by combining modelling, X-ray crystallography and CryoEM. We show that YfjB is a DNA-binding protein that forms a dimer. Structure-based mutagenesis demonstrate that focus formation requires dimerization which is provided by a C-terminal domain which adopt an original structure. Most importantly, we reveal that YfjB induces a dramatic modification of chromosome gene expression in the transconjugant cell. The deletion of *yfjB* has no impact on the plasmid transfer efficiencies but delays the processing of the plasmid in the transconjugant cell. These data lead us to propose that YfjB acts to reprogram the physiology of the new host cell in a manner that facilitates the establishment of the F plasmid.

KEYWORDS: *F-plasmid, recipient cell, ParB protein, leading region*

REFERENCES

[1] Virolle, C., Goldlust, K., Djermoun, S., Bigot, S. & Lesterlin, C. (2020) Plasmid Transfer by Conjugation in Gram-Negative Bacteria: From the Cellular to the Community Level, *Genes (Basel).* **11**..

[2] Couturier, A., Virolle, C., Goldlust, K., Berne-Dedieu, A., Reuter, A., Nolivos, S., Yamaichi, Y., Bigot, S. & Lesterlin, C. (2023) Real-time visualisation of the intracellular dynamics of conjugative plasmid transfer, *Nat Commun.* **14**, 294.

Competition between conjugative plasmids by fertility inhibition protein

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ABSTRACT

Bacterial conjugation is the primary mechanism for genetic material exchange, involving the transfer of DNA—primarily conjugative plasmids—via direct contact between a donor and a recipient cell. Conjugative plasmids face competition from co-resident plasmids and have evolved diverse strategies to ensure their successful dissemination. Among these, fertility inhibition (FI) systems play a critical role by preventing the transfer of unrelated co-resident conjugative plasmids. Despite the identification of numerous FI systems, their molecular mechanisms and functional roles remain poorly characterized.

One such FI factor is the Tir protein, first identified in 1985 on the R100 plasmid of the IncFII incompatibility group. Tir is known to inhibit the transfer of the IncP plasmid RP4, but its mechanism of action has remained elusive. In this study, we uncover the molecular mechanism by which Tir exerts its inhibitory effect. We demonstrate that Tir is an inner membrane protein that selectively inhibits the transfer of co-resident IncP and IncW plasmids by targeting a key component of the Type IV secretion system. Furthermore, the presence of Tir homologues across diverse conjugative plasmids from various incompatibility groups highlights its widespread use as a competitive strategy to outcompete co-resident plasmids.

Uncovering the Role of IMEs in Antimicrobial Resistance and Horizontal Gene Transfer

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ABSTRACT

Integrative Mobilizable Elements (IMEs) are mobile genetic elements that integrate into bacterial genomes and excise to transfer to other bacteria. Unlike Integrative Conjugative Elements (ICEs), IMEs lack their own conjugation machinery, hijacking an ICE or plasmid Type IV Secretion System (T4SS) (1). This allows IMEs to excise, circularize, and mobilize autonomously, often without disrupting the ICE's own transfer process (2). Though widespread across bacterial species, IMEs remain relatively underexplored compared to other mobile genetic elements. Many IMEs integrate into the origin of transfer (*oriT*) or open reading frames of ICEs, using the ICE as both a host and facilitator for their mobilization (3). Besides mobility, IMEs frequently harbor genes conferring antimicrobial resistance (AMR), positioning them as key players in the horizontal spread of resistance among bacterial populations. Furthermore, the interaction between IMEs and T4SSs that enables their transfer remains an area of ongoing research.

In *Streptococcus suis*, a zoonotic pathogen, IMEs have been shown to play a major role in disseminating AMR genes. In silico analyses of 102 *S. suis* strains isolated from healthy or sick pigs, humans and wild boars, revealed that IMEs exhibit impressive diversity and plasticity. While ICEs were initially thought to be the primary vectors of AMR, it has been established that IMEs in *S. suis* carry a significant portion of these genes. IME_*SNF2* and IME_*PPI* in particular frequently carry both *tet*(O) and *erm*(B) genes. Analysis of the expression of their recombination module by quantitative PCR indicated that environmental factors, such as oxidative stress and UV exposure, play a role in their activation. This indicates that these elements may respond dynamically to external stimuli, allowing them to exploit conjugation systems under specific conditions. Understanding these environmental triggering signals could provide insights into how IMEs contribute to resistance genes spread inside bacterial communities.

KEYWORDS: *Integrative Mobilizable Elements, Streptococcus suis, Antimicrobial Resistance, Horizontal Gene Transfer*

REFERENCES:

[1] Guédon G., Libante V., Coluzzi C, Payot S and Leblond-Bourget N. The Obscure World of Integrative and Mobilizable Elements, Highly Widespread Elements that Pirate Bacterial Conjugative Systems. Genes. 2017;8:337. doi: 10.3390/genes8110337.

[2] Libante V, Sarica N, Mohamad Ali A, Gapp C, Oussalah A, Guédon G, Leblond-Bourget N. and Payot S. Mobilization of IMEs Integrated in the *oriT* of ICEs Involves Their Own Relaxase Belonging to the Rep-Trans Family of Proteins. 2020. doi: 10.3390/pathogens9010022.

[3] Libante V, Nombre Y, Coluzzi C, Staub J, Guédon G, Gottschalk M, Teatero S, Fittipaldi N, Leblond-Bourget N, Payot S. Chromosomal Conjugative and Mobilizable Elements in *Streptococcus suis*: Major Actors in the Spreading of Antimicrobial Resistance and Bacteriocin Synthesis Genes. Pathogens. 2019;9:22. doi: 10.3390/pathogens9010022.

Entry and Surface Exclusion of IncC Plasmids and SGI1: An Asymmetric Interplay

Nicolas RIVARD[1], Malika HUMBERT[1], Kévin HUGUET[2], Aurélien FAUCONNIER[1] and **Vincent BURRUS**[1]

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ABSTRACT

IncC conjugative plasmids propagate very efficiently within a broad range of Gammaproteobacteria and often carry multiple antibiotic-resistance genes. Hence, IncC plasmids are a threat to human and farmed animal health. This threat is heightened by the ability of IncC plasmids to mobilize unrelated integrative and mobilizable elements (IMEs), such as the *Salmonella* genomic island 1 (SGI1) and relatives, which often carry additional resistance genes. The interactions between IncC plasmids and SGI1-like IMEs are complex and involve regulatory crosstalk and alteration of the conjugative type IV secretion system (T4SS) encoded by the helper plasmids. By substituting three subunits of the IncC plasmid-encoded T4SS, SGI1 considerably improves the efficiency of conjugation and bypasses IncC entry exclusion, a poorly understood mechanism by which IncC plasmids inhibit the entry of highly similar elements into their host [1,2].

Recently, we discovered that IncC plasmids also promote surface exclusion, another mechanism of conjugative transfer inhibition that acts by disrupting mating pairs [3]. Like entry exclusion, the inner workings of surface exclusion remain elusive. Here, we describe the surface exclusion system of IncC plasmids. We identified the small lipoprotein Sfx as the effector in recipient cells. Mating assays using pairs of Sfx and TraN genes from related plasmids revealed surface exclusion groups, suggesting that the adhesin TraN in donors is the target. We also found that SGI1 and kin encode entry and surface exclusion systems with specificity patterns distinct from those of IncC plasmids. The substitution of TraN by SGI1 alters surface exclusion specificity, like the substitution of TraG changes entry exclusion specificity, favoring the spread of SGI1 between IncC$^+$ cells when DNA transfer should not occur.

The interplay between SGI1 and IncC plasmids gets increasingly complex and asymmetric as the parasitic IME interferes with the finely-tuned balance that enables the transmission of its helper plasmid.

KEYWORDS: *conjugation, DNA transfer, mobilization, entry exclusion, surface exclusion, IncC plasmids, SGI1*

REFERENCES

[1] Carraro, N., Durand, R., Rivard, N., Anquetil, C., Barrette, C., Humbert, M. and Burrus, V. (2017) *Salmonella* genomic island 1 (SGI1) reshapes the mating apparatus of IncC conjugative plasmids to promote self-propagation, *PLOS Genetics* 13: e1006705, http://dx.doi.org/10.1371/journal.pgen.1006705

[2] Humbert, M., Huguet, K.T., Coulombe, F. and Burrus, V. (2019) Entry Exclusion of Conjugative Plasmids of the IncA, IncC, and Related Untyped Incompatibility Groups, *Journal of Bacteriology* 201: e00731-18, http://dx.doi.org/10.1128/JB.00731-18

[3] Rivard, N., Humbert, M., Huguet, K.T., Fauconnier, A., Bucio, C.P., Quirion, E. and Burrus, V. (2024) Surface exclusion of IncC conjugative plasmids and their relatives, *PLOS Genetics* In press, http://dx.doi.org/10.1101/2023.05.15.540745

POSTERS

List of Posters

Posters exhibition Room Louis Majorelle – Maison des étudiants (MDE) Opening hours : 8:00 -17:00	
P1	Stabilizing the interaction between E.coli pKM101 T4SS ATPases for structural work **Natalie AL-OTAIBI** and Gabriel WAKSMAN *Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck College, London, UK*
P2	To kill but not to be killed: Inhibition of bactericidal X-T4SS-mediated trans-intoxication by XAC2611 **Camilla ADAN[1],** Gabriel OKA[1,2], Thiago SANTOS[1], William CENENS[1], Diorge SOUZA[1] and Chuck FARAH[1] *1 - Departament of Biochemistry, Universidade de São Paulo, São Paulo, Brazil* *2- Institut Européen de Chimie et Biologie – CNRS, University of Bordeaux, Pessac, France*
P3	Exploring oriT Binding Site Specificity in the MOBT Relaxase Family **Hicham Sekkouri ALAOUI[1,2],** Zoé BRUCHON[1], Haifa LAROUSSI[1], Louise THIRIET[1], Frédérique FAVIER[2], Claude DIDIERJEAN[2], Nathalie LEBLOND-BOURGET[1], Nicolas SOLER[1] *1 - Université de Lorraine, INRAE, DynAMic, Nancy, France* *2 - Université de Lorraine, CNRS, CRM2, Nancy, France*
P4	Type 4 Coupling Proteins as molecular targets to control antibiotic resistance spread **Itziar ALKORTA[1],** Kepa ARBÉ-CARTON[1], Nagore SANTOS-FERNÁNDEZ[1], Sofía RUIZ-CRUZ[1], Ana REY-SOGO[1], Lide ARANA[2], Sonsoles MARTÍN-SANTAMARÍA[3] and Carlos GARBISU[4] *1 - Department of Biochemistry and Molecular Biology, University of the Basque Country (UPV/EHU), Bilbao, Spain* *2 - Department of Applied Chemistry, University of the Basque Country (UPV/EHU), Donostia, Spain* *3 - Department of Structural and Chemical Biology, Centro de Investigaciones Biológicas Margarita Salas, CIB-CSIC, Madrid, Spain* *4 - Department of Conservation of Natural Resources, NEIKER-Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Derio, Spain*
P5	Study of the CagH, CagI and CagL protein interactions and its role in the biogenesis of Helicobacter pylori Cag type IV secretion system pilus **Alpay AYDIN[1],** Priscillia LAGOUTTE[1], Marine BLANC[1], Wolfgang FISHER[2] and Laurent TERRADOT[1] *1 - UMR 5086 Molecular Microbiology and Structural Biochemistry CNRS-Université de Lyon, Institut de Biologie et Chimie des Protéines, Lyon, France* *2 - Max von Pettenkofer-Institut für Hyiene und Medizinische Mikrobiologie, Ludwig Maximilians Universität, München, Germany*
P6	Elucidation of the structural and mechanistic basis of the pilus subunit-incorporation cycle in conjugative T4SSs **Magnus BLOCH[1]** and Gabriel WAKSMAN[1,2] *1 - Institute of Structural and Molecular Biology, Birkbeck, University of London, London, UK* *2 - Institute of Structural and Molecular Biology, University College London, London, UK*

P7	The multifunction Coxiella effector Vice stimulates macropinocytosis and interferes with the ESCRT machinery **Matteo BONAZZI[1],** Arthur BIENVENU[1], Mélanie BURETTE[1], Franck CANTET[1], Chantal CAZEVIEILLE[2], Stacey GILK[3], Delphine MURIAUX[1] and Eric MARTINEZ[1] *1 - Institut de Recherche en Infectiologie de Montpellier (IRIM), CNRS, Université de Montpellier, France* *2 - Institut des Neurosciences de Montpellier (INM), Université de Montpellier, INSERM, France* *3 - Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA*
P8	Cryo-EM structure of the core complex of the X-T4SS from the opportunistic pathogen Stenotrophomonas maltophilia **Michella BRESCIA[1]**, Germán SGRO[2], Santiago JUSTO[1] and Chuck FARAH[1] *1 - Department of Biochemistry, University of São Paulo, São Paulo, Brazil* *2 - Department of Biomolecular Sciences, University of São Paulo, Ribeirão Preto, Brazil*
P9	Species-specific components of the Helicobacter pylori Cag type IV secretion system **Kaeli BRYANT[1]**, Arwen FRICK-CHENG[2], Lauren SOLECKI[1], Heather KROH[1], Hayes MCDONALD[3,4], Borden LACY[1,5], Mark MCCLAIN[6], Melanie OHI[2,7], and Timothy COVER[1,5,6] *1 - Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, U.S.A* *2 - Life Sciences Institute, University of Michigan, Ann Arbor, U.S.A* *3 - Mass Spectrometry Research Center, Vanderbilt University School of Medicine, Nashville, U.S.A* *4 - Department of Biochemistry, Vanderbilt University, Nashville, U.S.A* *5 - Veterans Affairs Tennessee Valley Healthcare System, Nashville, U.S.A* *6 - Department of Medicine, Vanderbilt University School of Medicine, Nashville, U.S.A* *7 - Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, U.S.A*
P10	T4SS Drives Lipid Mixing During Substrate Exchange **David CHETRIT[1,2]**, Craig ROY[3] and Erdem KARATEKIN[1,2] *1 - Department Cellular and Molecular Physiology, Yale School of Medicine, New Haven, Connecticut, USA* *2 - Nanobiology Institute, Yale University, West Haven, Connecticut, USA.* *3 - Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, Connecticut, USA*
P11	Activation of Integrative and Conjugative Element ICEclc is influenced by the oxidative stress response heterogeneity, at single cell and lineage level **Anthony CONVERS[1]**, Vladimir SENTCHILO[1], Helena TODOROV[1] and Jan Roelof van der MEER[1] *Department of Fundamental Microbiology, UNIL, Lausanne, Switwerland, UNIL, Lausanne, Switzerland*
P12	Uncovering the molecular architecture of a Gram-positive type 4 secretion system **Kieran DEANE-ALDER[1]**, Josy TER BEEK[1,2], Annika BREIDENSTEIN[1,2] and Ronnie BERNTSSON[1,2] *1 - Department of Medical Biochemistry & Biophysics, Umeå University, Sweden* *2 - Wallenberg Centre for Molecular Medicine & Umeå Centre for Microbial Research, Umeå University, Sweden*
P13	Mechanistic Insights and Inhibition Strategies of DNA Transfer during Bacterial Conjugation **Mario DELGADILLO-GUEVARA[1,2]**, Shubha UDUPA[2], Debnath GHOSAL[2], and Marc ERHARDT[1,3] *1 - Institute of Biology, Humboldt-Universität zu Berlin, Berlin, Germany* *2 - University of Melbourne, Melbourne, Australia* *3 - Max Planck Unit for the Science of Pathogens, Berlin, Germany*

P14	Characterization of OrfA, the peptidoglycan hydrolase involved in ICESt3 conjugation in Streptococcus thermophilus **Rachida ESSMIMIH**, Stéphane BERTIN, Louise THIRIET, Nathalie LEBLOND, Yvonne ROUSSEL *Université de Lorraine, INRAE, DynAMic, Nancy, France*
P15	Sentinel plasmids: a novel approach to trace horizontal gene transfer in natural environments **Andrea FERNÁNDEZ-GÓMEZ**[1,2], Danielle MOONEY[2], Dolores GUZMÁN-HERRADOR[1,2] and Matxalen LLOSA[1,2] *1 - Departamento de Biología Molecular, Universidad de Cantabria (UC), Santander, Spain* *2 - Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC), UC-CSIC-SODERCAN, Santander, Spain*
P16	The VirB type IV secretion system is localized at the growth pole in Brucella abortus **Charline FOCANT[1,2]** and Xavier DE BOLLE[1,2] *1 - Research Unit in Biology of Microorganisms (URBM), University of Namur, Belgium* *2 - Namur Research Institute for Life Sciences (NARILIS), University of Namur, Belgium*
P17	Conjugative sabotage: new fertility inhibitors against broad-host-range plasmids **Daniel GARCÍA-LÓPEZ,** Sheila GONZÁLEZ-GUTIÉRREZ, Fernando DE LA CRUZ and M. Pilar GARCILLÁNBARCIA *Instituto de Biomedicina y Biotencología de Cantabria, UC-CSIC, Santander, Spain*
P18	Cryo EM elucidation of the TraI relaxase dimer loading onto oriT **Danylo GORENKIN**, Ruina LIANG and Aravindan ILANGOVAN *School of Biological and Behavioural Sciences, Queen Mary University of London, UK*
P19	Conjugation to Gram-positive bacteria as the basis for their genetic modification **Pablo GURIDI-FERNANDEZ**, Dolores L GUZMAN-HERRADOR, Andrea FERNANDEZ-GOMEZ and Matxalen LLOSA *IBBTEC, University of Cantabria, Santander, Spain*
P20	PO-CBR for Delimiting Integrated Mobile Elements transferred by Conjugation **Toufik HAMADOUCHE[1,2],** Gérard GUEDON[1], Thibaut LIMBACH[3], Jean LIEBER[2] and Nathalie LEBLOND-BOURGET[1] *1 - DynAMic, Université de Lorraine, INRAE, Nancy, France* *2 - LORIA, Université de Lorraine, CNRS, Inria, Nancy, France* *3 - Faculté des Sciences et Technologies, Vandoeuvre-Lès-Nancy, France*
P21	Studies on DNA binding mechanism of TraA relaxase from a gram-positive Type IV secretion system **Lena HIRTENFELDER[1]**, Kirill Kuhlmann[1], Claudia MICHAELIS[2], Tamara BERGER[1], Anna REISENBICHLER[1], Michelle BOELCKE[2], Elisabeth GROHMANN[2], Walter KELLER[1] and Tea PAVKOV-KELLER[1] *1 - Institute of Molecular Biosciences, University of Graz, Graz, Austria* *2 - Faculty of Life Sciences and Technology, Department of Microbiology, Berliner Hochschule für Technik, Berlin, Germany*
P22	Exploring novel protein-protein interactions and functions of selected Helicobacter pylori Cag Type 4 Secretion System (CagT4SS) outer proteins **Christine JOSENHANS[1]**, Felix METZ[1], Johanna BEILMANN[1], Simon BATS[1], Wolfgang FISCHER[1], Sebastian SUERBAUM[1] and Laurent TERRADOT[2] *1 - Max von Pettenkofer-Institut, Chair for Medical Bacteriology and Hygiene, Ludwig Maximilian University Munich, München, Germany* *2 - UMR5086, IBCP, Lyon, France*

P23	Revisiting effector functions: Involvement of a putative T4SS effector in the envelope integrity of Brucella **Maren KETTERER,** Petra CHIQUET, Mara ESPOSITO, Jaroslaw SEDZICKI, Maxime QUEBATTE and Christoph DEHIO *Biozentrum, University of Basel, Basel, Switzerland*
P24	Chromosomal Factors Essential for F Plasmid Conjugative Transfer **Kouhei KISHIDA**, Ren KUMAGAI, Sakura IJIMA, Yoshiyuki OHTSUBO and Yuji NAGATA *Graduate School of Life sciences, Tohoku University, Sendai, Japan*
P25	Investigating the structure and interaction of TrwB/VirD4 within the T4SS **Julien KOWALEWSKI[1]** and Gabriel WAKSMAN[1,2] *1 - Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck College, London, UK* *2 - Institute of Structural and Molecular Biology, Division of Biosciences, University College of London, London, UK*
P26	Structural studies on TraF, a transmembrane protein from a G+ Type IV secretion system **Kirill KUHLMANN[1]**, Tamara BERGER[1], Claudia MICHAELIS[2], Amrutha STALLINGER[1], Elisabeth GROHMANN[2], Walter KELLER[1] and Tea PAVKOV-KELLER[1] *1 - Institute of Molecular Biosciences, University of Graz, Graz, Austria* *2 - Faculty of Life Sciences and Technology, Department of Microbiology, Berliner Hochschule für Technik, Berlin, Germany*
P27	Establishment of in vivo photo-crosslinking in Legionella **Ann-Kathrin KUWERTZ**, Sarah TRENZ and Samuel WAGNER *Interfaculty Institute of Molecular Microbiology and Infection Medicine (IMIT), University of Tübingen, Tübingen, Germany*
P28	Deciphering the repeat II domain structure and function of CagY from Helicobacter pylori **Priscillia LAGOUTTE[1]**, Alpay AYDIN[1], Marine BLANC[1], Wolfgang FISCHER[2] and Laurent TERRADOT[1] *1 - UMR 5086 Molecular Microbiology and Structural Biochemistry CNRS-Université de Lyon, Institut de Biologie et Chimie des Protéines, Lyon, France* *2 - Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie Medizinische Fakultät I LMU München, München, Germany*
P29	Exploration of DNA processing mediated by MOBT relaxases during bacterial conjugation **Haifa LAROUSSI[1],** Robine MAFFO-WOULEFACK[1], Louise THIRIET[1], Emilie CLÉMENT[2], Nathalie LEBLOND-BOURGET[1] and Nicolas SOLER[1] *1 - Université de Lorraine, INRAE, DynAMic, Nancy, France* *2 - Université de Lorraine, CNRS, IAM, Nancy, France*
P30	Biochemical Characterisation of TraI Relaxase Dimer Loading onto oriT during Bacterial Conjugation **Ruina LIANG**, Danylo GORENKIN and Aravindan ILANGOVAN *School of Biological and Behavioural Sciences, Queen Mary University of London, UK*
P31	Uncovering the Role of IMEs in Antimicrobial Resistance and Horizontal Gene Transfer **Virginie LIBANTE[1]**, Manon DECHENE-TEMPIER[1,2], Charlène SAGRILLO[1], Johan STAUB[1], Corinne MAROIS-CREHAN[2], Nathalie LEBLOND-BOURGET[1], and Sophie PAYOT[1] *1 - DynAMic, Université de Lorraine, INRAE, Nancy, France* *2 - Unité Mycoplasmologie, Bactériologie et Antibiorésistance, Anses, Laboratoire de Ploufragan-Plouzané-Niort, Ploufragan, France*

P32	Establishing proximity biotinylation as tool to investigate Dot/Icm T4SS effectors in Legionella pneumophila **Daniel LOCKWOOD[1]**, Mohammad AREFIAN[2], Jack PENNY[2], Prof. Ben COLLINS[2], Prof. Miguel VALVANO and Dr. Gunnar SCHROEDER[1] *1 - Welcome-Wolfson Institute of Experimental Medicine, Queen's University Belfast, Belfast, UK* *2 - School of Biological Sciences, Queen's University Belfast, Belfast, UK*
P33	Elucidating assembly and function of VirB8 cell wall subunits refines the DNA translocation model in Gram-positive T4SSs **Robine MAFFO-WOULEFACK[1]**, Abbas MOHAMAD ALI[1], Haifa LAROUSSI[1], Julien CAPPÈLE[2], Felipe ROMERO-SAAVEDRA[3], Nancy RAMIA[1], Emilie ROBERT[1#], Sandrine MATHIOT[2], Nicolas SOLER[1], Yvonne ROUSSEL[1], Rémi FRONZES[4], Johannes HUEBNER[3], Claude DIDIERJEAN[2], Frédérique FAVIER[2*], Nathalie LEBLOND-BOURGET[1*] and Badreddine DOUZI[1] *1 - Université de Lorraine, INRAE, DynAMic, Nancy, France* *2 - Université de Lorraine, CNRS, CRM2, Nancy, France* *3 - Division of Pediatric Infectious Diseases, Dr. von Hauner Children's Hospital, Ludwig. Maximillian's University, Munich, Germany* *4 - Institut Européen de Chimie et Biologie, University of Bordeaux, Pessac, France* *5 - CNRS UMR 5234 Microbiologie Fondamentale et Pathogénicité, Bordeaux, France* *#Current address: Université de Lorraine, INRAE, IAM, Nancy, France*
P34	Formation, purification, and structural analysis of protein-protein and protein-DNA complexes from the conjugative machinery of the R388 plasmid **Tamara MENGUIANO**, Elena CABEZÓN and Ignacio ARECHAGA *Departamento de Biología Molecular, Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC), Universidad de Cantabria-CSIC, Santander, Spain*
P35	Characterization of the Xanthomonas citri T4SS subunits VirB8 and VirB6 **José Edwin NECIOSUP QUESÑAY**, Guilherme MEDEIROS, Livia BARROS, Chuck FARAH and Roberto SALINAS *Department of Biochemistry, institute of Chemistry, University of São Paulo, São Paulo, Brazil*
P36	Molecular interactions required for secretion of *Helicobacter pylori* CagA **Chiamaka OKOYE[1],** Hayes MCDONALD[2], Mark MCCLAIN[3] and Timothy COVER[1,3,4] *1 - Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN, USA* *2 - Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, USA* *3 - Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA* *4 - Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN, USA*
P37	Deciphering the Secretome of Brucella using Non-Canonical Amino Acid Tagging (BONCAT) **Joanne BRAUNWEILER ,** Maren KETTERER , Muhammad Naadir GANIEF , Christoph DEHIO *Biozentrum, University of Basel, Basel, Switzerland*
P38	Structural Characterisation of the Recipient Cell Detection Mechanism by the Type IV Secretion System **Pierre PAILLARD**, Manon THOMET, Quentin ROUGER and Kévin MACÉ *Institute Genetics & Development of Rennes (IGDR), Rennes, France*

P39	Prevalence and diversity of Integrative and Conjugative or Mobilizable Elements carrying antimicrobial resistance genes in Streptococcus suis **Sophie PAYOT**[1], Manon DECHENE-TEMPIER[1,2], Stéphanie BOUGEARD[3], Valentin LOUX[4,5], Hélène CHIAPELLO[4], Virginie LIBANTE[1], Corinne MAROIS-CREHAN[2] and Nathalie LEBLOND-BOURGET[1] *1 - DynAMic, Université de Lorraine, INRAE, Nancy, France* *2 - Unité Mycoplasmologie, Bactériologie et Antibiorésistance, Anses, Laboratoire de Ploufragan-Plouzané-Niort, Ploufragan, France* *3 - Unité Épidémiologie, santé et bien-être, Anses, Laboratoire de Ploufragan-Plouzané-Niort, Ploufragan, France* *4 - MaIAGE, Université Paris-Saclay, INRAE, Jouy-en-Josas, France* *5 - BioinfOmics, MIGALE bioinformatics facility, Université Paris-Saclay, INRAE, Jouy-en-Josas, France*
P40	Type IV secretion system drives interbacterial competition in the plant pathogen *Xanthomonas* **Chloé PEDUZZI[1],** Jules BUTCHACAS[2,3], Veronica ROMAN-REYNA[5], François NIKIS[1], Jonathan JACOBS[2,3], Ralf KOEBNIK[4] and Claude BRAGARD[1] *1 - Earth & Life Institute, applied microbiology-plant health UCLouvain, Louvain-la-Neuve, Belgium* *2 - Department of Plant Pathology, The Ohio State University, Columbus, Ohio, U.S.A.* *3 - Infectious Diseases Institute, The Ohio State University, Columbus, Ohio, U.S.A.* *4 - Plant Health Institute of Montpellier (PHIM),* *Institut de recherche pour le développement (IRD), Montpellier, France* *5 - Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, Pennsylvania, U.S.A.*
P41	Structural and functional insights into OrfD, the VirB4-like component of ICESt3 from Streptococcus thermophilus **Lola PERREY[1],** Robine MAFFO-WOULEFACK[1], Pierre PAILLARD[1,2,3], Haifa LAROUSSI[1], Nathalie LEBLOND-BOURGET[1], Badreddine DOUZI[1] *1 - Université de Lorraine, INRAE, DynAMic, Nancy, France* *2 - IGDR- Université de Rennes 1 - UMR CNRS 6290 – IGDR, Rennes – France* *3 - Institut de Génétique et Développement de Rennes - Université de Rennes I, Rennes – France*
P42	Optimal secretion of effector through the Dot/Icm secretion system depends on the bipolar diguanylate cyclase Lpp0809 **Kévin PICQ**, Julie ALLOMBERT, Corentin JABOULAY, Claire ANDREA, Patricia DOUBLET and Anne VIANNEY *CIRI (Centre International de Recherche en Infectiologie), Team Legiopath, Inserm U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France*
P43	Effect of Emerging pollutants at environmentally relevant concentrations on the transference of antibiotic-resistant genes **Ana REY-SOGO**[1], Alice MACCHIA[1], Itziar ALKORTA[1] and Hélène BUDZINSKI[2] *1 - Department of Biochemistry and Molecular Biology, University of the basque country (UPV/EHU), Leioa, Spain* *2 - EPOC-LPTC, university of Bordeaux, Talence, France*

P44	Legionella pneumophila LpDot1 interferes with host nuclear functions by altering paraspeckles **Monica ROLANDO[1*]**, Sonia NICCHI[1], Sonia MONDINO[2], Hanno SCHOELER[1], Jessica MARTYN E.[1], Giai Giannetto QUENTIN[3], Rayen ELY[3], Mariette MATONDO[3], Alejandro BUSCHIAZZO[2] and Carmen BUCHRIESER[1*] *1 - Institut Pasteur, Université Paris Cité, Biologie des Bactéries Intracellulaires, Paris, France* *2 - Laboratory of Molecular & Structural Microbiology, Institut Pasteur de Montevideo, Montevideo, Uruguay* *3 - Unité de spectrométrie de masse et Protéomique, CNRS USR 2000, Institut Pasteur, Paris, France* *4 - Citech, Imagopole-UTechS-PBI Photonic BioImaging, Institut Pasteur, Paris, France*
P45	Type 4 secretion system protein-protein interaction and structure prediction using PPIFold **Quentin ROUGER**, Pierre PAILLARD, Manon THOMET and Kévin MACE *Institute Genetics & Development of Rennes (IGDR), Rennes, France*
P46	Characterization of the ICEEc1 coupling protein from Escherichia coli ECOR31 **Sofia RUIZ CRUZ** and Itziar ALKORTA *Biochemistry and Molecular Biology Department, Faculty of Science and Technology, University of the Basque Country, Leioa, Spain*
P47	Uncovering the Role of IMEs in Antimicrobial Resistance and Horizontal Gene Transfer **Charlène SAGRILLO[1]**, Manon DECHENE-TEMPIER[1,2], Johan STAUB[1], Corinne MAROIS-CREHAN[2], Nathalie LEBLOND-BOURGET[1], Sophie PAYOT[1] and Virginie LIBANTE[1] *1 - DynAMic, Université de Lorraine, INRAE, Nancy, France* *2 - Unité Mycoplasmologie, Bactériologie et Antibiorésistance, Anses, Laboratoire de Ploufragan-Plouzané-Niort, Ploufragan, France*
P48	Characterization of the dynamics of a Type IV Secretion System (T4SS) substrate and interaction with ATPase domains **Roberto SALINAS**[1], Luis PIZZOLATO-CEZAR[2], Hugo van INGEN[3] and Shaker FARAH[2] *1 - Department of Biochemistry, University of São Paulo, São Paulo, Brazil* *2 - Department of NMR Spectroscopy, Utrecht University, Utrecht, the Netherlands*
P49	Structure of the type IV secretion system encoded by the E.coli F plasmid **Manoj SAXENA[1]**, and Gabriel WAKSMAN[1,2] *Birkbeck College, University of London, London, UK*
P50	Disruption of the nucleoli and translation by a *Legionella* Dot/Icm T4SS effector **Gunnar SCHROEDER[1],** Daniel LOCKWOOD[1*], Vikrant KASHYAP[1*], Alejandro FERNANDEZ-VEGA GRANADO[2*], Guan-Bo WANG[1], Arshad RIZVI[1], Flavia VIANA[1], Mohammad AREFIAN[3], Jack PENNY[3], Raissa WIBAWA[4], Erin GIBSON[1], Isabel RODRÍGUEZ-ESCUDERO[2], Elizabeth HARTLAND[4], Ben COLLINS[3] and Victor CID[2] *1 - Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK* *2 - Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad Complutense de Madrid and Instituto Ramón y Cajal de Investigaciones Sanitarias, Madrid, Spain* *3 - School of Biological Sciences, Queen's University Belfast, Belfast, UK* *4 - Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Australia*

P51	Elucidating the function and constituents of an anomalous T4SS in Rickettsia **Allison SCOTT**, Cassandra VONDRAK, Allen SANDERLIN, Brandon SIT and Rebecca LAMASON *Department of Biology, Massachusetts Institute of Technology, Cambridge, USA*
P52	Investigating the molecular basis of effector delivery through the bacterial Type IV Secretion System **Doulin SHEPHERD,** Matthew JOHNSON, Manasi MUDALIYAR and Debnath GHOSAL *Department of Biochemistry and Pharmacology, The University of Melbourne, Melbourne, Australia*
P53	Revealing membrane insertion mechanisms of Legionella's integral membrane effector proteins in host cells **Sarah-Maria TRENZ**, Sophie SCHMINKE and Samuel WAGNER *Interfaculty Institute of Microbiology and Infection Medicine (IMIT), University of Tübingen, Tübingen, Germany*
P54	NMR solution structure of OrfM from ICESt3 of Streptococcus thermophilus **Pascale TSAN[1],** Julien CAPPELE[1], Haifa LAROUSSI[2], Emilie CLEMENT[2], Dunkan BEGUE[1], Nathalie LEBLOND-BOURGET[2], Nicolas SOLER[2], Claude DIDIERJEAN[1] and Frédérique FAVIER[1] *1 - CRM2, Université de Lorraine, CNRS, Nancy, France* *2 - DynAMic, Université de Lorraine, INRAE, Nancy, France*
P55	Visualizing the structure and dynamics of the horizontal gene transfer during bacterial conjugation **Shubha UDUPA[1]**, Mario DELGADILLO[1,2], Matthew JOHNSON[1], Marc ERHARDT[2], and Debnath GHOSAL[1] *1 - Department of Biochemistry and Pharmacology, University of Melbourne, Melbourne, VIC, Australia* *2 - Institut für Biologie - Molecular Microbiology, Humboldt-Universität zu Berlin, Berlin, Germany*
P56	Cryo-EM structure of the F plasmid relaxosome provides a molecular basis for DNA recruitment and processing in bacterial conjugation **Sunanda WILLIAMS[1]**, Gabriel WAKSMAN[1,2] *1 - Institute of Structural and Molecular Biology, School of Natural Sciences, Birkbeck College, London, UK* *2 - Institute of Structural and Molecular Biology, Division of Biosciences, University College of London, London, UK*
P57	Structural Studies of X-Tfe Recognition by VirD4 Coupling Protein of a Type IV Secretion System **Daniela ZAPATA,** Luis CEZAR, Gabriel OKA, Roberto SALINAS, Chuck FARAH *Departamento de Bioquímica, Universidade de São Paulo, São Paulo, Brazil*
P58	TurboID as a tool to decipher the role of TraC/VirB5 in bacterial conjugation **Leticia MATILLA-CUENCA[1]** and Gabriel WAKSMAN[1,2] *1 - Institute of Structural and Molecular Biology, Birkbeck, London, UK* *2 - Institute of Structural and Molecular Biology, University College London, London, UK*

Abstracts of Posters

Stabilizing the interaction between *E.coli* pKM101 T4SS ATPases for structural work

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ABSTRACT

Conjugative Type IV Secretion Systems (T4SS) are versatile molecular machines responsible for the transfer of genetic material, such as plasmids, between donor and recipient cells, playing a key role in horizontal gene transfer. In Gram-negative bacteria, the T4SS spans both the inner and outer membranes and includes an extracellular filament known as the conjugative pilus, which facilitates DNA transfer between cells. The system comprises 12 proteins (VirB1-VirB11 and VirD4), with three ATPases (VirB4, VirB11, and VirD4) supplying the energy required for its function. T4SS operates in two distinct modes: pilus-biogenesis, where the VirD4 ATPase is non-essential, and DNA transfer, which requires VirD4.

Recent advancements in cryo-electron microscopy (cryo-EM) enabled the determination of the most complete high-resolution structure of the 2.8 megadalton T4SS complex from the R388 plasmid[1]. This structure offers crucial insights into the system but represents the pilus-biogenesis mode due to the absence of key elements necessary for DNA transfer, such as the VirD4 ATPase and a continuous central channel for DNA transfer. Additionally, the VirB11 ATPase, essential for pilus biogenesis, is missing, potentially indicating a snapshot of the T4SS in an early, closed state before pilus formation.

The VirB11 ATPase does not remain attached to the VirB4 ATPase upon introduction of detergent, thus requiring in-situ methods for studying this interaction. In this study, we utilize different in-situ approaches of studying VirB11-VirB4 binding, some of which attempt to capture the interaction in its native state (cryo-FIB milling of bacterial membranes overexpressing T4SS[2]), while others stabilize the interaction in-situ through crosslinking (BPA residue-directed crosslinking[3]), ultimately resulting in a sample suitable for cryo-EM structural work.

KEYWORDS: *cryo-EM, pKM101, ATPase, crosslinking, cryo-FIB, VirB11, VirB4, TraB, TraF*.

REFERENCES

[1] Mace, K. *et al.* Cryo-EM structure of a type IV secretion system. *Nature* **607**, 191-196 (2022). https://doi.org:10.1038/s41586-022-04859-y

[2] You, X. *et al.* In situ structure of the red algal phycobilisome–PSII–PSI–LHC megacomplex. *Nature* (2023). https://doi.org:10.1038/s41586-023-05831-0

[3] Joiner, C. M., Breen, M. E. & Mapp, A. K. Electron-deficient p-benzoyl-l-phenylalanine derivatives increase covalent chemical capture yields for protein-protein interactions. *Protein Sci* **28**, 1163-1170 (2019). https://doi.org:10.1002/pro.3621

To kill but not to be killed: Inhibition of bactericidal X-T4SS-mediated *trans*-intoxication by XAC2611

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ABSTRACT

We have previously demonstrated that immunity proteins (X-Tfis) of bactericidal X-T4SSs provide defense primarily against *cis*-intoxication (suicide) by their cognate X-Tfe effectors rather than *trans*-intoxication (fratricide) from sister cells [1]. The observation that *X. citri* wild type and mutant cells lacking eight X-Tfe/X-Tfi pairs (Δ8Δ2609) grow harmoniously in co-culture raises the question as to the molecular mechanism that protects *X. citri* cells from attacking their sister cells while at the same time efficiently targeting cells from other gram-negative species. Here, we elucidate the role of the conserved lipoprotein XAC2611 in inhibiting *trans*-intoxication in *X. citri* from neighboring sister cells. Using time-lapse fluorescence microscopy, we observed that *X. citri* mutants lacking XAC2611 exhibited contact-dependent fratricide. No killing was observed for the double mutant lacking both XAC2611 and VirB5, indicating that a functional X-T4SS is essential for this process. To further support the hypothesis that X-T4SS-dependent *trans*-intoxication is due to the absence of XAC2611, we constructed the Δ8Δ2609ΔXAC2611 *X. citri* strain from the Δ8Δ2609 *X. citri* strain. We observe that wild-type *X. citri* can kill Δ8Δ2609ΔXAC2611 cells. A XAC2611-msGFP chimera is observed to be localized on the *X. citri* envelope. Based on the prototypical T4SS model, which posits that VirB5 is located at the pilus tip for contact with potential recipient cells [2], we hypothesized that XAC2611 may inhibit *trans*-intoxication by directly interacting with this lipoprotein. Pull-down experiments using lysates from *E. coli* expressing these proteins demonstrated a direct interaction between N-terminal-6xHis-XAC2611$_{55-164}$ and VirB5$_{51-275}$. We also employed size-exclusion chromatography to characterize the resulting soluble protein complexes. We propose that XAC2611 blocks X-T4SS-dependent transfer of X-Tfes toxins between *X. citri* cells by direct interaction with VirB5. This mechanism is most likely related to previously described surface and entry exclusion mechanisms that prevent unnecessary substrate plasmid transfer between sister bacteria.

KEYWORDS: *X-T4SS, Xanthomonas, bacterial competition, fratricide.*

REFERENCES

[1] Oka, Gabriel U., et al. "Xanthomonas immunity proteins protect against the cis-toxic effects of their cognate T4SS effectors." EMBO Reports 25.3 (2024): 1436-1452.

[2] Macé, Kévin, and Gabriel Waksman. "Cryo-EM structure of a conjugative type IV secretion system suggests a molecular switch regulating pilus biogenesis." The EMBO Journal (2024): 1-20.

Exploring *oriT* Binding Site Specificity in the MOB_T Relaxase Family

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ABSTRACT

Integrative and Conjugative Elements (ICEs) are crucial drivers of bacterial evolution, promoting the dissemination of genetic traits such as antibiotic resistance, virulence factors, and novel metabolic capabilities. ICEs encode the machinery necessary for their own excision, horizontal transfer via conjugation, and integration into the host genomes. Relaxase proteins play a central role in initiating DNA transfer, by recognition and nicking of the origin-of-transfer (*oriT*) sequence. In Gram-positive bacteria, the ICE*St3*/Tn*916*/ ICE*Bs1* superfamily encodes a unique class of relaxases, known as MOB_T, which are related to Rep_*trans* rolling-circle replication initiators[1,2]. This study investigates the molecular mechanisms by which MOB_T relaxases recognize and interact with their DNA substrates.

Using recent insights into the *oriT* binding site of the ICE*St3* relaxase (RelSt3), we examined the DNA-binding behavior of related MOB_T relaxases. Electrophoretic mobility shift assays and endonuclease activity assays were conducted with various *oriT* sequences, including *oriT*-ICE*St3*, *oriT*-Tn*916* and *oriT*-ICE_*515*_*tRNA*^{Lys}. These sequences share conserved features, such as GC-rich regions flanking a CTAA *nic* site. In the case of ICE*St3*, a binding site made of two inverted repeat sequences, termed *bind* site, is positioned approximately 60 base pairs from the *nic* site[3].

Despite the structural and genomic similarities among the relaxases RelSt3 (from ICE*St3*), Orf20 (from Tn*916*) and Rel515 (from ICE_*515*_*tRNA*^{Lys}), including an N-terminal helix-turn-helix (HTH) motif, we observed distinct patterns of DNA interaction. Notably, Orf20 did not bind to ICE*St3*-like binding sites. However, all three relaxases exhibited single-stranded endonuclease activity on various substrates, including *oriT*-ICE*St3*. These findings point out a potential functional divergence within the MOB_T relaxase family, suggesting different DNA recognition mechanisms.

KEYWORDS: *Gram-positive, Integrative Conjugative Elements (ICE), relaxase, MOB_T, oriT*

REFERENCES:

1. Carr SB, Phillips SEV, Thomas CD. Structures of replication initiation proteins from staphylococcal antibiotic resistance plasmids reveal protein asymmetry and flexibility are necessary for replication. Nucleic Acids Res. 2016;44(5):2417-2428. doi:10.1093/nar/gkv1539

2. Soler N, Robert E, Chauvot De Beauchêne I, et al. Characterization of a relaxase belonging to the MOB_T family, a widespread family in Firmicutes mediating the transfer of ICEs. Mob DNA. 2019;10(1):18. doi:10.1186/s13100-019-0160-9

3. Laroussi H, Aoudache Y, Robert E, et al. Exploration of DNA processing features unravels novel properties of ICE conjugation in Gram-positive bacteria. Nucleic Acids Res. 2022;50(14):8127-8142. doi:10.1093/nar/gkac607

Type 4 Coupling Proteins as molecular targets to control antibiotic resistance spread

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ABSTRACT

The abuse and misuse of antibiotics has led to a situation where available antibiotics become ineffective because pathogenic bacteria acquire antibiotic resistance genes, becoming multiresistant to most known antibiotics. Bacterial conjugation is the main process by which bacteria acquire these resistances. However, it is a difficult biological process to study due to its intrinsic variability and the large number of variables involved. Therefore, in this work we present a massive conjugation platform or "Conjugomics"[1], which once calibrated, allows to carry out massive conjugation assays. This platform allows to study the effect of different compounds or substances on the conjugation frequency. More specifically, in this work, the effect of some potential conjugation inhibitors from an *in silico* assay[2] have been studied on the conjugation frequency of three representative plasmids (i.e., R388, R1 and pKM101). In addition to these compounds, linoleic acid was used as a positive control since it has been described as an inhibitor of F-type plasmid conjugation, as is the case for plasmids R388 and R1[3]. Some of the compounds studied have shown inhibition of conjugation of the R388 plasmid; in the R1 plasmid all compounds inhibit its conjugation; while for the pKM101 plasmid, in no case has inhibition of conjugation been observed.

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KEYWORDS: *Bacterial conjugation, antibiotic resistance, conjugative plasmids*

REFERENCES

[1] Arbé-Carton, K., Rey-Sogo, A., Santos-Fernández, N., Altube, O., Garbisu, C., Arana, L. and Alkorta, I. (2023) Development of a high-throughput platform to measure plasmid transfer frequency, Front Cell Infect Microbiol. Oct 11;13:1269732. doi: 10.3389/fcimb.2023.1269732.

[2] Álvarez-Rodríguez, I., Arana, L., Ugarte-Uribe, B., Gómez-Rubio, E., Martín-Santamaría, S., Garbisu, C. and Alkorta, I. (2020) Type IV Coupling Proteins as Potential Targets to Control the Dissemination of Antibiotic Resistance, Front Mol Biosci. Aug 12;7:201. doi: 10.3389/fmolb.2020.00201.

[3] Fernandez-Lopez, R., Machón, C., Longshaw, C.M., Martin, S., Molin, S., Zechner, E.L., Espinosa, M., Lanka, E. and de la Cruz, F. (2005) Unsaturated fatty acids are inhibitors of bacterial conjugation. Microbiology Nov;151(Pt 11):3517-3526. doi: 10.1099/mic.0.28216-0. PMID: 16272375.

Study of the CagH, CagI and CagL protein interactions and its role in the biogenesis of *Helicobacter pylori* Cag type IV secretion system pilus

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ABSTRACT

Helicobacter pylori is a Gram-negative bacteria and human pathogen that colonizes the stomachs of half the world's population. *H. pylori* can cause gastritis, peptic ulcers and gastric cancer. Gastric cancer is responsible for 800,000 deaths per year and the vast majority of these cases are caused by *H. pylori* infection. The most virulent strains inject the oncoprotein CagA into the host cells, which is delivered via Cag Type IV Secretion System (CagT4SS) encoded by cag pathogenicity island (cagPAI).

The cagPAI contains 27 proteins of which only 22 are necessary for the injection of CagA and/or the induction of inflammation. Encoded by a single operon, CagH, CagI and CagL are essential proteins for the biogenesis of a functional pilus and the translocation of CagA. These proteins were shown to co-purify together and display significant sequence similarity [1]. CagI and CagL interact together and with host proteins such as integrin $\alpha 5$ $\beta 1$ (Pham et al., 2012 [2]. CagI and CagL play an important role in CagA delivery and ligands targeting CagI can inhibit CagA translocation [3]. From a structural point of view, CagL and CagI are helical proteins with a conserved C-terminal helical bundle. Little is known about CagH structure, how the three proteins interact and the exact function of the putative complex(es). Understanding the dynamics of these 3 proteins in *H. pylori* is important to further our grasp of CagT4SS machinery and function. We will present our study on protein-protein interactions between the three proteins using different approaches that include, bacterial two-hybrid (BACTH), co-expression in *Escherichia coli* and also co-purification from *H. pylori* cultures.

KEYWORDS: *Helicobacter pylori,* pilus, BACTH

REFERENCES

[1] Shaffer CL, Gaddy JA, Loh JT, et al. Helicobacter pylori exploits a unique repertoire of type IV secretion system components for pilus assembly at the bacteria-host cell interface. *PLoS Pathog*. 2011;7(9):e1002237. doi:10.1371/journal.ppat.1002237

[2] Koelblen T, Bergé C, Cherrier MV, et al. Molecular dissection of protein-protein interactions between integrin α5β1 and the Helicobacter pylori Cag type IV secretion system. *FEBS J*. 2017;284(23):4143-4157. doi:10.1111/febs.14299

[3] Blanc M, Lettl C, Guérin J, et al. Designed Ankyrin Repeat Proteins provide insights into the structure and function of CagI and are potent inhibitors of CagA translocation by the Helicobacter pylori type IV secretion system. *PLoS Pathog*. 2023;19(5):e1011368. Published 2023 May 8. doi:10.1371/journal.ppat.1011368

Elucidation of the structural and mechanistic basis of the pilus subunit-incorporation cycle in conjugative T4SSs

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ABSTRACT

Conjugative Type IV secretion systems (T4SSs) are large DNA injection nanomachines consisting typically of 1) a double membrane-spanning transfer apparatus, 2) various coupling proteins involved in substrate recruitment, and 3) a conjugative pilus. The conjugative pilus is essential for conjugative transfer of the single-stranded DNA substrate during bacterial conjugation [1] and is assembled by the transfer apparatus in a poorly understood process.

Based on structural and functional characterization of the R388 T4SS [2], we propose a model of pilus biogenesis: Monomers of the major pilus subunit (VirB2) are recruited and translocated to positions between the upper and lower parts of the transfer apparatus Stalk segment, facilitating assembly of VirB2 monomers into a five-start helical polymer [3]. The lower part of the Stalk segment (a pentamer of VirB6) serves as a platform for iterative cycles of pilus subunit recruitment and assembly, while the upper part (a pentamer of VirB5) locates at the tip of the nascent pilus.

We will probe the proposed pilus subunit-incorporation model using amber-suppression technologies to site-specifically incorporate p-benzoyl-L-phenylalanine (Bpa) at key positions of specific subunits (e.g. VirB2, VirB5, VirB6) followed by UV-induced crosslinking and MS analysis to detect and characterise transient subunit interactions during *in vivo* operation of the native conjugative machinery.

KEYWORDS: *Conjugative pilus, R388, amber suppression, Bpa, XL-MS*

REFERENCES

[1] Waksman, G. (2019) From conjugation to T4S systems in Gram-negative bacteria: a mechanistic biology perspective, *EMBO Rep.* Feb;20(2):e47012. doi: 10.15252/embr.201847012.

[2] Macé, K. and Vadakkepat, A.K., Redzej, A., Lukoyanova, N., Oomen, C., Braun, N., Ukleja, M., Lu, F., Costa, T.R.D., Orlova, E.V., Baker, D., Cong, Q., Waksman, G. (2022) Cryo-EM structure of a type IV secretion system, *Nature* Jul;607(7917):191-196. doi: 10.1038/s41586-022-04859-y.

[3] Vadakkepat, A.K., Xue, S., Redzej, A., Smith, T.K., Ho, B.T., Waksman, G. (2024) Cryo-EM structure of the R388 plasmid conjugative pilus reveals a helical polymer characterized by an unusual pilin/phospholipid binary complex, *Structure* Sep 5;32(9):1335-1347.e5. doi: 10.1016/j.str.2024.06.009.

The multifunction *Coxiella* effector Vice stimulates macropinocytosis and interferes with the ESCRT machinery

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ABSTRACT

Coxiella burnetii, the causal agent of the Q fever, is an obligate intracellular pathogen that replicates within host cells. This lifestyle relies on the formation of *Coxiella*-containing vacuoles (CCVs), specialized vacuoles that provide a shielded environment for bacterial replication, enabling *C. burnetii* to evade host immune defences and establish persistent infections[1]. CCV development hinges on bacterial effector proteins translocated into host cells through a Type 4 Secretion System (T4SS), which interact with host proteins to subvert membrane trafficking. Expanding on the earlier identification of CvpB as a *C. burnetii* Lipid-Interacting Effector (LIE) that manipulates PI(3)P metabolism[2], we have conducted further investigations into the lipid composition of CCVs. Use of lipid binding probes revealed that PI(3)P, PI(4)P and LBPA were actively enriched at the CCV by T4SS effectors. We have identified the effector Vice as an additional LIE responsible for LPBA enrichment at the CCV. Vice interacts with phosphoinositides and late endosomal membranes, enriched in phosphatidylserine (PS) and lysobisphosphatidic acid (LBPA)[3]. CCVs generated by the *vice*::Tn mutants are negative for LBPA, showed a strong defect in size and an altered morphology as compared to wt CCVs. Strikingly, ectopic expression of Vice in eukaryotic cells leads to the formation of compartments reminiscent of early CCVs in both morphology, protein and lipid composition. The biogenesis of these Vice-induced compartments (VICs) is driven by an intriguing dual role of Vice. Initially, the effector localizes at the plasma membrane of eukaryotic cells, triggering the internalization of large vacuoles through the induction of macropinocytosis. Subsequently, Vice stabilizes VICs by disrupting the ESCRT machinery, impeding the formation of intraluminal vesicles (ILVs), with consequences on the release of extracellular vesicles (EVs). Taken together, our findings highlight Vice as a pivotal *C. burnetii* effector capable of exploiting two major cellular pathways to shape the bacterial replicative environment[3].

KEYWORDS: *At least 3* keywords are required.
Host-pathogen interactions, effector proteins, *Coxiella burnetii*, intracellular traffic

REFERENCES

1. Burette, M. & Bonazzi, M. From neglected to dissected: How technological advances are leading the way to the study of Coxiella burnetii pathogenesis. *Cell Microbiol* **22**, e13180 (2020).

2. Martinez, E. *et al.* Coxiella burnetii effector CvpB modulates phosphoinositide metabolism for optimal vacuole development. *Proc National Acad Sci* **113**, E3260–E3269 (2016).

3. Bienvenu, A. *et al.* The multifunction Coxiella effector Vice stimulates macropinocytosis and interferes with the ESCRT machinery. *Proc. Natl. Acad. Sci.* **121**, e2315481121 (2024).

Cryo-EM structure of the core complex of the X-T4SS from the opportunistic pathogen *Stenotrophomonas maltophilia*

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ABSTRACT

Among the secretory systems, the Type IV Secretion System from Xanthomonadales order (X-T4SS) is known as a system primarily associated with the contact-dependent killing mechanism by delivering toxins to Gram-negative rival species [1, 2]. This system consists of a 12 proteins machinery that spans the double bacterial membrane. *Stenotrophomonas maltophilia*, a multi-drug-resistant opportunistic pathogen, has a functional X-T4SS. It was shown that this system remains active even when components are replaced by homologous proteins from *Xanthomonas citri* highlighting the structural functional conservation inside the Xanthomonadales order [2]. Understanding the T4SS structure from different species from this order is crucial for deciphering its evolutionary adaptation and bactericidal mechanisms. We obtained a cryo-electron microscopy map of one of the major assemblies of the X-T4SS, the core complex from *Stenotrophomonas maltophilia*. This structure of ~1.3 MDa consisting of 14 copies of VirB7, VirB9, and VirB10, was solved at 3.37 Å. We present a comparison of the *S. maltophilia* core complex with that of the homologous structure from *Xanthomonas citri* [3].

KEYWORDS: *Cryo-EM, bacterial competition, core complex, X-T4SS, Stenotrophomonas maltophilia, Xanthomonas citri*

REFERENCES

[1] Souza, D. P., Oka, G. U., Alvarez-Martinez, C. E., Bisson-Filho, A. W., Dunger, G., Hobeika, L., ... & Farah, C. S. (2015). Bacterial killing via a type IV secretion system. *Nature communications*, *6*(1), 6453.

[2] Bayer-Santos, E., Cenens, W., Matsuyama, B. Y., Oka, G. U., di Sessa, G., del Valle Mininel, I., Alves, T. L., Farah, C. S. (2019). The opportunistic pathogen *Stenotrophomonas maltophilia* utilizes a type IV secretion system for interbacterial killing. PLoS Pathogens, 15(9). https://doi.org/10.1371/journal.ppat.1007651

[3] Sgro, G. G ., Costa, T. R. D., Cenens, W., Souza, D. P., Cassago, A., Coutinho de Oliveira, L., Salinas, R. K., Portugal, R. V., Farah, C. S., Waksman, G. (2018). Cryo-EM structure of the bacteria-killing type IV secretion system core complex from *Xanthomonas citri*. Nature Microbiology, 3(12), 1429–1440. https://doi.org/10.1038/s41564-018-0262-z

Species-specific components of the *Helicobacter pylori* Cag type IV secretion system

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ABSTRACT

Helicobacter pylori strains containing the *cag* pathogenicity island deliver substrates, including the oncoprotein (CagA) into gastric cells through the Cag type IV secretion system (T4SS)[1]. The Cag T4SS outer membrane core complex (OMCC) contains multiple copies of five proteins (including two species-specific proteins)[2]. In this study, we sought to identify additional components of the Cag T4SS. Through modification of the previously described OMCC immunopurification method (targeting hemagglutinin (HA)-tagged CagF and CagA) and optimizing mass spectrometric methods, we isolated the five known OMCC components along with additional Cag proteins present in lower abundance. The latter Cag proteins were not isolated by immunopurification of HA-tagged control proteins unrelated to the Cag T4SS. The relative abundance of four Cag proteins (CagW, CagL, CagI, and CagH), which do not exhibit sequence relatedness to T4SS components in other bacterial species, was enriched in immunopurified samples compared to unprocessed *H. pylori* lysate. Fractionation of immunopurified samples by gel filtration chromatography revealed that CagW, CagL, CagI and CagH co-purified with OMCC components, consistent with a physical association. These four Cag proteins were copurified with HA-CagF and CagA in immunopurifications from a Δ*cag3* mutant strain (lacking peripheral components of the OMCC), but not from a Δ*cagX* mutant strain (defective in OMCC assembly) or a Δ*cagA* mutant (from which the OMCC cannot be copurified using the HA-CagF approach). Analysis of *cagW*, *cagL*, *cagI* and *cagH* mutant strains by negative-stain electron microscopy indicated that these proteins are not required for OMCC assembly. In summary, by using multiple complementary methods, we have identified four species-specific components of the Cag T4SS (CagW, CagL, CagI, and CagH) that co-purify with the Cag T4SS OMCC.

KEYWORDS: *Helicobacter pylori*, *mass spectrometry, protein purification*

REFERENCES

[1] Tran, S.C., Bryant, K.N., and Cover, T.L. (2024) The *Helicobacter pylori cag* pathogenicity island as a determinant of gastric cancer risk, *Gut Microbes* 16(1), https://doi.org/10.1080/19490976.2024.2314201

[2] Chung, J.M., Sheedlo, M.J., Campbell, A.M., Sawhney, N., Frick-Cheng, A.E., Lacy, D.B., Cover, T.L. and Ohi, M.D. (2019) Structure of the *Helicobacter pylori* Cag type IV secretion system, *eLife* 8(e47644), https://doi.org/10.7554/eLife.47644

T4SS Drives Lipid Mixing During Substrate Exchange

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ABSTRACT

Members of the large family of type IV secretion systems (T4SS) are utilized by bacteria for pathogenesis and the exchange of genetic material. Some T4SSs specialize in the translocation of effector proteins into the host-cell cytosol and play a central role in human diseases, while others are primarily used for plasmid exchange among bacteria, promoting the spread of fitness-enhancing traits. Although several structural models of T4SSs in their inactive (non-translocating) states exist, the mechanisms that activate T4SSs and facilitate substrate translocation remain unclear[1,2]. Pathogens such as *Legionella pneumophila* (*L. pneumophila*) interact with a broad range of host membranes, from amoebae to humans, using their T4SS, suggesting that a specific receptor may not be required for activation. No needle-like projection, which could pierce the target membrane and form a conduit for substrate translocation, has been observed in the T4SS of *L. pneumophila* or other pathogens. Thus, the activation of T4SSs and their method of delivering substrates into host cells have remained outstanding questions for decades. We hypothesized that contact with host membrane lipids could activate T4SSs and that substrate delivery might involve the formation of a lipidic intermediate, resulting in lipid exchange. We found that lipid exchange accompanies T4SS activity in both *L. pneumophila* and *Escherichia coli* (*E. coli*). The exchange depends on direct contact, can occur between bacterial cells and liposomes, and is modulated by membrane fusion modulators. It occurs prior to or during substrate delivery and does not require the presence of the T4SS ATPases, but primarily depends on the conserved core complex protein DotG, a central component of the T4SS family. Our findings suggest that lipid exchange may be widespread among T4SSs and that the process could be a consequence of T4SS priming, with the formation of a lipid-based intermediate between donor and recipient cells being crucial for the efficient transfer of substrate molecules.

KEYWORDS:
T4SS, Host-pathogen interactions, *L. pneumophila,* Dot/Icm, *Membrane contact, Lipid Mixing, DotG*

REFERENCES

[1] Chetrit D, Hu B, Christie PJ, Roy CR, Liu J. A unique cytoplasmic ATPase complex defines the Legionella pneumophila type IV secretion channel. Nat Microbiol. 2018 Jun;3(6):678-686. doi: 10.1038/s41564-018-0165-z. Epub 2018 May 21. PMID: 29784975; PMCID: PMC5970066.

[2] Macé K, Vadakkepat AK, Redzej A, Lukoyanova N, Oomen C, Braun N, Ukleja M, Lu F, Costa TRD, Orlova EV, Baker D, Cong Q, Waksman G. Cryo-EM structure of a type IV secretion system. Nature. 2022 Jul;607(7917):191-196. doi: 10.1038/s41586-022-04859-y. Epub 2022 Jun 22. PMID: 35732732; PMCID: PMC9259494.

Activation of Integrative and Conjugative Element ICE*clc* is influenced by the oxidative stress response heterogeneity, at single cell and lineage level

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ABSTRACT

Bacteria adaptability is closely associated with horizontal gene transfer (HGT), often facilitated by mobile genetic elements that contribute to the plasticity of bacterial genomes. The mechanisms responsible for HGT have been well documented, yet the corresponding regulatory system(s) and the impact of environmental signals on gene transfer remain less well understood.

Here, we work with the Integrative and Conjugative Element ICE*clc* and study the environmental signal(s) influencing its activation. ICE*clc* carries the genes responsible for the degradation of pollutants, notably the aromatic compound 3-chlorobenzoate (3CBA). Its activation is constrained to a small subset of cells, the 'transfer competent' (tc) cells and is highest in stationary phase when grown on 3CBA (2-5% of cells) [1]. Interestingly, cells grown on the related compound benzoate exhibit minimal tc cells formation. The growth with 5 mM 3CBA is also inducing a high level of intracellular oxidative stress, which is not observed with benzoate.

Our main hypothesis is that the high ROS level detected in 3CBA-grown cells is influencing tc cells formation, either directly through activation of ICE*clc* regulators, or indirectly (through other cell's factors such as RpoS [2]). Specifically, we propose that cells experiencing higher-than-average oxidative stress are more likely to initiate the tc program. To test this hypothesis, we used double-reporters strains, with one fluorescent gene fused to an ICE*clc* promoter, as proxy for its activation, and the other fused to one of six selected oxidative stress response promoters. Both fluorescence were then quantified at single-cell level by flow-cytometry as a function of growth substrate, and in time-lapse microscopy to assess a potential temporal link between oxidative stress and tc appearance. Our results show that tc cells preferentially appear in cells with a higher oxidative stress response and could be linked to a higher oxidative stress response experienced by their lineage ancestors.

KEYWORDS: *ICE regulation, bistability, oxidative stress, single-cell, flow cytometry, time-lapse microscopy*.

REFERENCES

[1] Sulser, Sandra, Andrea Vucicevic, Veronica Bellini, Roxane Moritz, François Delavat, Vladimir Sentchilo, Nicolas Carraro, and Jan Roelof van der Meer. 2022. "A Bistable Prokaryotic Differentiation System Underlying Development of Conjugative Transfer Competence." Edited by Ivan Matic. *PLOS Genetics* 18 (6): e1010286. https://doi.org/10.1371/journal.pgen.1010286.

[2] Miyazaki, R, M Minoia, N Pradervand, S Sulser, and F Reinhard. 2012. "Cellular Variability of RpoS Expression Underlies Subpopulation Activation of an Integrative and Conjugative Element." *PLoS Genet* 8 (7): 1002818. https://doi.org/10.1371/journal.pgen.1002818.

Uncovering the molecular architecture of a Gram-positive type 4 secretion system

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ABSTRACT

Gram-positive (G+) bacteria are major contributors to the burden of antimicrobial resistance. The spread of resistance is facilitated by the transfer of resistance genes through conjugal type 4 secretion systems (T4SSs), which transport single-stranded DNA from host to donor cells. Recent advances in cryogenic electron microscopy have enabled a high-resolution structure of the T4SS from the Gram-negative (G-) *E. coli* R388 plasmid (1). However, no overall structure is available for any T4SS of G+ origin, though we have characterized some components of the T4SS encoded by the pCF10 plasmid in the G+ organism *Enterococcus faecalis* (2). Here we present three ongoing approaches to elucidate a more complete structure.

Firstly, we used recent developments in bioinformatics tools to build an *in silico* structure model of the core channel. As the membrane-associated subunits of the T4SS from pCF10 have little sequence identity to any proteins from G- systems, we employed AlphaFold3 (AF3) and Foldseek (3) in a 'divide and conquer' approach. These results indicate that the central channel might be more structurally conserved than previously predicted. Combining the AF3 predictions, experimental data, and homology to G- systems, we constructed a model that can be used as a starting point for future experiments.

Secondly, we have endogenously expressed affinity-tagged pCF10 T4SS and purified the assembled native channel. Using negative stain EM, we obtained the first low-resolution 2D images of a G+ T4SS. Pleasingly, the channel is intact and similar to the assembly predicted by AF3. Efforts to obtain a high-resolution structure using cryo-EM are ongoing.

Thirdly, we present the basic groundwork towards using cryogenic electron tomography (cryo-ET) to study the full pCF10-encoded T4SS *in situ*. Together, these approaches allow us to better understand the structure and function of the pCF10 T4SS, and G+ T4SS in general.

KEYWORDS: *cryo-EM, cryo-ET, AlphaFold*

REFERENCES

[1] Macé K, Vadakkepat AK, Redzej A, Lukoyanova N, Oomen C, Braun N, et al. (2022) Cryo-EM structure of a type IV secretion system. *Nature*. 607(7917):191–6.

[2] Sun WS, Torrens G, Ter Beek J, Cava F, Berntsson RPA. (2024) Breaking barriers: pCF10 type 4 secretion system relies on a self-regulating muramidase to modulate the cell wall. *mBio*. 15(8):e00488-24.

[3] van Kempen M, Kim SS, Tumescheit C, Mirdita M, Lee J, Gilchrist CLM, et al. (2024) Fast and accurate protein structure search with Foldseek. *Nat Biotechnol*. 42(2):243–6.

Mechanistic Insights and Inhibition Strategies of DNA Transfer during Bacterial Conjugation

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ABSTRACT
Conjugation is a major pathway for horizontal gene transfer, significantly contributing to the spread of antimicrobial resistance (AMR) genes. This process requires the Type IV secretion system (T4SS), a complex machinery integrated into the cell envelope that enables unidirectional transfer of DNA between donor cells carrying conjugative plasmids and recipient cells. Despite recent advances in the understanding of T4SS-mediated DNA transfer, the detailed structural dynamics of substrate transfer remain elusive.
This project focuses on the relaxase TraI of the F-like conjugative plasmid pED208, a key enzyme in DNA processing and transfer. In this study, we developed tools to facilitate both the structural and functional characterization of T4SS during active DNA transfer. Specifically, we generated translational and transcriptional fusion reporters that enable the capture and visualization of the secretion machinery in its active DNA transfer state using a combination of fluorescence microscopy and cryo-electron tomography.
Our study aims to deepen the understanding of the overall bacterial conjugation mechanism by identifying key structural aspects involved in the process of DNA transfer. This will ultimately contribute to the development of novel strategies for inhibiting critical components of bacterial conjugation and aid in the development of strategies to control the spread of AMR genes.

KEYWORDS: *Bacterial conjugation, plasmid transfer, TraI, relaxase, cryo-ET.*

Characterization of OrfA, the peptidoglycan hydrolase involved in ICE*St3* conjugation in *Streptococcus thermophilus*

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ABSTRACT

Horizontal gene transfer is a key mechanism in bacterial genome evolution, enabling adaptation by gene exchange between cells. Bacterial conjugation, which contributes significantly to the spread of antibiotic resistance, requires direct contact between donor and recipient cells. In the DynaMIc lab, one of our working models of conjugative elements is ICE*St*3, an integrative conjugative element commonly found in *Streptococcus thermophilus*, a Firmicute Gram-positive bacterium. Conjugation of ICE*St3* requires the production of a type IV secretion systems (T4SS) and other proteins among which the peptidoglycan hydrolase OrfA is supposed to perforate the bacterial cell wall of the conjugating cells.

By database enquiries and sequence alignments, we found that the 370-amino acid residue OrfA protein harbors an N-terminal "lysozyme-like" domain with the potential catalytic Glu114 key-residue and a C-terminal CHAP (Cysteine/Histidine-Dependent Amidohydrolase/Peptidase) domain with the potential catalytic Cys267 key-residue. To investigate the role of these two domains in the conjugative process of ICE*St3*, we constructed *S. thermophilus* strains where *orfA* was mutated to produce variants OrfAE114Q or OrfAC267A. We tested their abilities to function in conjugation assays. The lack of detectable transfer obtained with both mutants, demonstrates the essential role of both "lysozyme-like" and CHAP domain in conjugation of ICE*St3*. This shows that OrfA is critical for T4SS functionality. We are now leading further studies to understand how both catalytic domains function and where OrfA acts in the bacterial cell.

A bioinformatics analysis was conducted within the *Streptococcus* genus to explore the diversity of peptidoglycan hydrolases present in the elements of ICE*St3* and Tn916 families. Peptidoglycan hydrolases associated with ICE*St3* elements were highly diverse and consistently possess a C-terminal CHAP domain, whereas those within the Tn916 family were similar and harbor a Nlpc_P60 domain. Furthermore, the peptidoglycan hydrolases within ICE*St3* elements exhibited a higher degree of diversity compared to those in Tn916 elements. We are expanding our research to further investigate the relationship between the substrate cleavage activities of these hydrolases and the range of host species where elements can transfer to. This will help us better understand the evolutionary and functional dynamics driving the spread of ICE*St3* elements.

KEYWORDS: *peptidoglycan hydrolase, bacterial conjugation, Streptococcus thermophilus, T4SS*

Sentinel plasmids: a novel approach to trace horizontal gene transfer in natural environments

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ABSTRACT

Horizontal gene transfer (HGT) is a key process in microbial evolution, particularly relevant to human health due to its role in spreading antibiotic resistance genes. However, understanding HGT in natural environments, such as the human gut microbiota, is challenging, largely because many microorganisms are difficult to culture and these events can be transient.

CRISPR spacer acquisition has been previously used to detect incoming mobile genetic elements (MGEs)[1]. Inspired on this work, we have developed conjugative Sentinel Plasmids (SP) to trace the movement of a single MGE. SP carry *Escherichia coli cas1* and *cas2* genes, enabling autonomous CRISPR spacer acquisition. These plasmids also contain a minimal CRISPR array consisting of 60 nucleotides from the leader sequence of *E. coli* K12 CRISPR array I and a single repeat, which has been shown to be sufficient for spacer acquisition[2]. Additionally, a variant was created with a randomized leader sequence to assess if the lack of an identical genomic array might provide a competitive advantage. Both variants were tested in different *E. coli* strains, and spacer acquisition was observed in all of them. Sanger sequencing confirmed the capture of spacers from both the host genome and plasmids.

Once validated in *E. coli*, the next step is to test SP spacer acquisition in transconjugants of intergeneric conjugation assays. To this end, we are using promiscuous replicons and promoters. Spacer acquisition patterns will be analyzed to determine the origin, frequency, and distribution of spacers. In the long term, the use of SP in natural environments will allow the tracing of HGT in bacterial communities, including the microbiota.

This approach offers a powerful tool to map long range HGT events in complex ecosystems, with potential applications in understanding antibiotic resistance spread and microbial ecology in natural environments.

KEYWORDS: *Spacer acquisition, mobile genetic elements, bacterial conjugation.*

REFERENCES

[1] Munck, C., Sheth, R. U., Freedberg, D. E. & Wang, H. H. Recording mobile DNA in the gut microbiota using an Escherichia coli CRISPR-Cas spacer acquisition platform. *Nat. Commun*. 11, 95 (2020).

[2] Yosef, I., Goren, M. G. & Qimron, U. Proteins and DNA elements essential for the CRISPR adaptation process in *Escherichia coli*. *Nucleic Acids Res.* **40**, 5569-5576 (2012).

The VirB type IV secretion system is localized at the growth pole in *Brucella abortus*

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ABSTRACT

Brucella abortus is an intracellular pathogenic alpha-proteobacterium, causing bovine brucellosis. It resides in three types of vacuoles (endosomal, replicative and autophagic, respectively eBCV, rBCV and aBCV where BCV stands for *Brucella*-containing vacuole) [1]. To reach its replication niche (rBCV), *Brucella* requires a type IV secretion system (T4SS) named VirB. VirB, formed by a complex of ten multimeric proteins, injects effector proteins into the host cell [2]. Our team previously demonstrated that unipolar growth of *B. abortus* begins in eBCVs [3], where T4SS expression is induced by starvation and acidic pH. We hypothesized that envelope generation at the growth pole could be accompanied by VirB incorporation into the envelope. To follow the biogenesis of the T4SS, translational fusions for different VirB subunits with mNeonGreen were constructed. The residual virulence during infection was tested to determine if the fusions preserve VirB function, which was the case for most of the C-terminal fusions since these strains were able to replicate intracellularly. Strains with a functional T4SS were analyzed by fluorescence microscopy during macrophage infection to localize the fused VirB subunits. We were able to localize the two ATPases of the system, VirB4 and VirB11, and also VirB3 from the Inner Membrane Complex, strictly to the growth pole. VirB3 and VirB4 show a circular subpolar pattern, reminiscent of the RgsE/GPR localization. VirB11, the last subunit recruited to the T4SS, shows a focalized localization at the growth pole. These patterns of localization are independent of the presence of a O-chain in the lipopolysaccharide. A vast majority (90 to 96%) of the bacteria detected in macrophages display of polarly localized VirB, arguing against population heterogeneity based on T4SS expression. These data suggest VirB is recruited to the growth pole, opening new avenues to study connections between envelope growth and virulence mechanisms in *B. abortus*.

KEYWORDS*: Brucella, VirB, pole, growth, infection, BCV*

REFERENCES

[1] Celli, J. The intracellular life cycle of *Brucella* spp. *Microbiol. Spectr.* 7, 1–11 (2019).

[2] O'Callaghan, D., *et al.* A homologue of the *Agrobacterium tumefaciens* VirB and *Bordetella pertussis* Ptl type IV secretion systems is essential for intracellular survival of *Brucella suis*. *Molecular microbiology*, *33*(6), 1210-1220. (1999).

[3] Deghelt, M., *et al*. G1-arrested newborn cells are the predominant infectious form of the pathogen *Brucella abortus*. *Nat. Commun.* 5, 1–12 (2014).

Conjugative sabotage: new fertility inhibitors against broad-host-range plasmids

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ABSTRACT

Plasmids are key players in the acquisition and spread of antimicrobial resistance genes. Given the global concern over the rise of multidrug-resistant strains, it is essential to explore strategies that prevent their transmission without exerting selective pressure [1]. In nature, we can find such anticonjugative strategies and exploit them to our advantage. One of these strategies is the fertility inhibition, where a plasmid prevents the transmission of other unrelated co-resident plasmids [2]. Despite its potential, many questions remain unanswered regarding this phenomenon. In this work, using bioinformatic tools, we have expanded the catalog of fertility inhibition factors, previously described as isolated examples, providing a broader view of their distribution in plasmids and chromosomes. Using mating assays, we have validated the specificity of different factors against a set of conjugative plasmids. Besides, we selected those that inhibit plasmids from the Plasmid Taxonomic Units W (PTU-W) and P1 (PTU-P1) to clarify their mechanism of action. We studied whether they block the Type 4 Secretion System, or they target the components of the relaxosome. We also generated and analyzed a collection of mutant plasmids capable of escaping fertility inhibition, with the aim of unravelling the molecular basis of this phenomenon.

KEYWORDS: *Conjugation, Fertility inhibition, Plasmid interactions.*

REFERENCES

[1] Baquero, F., Coque, T. M. & Cantón, R. (2014) Counteracting antibiotic resistance: breaking barriers among antibacterial strategies. *Expert Opin. Ther. Targets* 18, 851–861. http://dx.doi.org/10.1517/14728222.2014.925881

[2] Getino, M. & De La Cruz, F. (2018) Natural and Artificial Strategies To Control the Conjugative Transmission of Plasmids. *Microbiol. Spectr*. 6, 6.1.03. https://doi.org/10.1128/microbiolspec.mtbp-0015-2016

Cryo EM elucidation of the TraI relaxase dimer loading onto *oriT*

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ABSTRACT

Horizontal Gene Transfer (HGT) in the major facilitator towards the spread of antimicrobial resistance among bacteria. Within bacterial conjugation, the principal contributing process towards HGT, the function and assembly of the relaxosome complex notably constitutes the regulatory role in the transfer of plasmid DNA across the membrane-bound Type IV Secretion System (T4SS).

Relaxosome constituent proteins are encoded and transcriptionally regulated alongside T4SS genes within the Tra operon. They assemble on the origin of transfer (oriT) and mediate two functions of the relaxase (TraI) that is decisive in the initiation of conjugation by nicking and unwinding of plasmid DNA. TraI is a bifunctional protein featuring transesterase and helicase domains that undertake single-strand nicking of oriT at its specific nic site and unwinding of double-stranded DNA via ATP-dependent helicase activity. These domains function in a mutually exclusive mechanism that relies on the formation of a TraI dimer.

Current research has characterised the conformation of TraI when loaded onto single-strand DNA[1], however the mechanism behind TraI loading as a dimer displaying negative cooperativity has remained elusive. For the first time, utilising Cryogenic Electron Microscopy (Cryo EM), we show the assembly of the TraI dimer on oriT. Our breakthroughs, investigating the loading of TraI helicase onto supercoiled plasmid DNA, suggest the presence of a structurally arbitrated sequential loading mechanism for the two relaxases composing the TraI dimer.

KEYWORDS: *Relaxase, TraI, Cryo-EM.*

REFERENCES

[1] Ilangovan, A., Kay, C.W.M., Roier, S., El Mkami, H., Salvadori, E., Zechner, E.L., Zanetti, G. and Waksman, G. (2017). Cryo-EM Structure of a Relaxase Reveals the Molecular Basis of DNA Unwinding during Bacterial Conjugation. Cell, 169(4), pp.708-721.e12. doi: https://doi.org/10.1016/j.cell.2017.04.010.

Conjugation to Gram-positive bacteria as the basis for their genetic modification

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ABSTRACT

Bacterial conjugation involves the transfer of genetic information from a donor to a recipient cell, allowing the spread of, for instance, antibiotic resistance. Conjugative plasmids are guided by a relaxase through a type IV secretion system (T4SS) into the recipient cell. The mechanism has been extensively studied among Gram-negative bacteria; Gram-positive are not as explored.

We apply conjugation as a gateway to introduce desired modifications in Gram-positive bacteria of medical, environmental, or industrial relevance. Since the natural conjugation mechanisms of these bacteria are not always accessible, we propose using *E.coli* as a donor bacterium. We use the broad host range conjugative system of plasmid RP4, either with its own transfer machinery or serving as a helper to transfer mobilizable plasmid RSF1010. The latter has a relaxase (MobA) that is more amenable to modifications when compared to the RP4 TraI relaxase.

Here we show the results of conjugation to recalcitrant Gram-positive bacteria. In addition to laboratory strains, we have focused on wild bacteria whose modification could be advantageous compared to heterologous expression in model organisms. We have successfully conjugated, to our knowledge for the first time, certain strains of lactobacilli [1] and other lactic acid bacilli, bifidobacteria, and staphylococci. Our current focus is *Streptomyces*, a genus of industrial interest due to its rich secondary metabolism. Although conjugation to *Streptomyces* with RP4 is well established, we have managed to carry it out in *S.lividans* and *S.coelicolor* using the MoA relaxase and derivatives of the RSF1010 plasmid.

Conjugation allows delivery of DNA as a prerequisite to attain genetic modification. In addition, our group has developed relaxase-Cas fusion proteins for directed modification of the recipient cell avoiding the need for gene expression [2]. Successful conjugation to wild-type strains may pave the way for future modifications and thus applications of these valuable bacteria.

KEYWORDS: *Conjugation, recalcitrant Gram-positive bacteria, directed genetic modification.*

REFERENCES

[1] Samperio, S., Guzmán-Herrador, D.L., May-Cuz, R., Martín, M.C., Álvarez, M.A., Llosa, M. (2021) Conjugative DNA Transfer From E. coli to Transformation-Resistant Lactobacilli. Front Microbiol. 2021 Feb 11;12:606629. doi: 10.3389/fmicb.2021.606629. PMID: 33643236; PMCID: PMC7905204.

[2] Guzmán-Herrador, D.L., Fernández-Gómez, A., Depardieu, F., Bikard, D. and Llosa, M. (2024). In vivo delivery of functional Cas:DNA nucleoprotein complexes into recipient bacteria through a Type IV Secretion System. Proc Natl Acad Sci USA (PNAS), in press.

PO-CBR for Delimiting Integrated Mobile Elements transferred by Conjugation

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ABSTRACT

Integrative Conjugative Elements (ICEs) and Integrative Mobilizable Elements (IMEs) are crucial drivers of bacterial plasticity. These mobile genetic elements integrate into bacterial genomes and disseminate adaptive traits, such as antibiotic resistance and virulence factors, via the T4SS conjugation machinery. To understand the role of ICEs and IMEs in bacterial evolution and adaptability, their delineation is essential. This study aims to develop an automated tool for the accurate detection of ICE and IME boundaries.

Our approach is based on case-based reasoning (CBR), an artificial intelligence approach, where a case is the representation of a problem-solving episode. CBR is often used to represent an expert's experience through a set of cases [1]. More specifically, our approach is based on process-oriented CBR, i.e., CBR in which cases represent processes. In this context, a case involves detailed procedures used to delineate a specific mobile element within the bacterial genome. Indeed, different case processes have been implemented to delimit numerous mobile elements, depending on the enzyme catalyzing integration and the targeted insertion gene. For example, our process case, CASE-1, employs data and integration characteristics from a known reference mobile element to define the boundaries of similar elements by determining their exact locations [2]. This approach enables precise modeling of delimitation processes and provides the flexibility to continually update our case base with new cases. PO-CBR also ensures transparent decision-making, essential for scientific validation and understanding the effectiveness of our approach.

Our findings confirm the effectiveness of our approach in delineating ICEs and IMEs in streptococcal genomes, revealing new genomic elements. The alignment of our delineation work with those reported in existing publications [3, 4] establishes the reliability of our method. These promising outcomes highlight its potential and encourage its application to more complex delineation tasks in other genera of the Bacillota phylum.

KEYWORDS: *Integrative Conjugative Elements, Integrative Mobilizable Elements, PO-CBR, Cases, mobile element delineation, Automated delineation.*

REFERENCES

[1] Lieber J, Bresson B. Case-Based Reasoning for Breast Cancer Treatment Decision Helping. In: Blanzieri E, Portinale L, editors. Advances in Case-Based Reasoning. Berlin, Heidelberg: Springer; 2000. p. 173–85.

[2] Lao J, Lacroix T, Guédon G, Coluzzi C, Payot S, Leblond-Bourget N, et al. ICEscreen: a tool to detect Firmicute ICEs and IMEs, isolated or enclosed in composite structures. NAR Genomics and Bioinformatics [Internet]. 2022 Dec 1 [cited 2024 Jun 26];4(4):lqac079. Available from: https://doi.org/10.1093/nargab/lqac079

[3] Ambroset C, Coluzzi C, Guédon G, Devignes MD, Loux V, Lacroix T, et al. New Insights into the Classification and Integration Specificity of Streptococcus Integrative Conjugative Elements through Extensive Genome Exploration. Front Microbiol. 2015;6:1483.

[4] Coluzzi C, Guédon G, Devignes MD, Ambroset C, Loux V, Lacroix T, et al. A Glimpse into the World of Integrative and Mobilizable Elements in Streptococci Reveals an Unexpected Diversity and Novel Families of Mobilization Proteins. Front Microbiol [Internet]. 2017 Mar 20 [cited 2024 Oct 1];8.

Studies on DNA binding mechanism of TraA relaxase from a gram-positive Type IV secretion system

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ABSTRACT

Multidrug-resistance among pathogenic bacteria is increasing rapidly. One mechanism by which these resistances get transferred between bacteria is conjugation through Type lV secretion systems (T4SS). In gram-negative bacteria these T4SS have been characterized well, while for gram-positive bacteria much is still unknown. In Enterococcus faecalis, the Tra complex, encoded on the plasmid pIP501, represents a T4SS system involved in the transfer of multidrug resistance across a broad range of bacteria. Tra consists of 15 transfer genes (TraA-TraO), which encode for proteins of the T4SS. Among these proteins is TraA, a relaxase that initiates the conjugation in the donor cell by binding at the oriT sequence, nicking of a single DNA strand and unwinding the plasmid. [1][2]

We investigate the structure of the N-terminal relaxase-domain of TraA (246 amino acids) with its specific DNA binding site. The obtained X-ray structure shows a complex with high similarity to the relaxase NES from conjugative plasmid pSK41 from Staphylococcus aureus, a multidrug-resistant gram-positive bacteria. [3] Comparison of the two structures showed a similar position of the putative catalytic residue, the DNA hairpin-like secondary structure as well as the complexed divalent ion located close to the active site. Based on this, new N-terminal variants of TraA were generated and the characterization of the interaction with DNA is ongoing. Due to the strong association of unspecific DNA with the protein, the production of variants proved to be challenging. The final aim of this work is to understand the DNA binding and transfer mechanism of TraA with the goal to prevent dissemination of antibiotic resistance by inhibiting the nicking activity as well as the transfer.

KEYWORDS: *relaxase, gram-positive, DNA-binding, pIP501, Enterococcus faecalis, TraA*

REFERENCES

[1] Kopec, J., Bergmann A., Fritz, G., Grohmann, E. and Keller, W. (2005) TraA and its N-terminal relaxase domain of the Gram-positive plasmid pIP501 show specific o*riT* binding and behave as dimers in solution, *Biochem*. J. 387, 401–409, doi: 10.1042/BJ20041178

[2] Kurenbach, B., Kopec, J., Mägdefrau, M., Andreas, K., Keller, W., Bohn, C., Abajy, M.Y., and Grohmann, E. (2006) The TraA relaxase autoregulates the putative type IV secretion-like system encoded by the broad-host-range *Streptococcus agalactiae* plasmid pIP501, *Microbiology*, 152, 000– 000, doi: 10.1099/mic.0.28468-0

[3] Edwards, J.S., Betts, L., Frazier, M.L., Pollet, R.M, Kwong, S.M., Walton, W.G., Ballentine III, W.K., Huang, J.J., Habibi, S., Del Campo, M., Meier, J.L., Dervane, P.B., Firth, N. and Redinbo, M.R. (2013) Molecular basis of antibiotic multiresistance transfer in Staphylococcus aureus, PNAS, vol. 110, no. 8, 2804–2809, doi : 10.1073/pnas.1219701110

Exploring novel protein-protein interactions and functions of selected *Helicobacter pylori* Cag Type 4 Secretion System (CagT4SS) outer proteins

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ABSTRACT

Background and Questions. The *Helicobacter pylori cag* pathogenicity island (*cag*PAI) is an important virulence factor of *H. pylori* and encodes a complex type IV secretion system (CagT4SS). Our earlier work has helped us to generate hypotheses concerning exposed, variable outer proteins that can interact with host factors. Recently, structural information on the CagT4SS has been substantially improved by cryo-EM. However, important details, in particular on protein interactions between the OMC and the surface "outer" complex of the CagT4SS, consisting of T4SS outer proteins, are missing. This also concerns functional details on putative active and inactive conformations of the export complex, and on the transport functions in general.

Methods and Results. Using bacterial two hybrid system (BACTH) and biochemical characterization methods, we have enhanced our knowledge on protein-protein interactions in the CagT4SS outer proteins. This includes interactions of outer membrane proteins, of the VirB2 homolog CagC and the outer protein CagN of yet unknown function. We have determined and quantitated homo-dimerization of CagC and CagN, elucidated novel interactions of all tested proteins by BACTH, and confirmed them using protein purification and interaction analysis, for instance by Octet biolayer interferometry analysis and high-resolution microscopy.

Conclusions. Novel interactions of *H. pylori* outer membrane proteins and CagT4SS outer proteins were found and further characterized using biochemical methods and microscopy. This will help to refine structural and functional details of the CagT4SS transport and translocation complex and machinery, also in contact with human cells.

KEYWORDS: *CagT4SS; protein interactions; Helicobacter pylori; outer membrane proteins; interactome*

Revisiting effector functions: Involvement of a putative T4SS effector in the envelope integrity of *Brucella*

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ABSTRACT

Intracellular pathogens encounter various stressful environments while trafficking towards their replicative niche inside the host cell. To successfully replicate, they have either to withstand the stress or manipulate the environment to their needs. We intended to understand the function of the putative Type 4 secretion system effector BspD of the zoonotic bacterium *Brucella* in infection. However, through *in silico* analyses, classical growth assays, morphological analyses, stress assays, as well as host cell infections we reveal that BspD is critical for envelope integrity of *Brucella*. We show that BspD is conserved in the Rhizobiales without evidence of co-evolution with the presence of a T4SS or a certain lifestyle. Further, we show that BspD is critical for *Brucella abortus* envelope integrity in the stationary phase and in the presence of the outer membrane destabilizing compound EDTA *in vitro*. Deletion of *bspD* leads to swelling, elongation, and plasmolysis. In infection the absence of BspD led to a survival defect, culminating in the formation of fewer and smaller microcolonies in a macrophage infection model. From our observations, we propose that BspD of *B. abortus* is critical for preserving the integrity of the bacterial envelope, particularly under stressful conditions, which may enhance *Brucella*'s ability to survive within host cells independently of its putative status as a Type 4 secretion system effector.

KEYWORDS: *Effector, function, Brucella, infection, envelope integrity*

Chromosomal Factors Essential for F Plasmid Conjugative Transfer

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ABSTRACT
Conjugative transfer is a phenomenon driven by *tra* genes encoded on self-transmissible plasmids, such as F plasmid. However, knowledge regarding chromosomal genes essential for conjugation remains limited. In this study, we experimentally identified *E. coli* chromosomal genes critical for conjugative transfer, based on structural analyses of T4SS. Tiago et al have shown that the pilus contains phosphatidylglycerol (PG), a type of phospholipid [1]. Using a PG synthesis-deficient mutant as the donor strain, we observed a complete loss of conjugative transfer capability and pilus formation. Additionally, the F plasmid's T4SS revealed cysteine-rich proteins with multiple predicted disulfide bonds [2], as suggested by AlphaFold2 predictions. These disulfide bonds are likely important for maintaining the structural stability and proper folding of T4SS components, which are essential for efficient plasmid transfer. Based on this observation, we targeted genes involved in disulfide bond isomerization. The deletion of these genes resulted in a marked reduction in the conjugative transfer efficiency of the F plasmid. These findings provide new insights into how T4SS assembles and functions during conjugative transfer, with potential applications in biotechnology, such as the development of targeted gene delivery systems, or in medicine for controlling the spread of antibiotic resistance.

KEYWORDS: Conjugative transfer, F-plasmid, Chromosomal factor

REFERENCES

[1] Tiago RD Costa, Aravindan Ilangovan, Marta Ukleja, Adam Redzej, Joanne M Santini, Terry K Smith, Edward H Egelman, Gabriel Waksman (2017) Structure of the bacterial sex F pilus reveals an assembly of a stoichiometric protein-phospholipid complex, *Cell* 166:1436-1444

[2] Kouhei Kishida, Yang Grace Li, Natsumi Ogawa-Kishida, Pratick Khara, Abu Amar M Al Mamun, Rachel E Bosserman, Peter J Christie (2024) Chimeric systems composed of swapped Tra subunits between distantly-related F plasmids reveal striking plasticity among type IV secretion machines, PLos Genetics 20: e1011088

Investigating the structure and interaction of TrwB/VirD4 within the T4SS

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ABSTRACT

The T4SS is a versatile bacterial mechanism that transfers DNA and proteins across cell membranes, facilitating processes such as bacterial conjugation or virulence. It plays a key role in spreading antibiotic resistance genes. TrwB/VirD4 is an essential hexameric transmembrane ATPase in the T4SS whose ATP hydrolysis activity is crucial for driving DNA translocation during conjugation. TrwB/VirD4 is believed to interact with other T4SS components embedded in the inner membrane, ensuring efficient DNA transfer [1]. However, its location within the T4SS still needs further investigation, as no clear structural evidence exists. We and others have identified interactions between TrwB/VirD4, TrwE/VirB10 and TrwK/VirB4 that could help us better understand the involvement of TrwB/VirD4 in the complex [2,3].

This project aims to solve the structure of the T4SS associated with TrwB/VirD4 by understanding specific interactions between the different components of the complex and stabilising the protein in place in the T4SS. To do so, we investigate different hypothesis using structure predictions, interaction assays, cryo-EM and crystallography. Crosslinking experiments together with structure predictions showed that TrwB/VirD4 interacts with TrwE/VirB10. However, further experiments are necessary to better understand and characterise in detail this interaction. To answer this, we aim to use both biophysical and structural approaches. Firstly, by using SPR or a similar method, we intend to validate the minimal motif of TrwE/VirB10 involved in the interaction with TrwB/VirD4. In the meantime, we aim to solve the structure of TrwB/VirD4 in the presence of TrwE/VirB10 motif using crystallography. On the other hand, to localise TrwB/VirD4 and solve its structure within the T4SS complex, we are trying to fuse a bulky domain to the protein that should help us identify the location of the protein using cryo-EM. In parallel, we investigate a manner to stabilise TrwB/VirD4 with the complex during the purification.

KEYWORDS: *Conjugation, Structural Biology, ATPase, Protein-protein interaction, Protein purification*

REFERENCES

[1] Garcillán-Barcia, M. P., de la Cruz, F. *TrwB, the DNA-Binding Coupling Protein Essential in Type IV Secretion.* Molecular Microbiology, 2002, **45**(4), 855-864. doi:10.1046/j.1365-2958.2002.03055.x

[2] Redzej, A., Ukleja, M., Connery, S., Trokter, M., Felisberto-Rodrigues, C., Cryar, A., Thalassinos, K., Hayward, R. D., Orlova, E. V., Waksman, G. *Structure of a VirD4 coupling protein bound to a VirB type IV secretion machinery.* EMBO J, 2017, 36: 3080-3095. doi:10.15252/embj.201796629

[3] Llosa M, Zunzunegui S, de la Cruz F. *Conjugative coupling proteins interact with cognate and heterologous VirB10-like proteins while exhibiting specificity for cognate relaxosomes*. Proc Natl Acad Sci U S A. 2003 Sep 2;100(18):10465-70. doi: 10.1073/pnas.1830264100

Structural studies on TraF, a transmembrane protein from a G+ Type IV secretion system

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ABSTRACT

Type IV secretion systems (T4SS) are large protein complexes which are capable of transferring DNA as well as proteins from one cell to another thus enabling the spread of plasmid-encoded antibiotic resistances within bacterial communities. The broad-host-range plasmid pIP501 from the gram-positive bacterium *Enterococcus faecalis* harbors a T4SS which encodes 15 transfer genes (*traA* to *traO*) organized in a 15kb-operon.[1] TraF is a 53 kDa protein, which is believed to assemble as a multimer as a part of the mating-pair formation (MPF) complex.

The aim of this work is to investigate the role of TraF within the MPF complex. Here, we present the crystal structure of the cytosolic N-terminal domain of $TraF_{pIP501}$ and investigate its putative function based on its high structural similarity to pseudokinases from T7SS like EssB from *Geobacillus thermodenitrificans*[2], and YukC from the T7SS of *Bacillus subtilis.*[3]

Based on crosslinking experiments, structure predictions of the full-length protein homo-multimer in combination with membrane mimetics were performed. A distinct two domain structure connected by long alpha-helical transmembrane bundles is proposed. Pull-down purification paired with mass spectrometry analysis shows that most of the Tra proteins are contained in the mating complex. Direct interactions between TraF and other Tra proteins are shown by BACTH assays.

TraF is an essential factor for the assembly of a functional conjugation system and therefore represents a promising target for therapeutics to inhibit the DNA transfer and prevent antimicrobial resistance dissemination.

KEYWORDS: *TraF, MPF complex, gram-positive, pIP501, Enterococcus faecalis*

REFERENCES [3]

[1] Berger T.M.I., Michaelis C., Probst I., Sagmeister T., Petrowitsch L., Puchner S., Pavkov-Keller T., Gesslbauer B., Grohmann E. & Keller W. (2022). Small Things Matter: The 11.6-kDa TraB Protein is Crucial for Antibiotic Resistance Transfer Among Enterococci, *Frontiers in Molecular Biosciences* 9, doi: 10.3389/fmolb.2022.867136

[2] Zoltner M, Norman D.G., Fyfe P.K., El Mkami H., Palmer T. & Hunter W.N. (2013). The Architecture of EssB, an Integral Membrane Component of the Type VII Secretion System, *Structure*, Volume 21, Issue 4, 595 – 603, doi: 10.1016/j.str.2013.02.007

[3] Tassinari, M., Doan, T., Bellinzoni, M., Chabalier, M., Ben-Assaya, M., Martinez, M., Gaday, Q., Alzari, P. M., Cascales, E., Fronzes, R., & Gubellini, F. (2022). The Antibacterial Type VII Secretion System of Bacillus subtilis: Structure and Interactions of the Pseudokinase YukC/EssB. *mBio*, *13*(5), doi: 10.1128/mbio.00134-22

Establishment of *in vivo* photo-crosslinking in *Legionella*

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ABSTRACT

Legionella pneumophila encodes for over 300 effector proteins which are injected by the Dot/Icm T4SS into the host cell during infection. Besides soluble effector proteins, the T4SS is also able to translocate transmembrane domain (TMD)-containing effector proteins (TMEs) which insert into various eukaryotic host membranes. It is still unclear how T4SS-TMEs get translocated. Depending on the TMD-hydrophobicity two mechanisms are proposed. TMEs with a high hydrophobic TMD are stated to use the two-step secretion pathway[1]. First, they get inserted into the inner bacterial membrane via the signal recognition particle (SRP) and Sec-translocon, followed by T4SS translocation. TMEs with a lower TMD-hydrophobicity most likely are directly targeted to the T4SS guided by possible housekeeping or T4SS-related chaperons[2].

In this study *in vivo* photo-crosslinking[3] is used to investigate possible interaction partners of four TMEs which differ in TMD-hydrophobicity to get an inside in the translocation mechanism. The most N-terminal TM-segment, which is most likely to get recognized by either the SRP or putative chaperons during the targeting process, was chosen for amber mutation to allow incorporation of the UV-reactive amino acid *p*Bpa. Sufficient *p*Bpa incorporation in the TMEs was achieved for *Legionella* growth on plate and in liquid culture. Further, *in vivo* photo-crosslinking of the effector $Ceg4_{F288X}$ showed a specific crosslink which was successfully immunopurified and will be analysed by mass spectrometry to identify the interaction partner. In addition, it was shown via the SplitNanoLuc-translocation assay that the amber mutated TMEs are translocated into macrophages during infection.

Overall, stable *in vivo* photo-crosslinking in *Legionella* was established, which can not only be used for further identification of direct interaction partners regarding targeting and translocation by the T4SS, but also could be extended to *Legionella*-infected macrophages to investigate eukaryotic protein interaction partners involved in membrane targeting and insertion of TMEs into host cell membranes.

KEYWORDS: *Legionella pneumophila, T4SS, transmembrane effectors, in vivo photo-crosslinking*

REFERENCES

[1] Malmsheimer, S., Grin, I., Bohn, E., Franz-Wachtel, M., Macek, B., Sahr, T., Smollich, F., Chetrit, D., Meir, A., Roy, C., Buchrieser, C., Wagner, S., (2024) The T4bSS of Legionella features a two-step secretion pathway with an inner membrane intermediate for secretion of transmembrane effectors, bioRxiv 2024.03.14.584949, https://doi.org/10.1101/2024.03.14.584949

[2] Krampen, L., Malmsheimer, S., Grin, I., Trunk, T., Lührmann, A., De Gier, J.-W., Wagner, S., (2018) Revealing the mechanisms of membrane protein export by virulence-associated bacterial secretion systems, Nat Commun 9, 3467, https://doi.org/10.1038/s41467-018-05969-w

[3] Farrell, I.S., Toroney, R., Hazen, J.L., Mehl, R.A., Chin, J.W., (2005) Photo-cross-linking interacting proteins with a genetically encoded benzophenone, Nat Methods 2, 377–384, https://doi.org/10.1038/nmeth0505-377

Deciphering the repeat II domain structure and function of CagY from *Helicobacter pylori*

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ABSTRACT

The more severe strains of the bacterial pathogen *Helicobacter pylori* produce a type IV secretion system (cagT4SS) to inject an oncoprotein CagA into gastric cells. CagA interacts with a large number of human signaling proteins, which disturbs cell functions and promotes tumor development. CagY, a large VirB10 homologue, belongs to the cagT4SS core complex and is also a pilus-associated protein. CagY is composed of three domains: a repeat I domain (RI), a repeat II domain (RRII) and B10 domain (B10). While the structure and localization of RRII is unknown, this domain can modulate TLR5 response [1] and allows antigenic evasion [2]. Here, our objective is to understand the role played by RRII in cagT4SS by determining its structure, its function and its partners of interaction during *H. pylori* infection.

First, a structural analysis was performed on RRII using Small Angle X-ray Scattering (SAXS). SAXS data showed that RRII is a monomeric protein with high flexibility. Prediction of RRII structure using AlphaFold2 showed that RRII was mainly composed of α helices which is coherent with previous CD experiments [3]. We also identified a structural motif (YRD) repeated fourteen times on RRII structure. YRD is composed of four antiparallel α helices stabilized by disulfide bridges. To confirm the model, one or two-three repetitions of YRD were produced, purified and the structure of one YRD solved by X-rays, confirmed AlphaFold model.
Furthermore, to understand the RRII's role during *H. pylori* infection we isolated RRII interactants without and with gastric epithelial cells, and identified them by mass spectrometry.

KEYWORDS: *H.pylori,* CagY, RRII domain

REFERENCES

[1] Tegtmeyer, N., Neddermann, M., Lind, J., Patchathundikandi, S.K., Sharafutdinov, I., Gutiérrez-Escobar, A.J., Brönstrup, M., Tegge, W., Hong, M., Rohde, M., Delahay, R.M., Vieth, M., Sticht, H. and Backert, S. Toll-like Receptor 5 Activation by the CagY Repeat Domains of Helicobacter pylori, Cell reports, 2020, Sep 15;32(11):108159, http://dx.doi.org/10.1016/j.celrep.2020.108159

[2] Aras, R.A., Fisher, W., Perez-Perez, G., Crosatti, M., Ando, T., Haas, R. and Blaser, M.J. Plasticity of Repetititve DNA Sequences within a Bacterial (Type IV) Secretion system Component, J Exp Med, 2003, 198(9):1349–1360, http://dx.doi.org/10.1084/jem.20030381

[3] Koelblen, T., Bergé, C., Cherrier, M.V, Brillet, K., Jimenez-Soto, L., Ballut, L., Tagagi, J., Montserret, R., Rousselle, P., Fisher, W., Haas, R., Fronzes, R. and Terradot, L. Molecular dissection of protein-protein interactions between integrin α5β1 and the Helicobacter pylori Cag type IV secretion system. FEBS J, 2017, Dec;284(23):4143-4157, http://dx.doi.org/10.1111/febs.14299

Exploration of DNA processing mediated by MOB_T relaxases during bacterial conjugation

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ABSTRACT

Integrated and Conjugative Elements (ICEs) are major players mediating bacterial conjugation, and are thus responsible for the spread of antibiotic resistance and virulence genes. During the initiation of conjugation, the DNA of the mobile element is processed by the relaxase, a transesterase that recognizes its origin of transfer (*oriT*) [1]. ICE*St3*/Tn*916* conjugative elements encode relaxases belonging to a unique family called MOB_T that is distantly related to *Rep-trans* proteins. Whereas the *nic* site of *oriT* is conserved with the *Rep-trans* proteins[2], the DNA binding site recognized by these MOB_T relaxases was still unknown. The aim of this study was to determine the sequence recognized by the relaxase of ICE*St3* (RelSt3) within the *oriT* region and to characterize the enzymatic features of MOB_T relaxases. We also studied two proteins encoded by ICE*St3*, OrfL and OrfM, which could be involved in the relaxosome complex.

To perform this work, we used multidisciplinary approaches combining *in vivo* (mating assays, bacterial two-hybrid) and *in vitro* (EMSA, SEC-MALS, ITC) techniques.

We identified a *bind* site of RelSt3 unexpectedly distant from the *nic* site. We revealed that the binding of RelSt3 on its *bind* site is required for efficient nicking activity[3]. We also characterized OrfL and OrfM as OB-fold proteins involved in ICE*St3* relaxosome. We demonstrated an interaction network between these accessory proteins and several DNA processing enzymes, especially the RelSt3 and the PcrA helicase.

In this study, we deciphered the role of a MOB_T relaxase in the initial and final stages of ICE conjugation. We also characterized OrfL and OrfM proteins as the first example of OB-fold accessory proteins involved in DNA processing during the first steps of conjugation.

Keywords: *DNA processing, bacterial conjugation, ICE, relaxase, OB-fold*

REFERENCES

[1]: De La Cruz, F., Frost, L.S., Meyer, R.J., and Zechner, E.L. (2010). Conjugative DNA metabolism in Gram-negative bacteria. FEMS Microbiol Rev 34, 18–40. https://doi.org/10.1111/j.1574-6976.2009.00195.x.

[2]: Soler N, Robert E, Chauvot De Beauchêne I, *et al*. Characterization of a relaxase belonging to the MOB_T family, a widespread family in Firmicutes mediating the transfer of ICEs. Mob DNA. 2019;10(1):18. doi:10.1186/s13100-019-0160-9

[3]: Laroussi H, Aoudache Y, Robert E, *et al.* Exploration of DNA processing features unravels novel properties of ICE conjugation in Gram-positive bacteria. Nucleic Acids Res. 2022;50(14):8127-8142. doi:10.1093/nar/gkac607

Biochemical Characterisation of TraI Relaxase Dimer Loading onto oriT during Bacterial Conjugation

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ABSTRACT

Horizontal gene transfer (HGT) plays a critical role in the dissemination of antimicrobial resistance among bacteria. Bacterial conjugation, a key driver of HGT, relies on the relaxosome complex to mediate the transfer of plasmid DNA through the Type IV Secretion System (T4SS). Within this system, the relaxase TraI (of the archetypical F/R1 system), a bifunctional enzyme with transesterase and helicase activities, initiates conjugation by nicking and unwinding the plasmid DNA at the origin of transfer (*oriT*).

Despite progress in understanding the relaxosome and T4SS, the mechanism of TraI relaxase loading as a dimer onto *oriT* remains unresolved. TraI was reported to function as a dimer, with its functionally distinct domains acting in a mutually exclusive manner to catalyse DNA nicking and unwinding. While previous studies have shed light on the conformation of TraI when bound to single-stranded DNA, the mechanism for loading two TraI molecules loading onto *oriT*, characterized by negative cooperativity, has remained elusive.

In this overall study, we employ biochemical assays (focus of this poster) and cryo-electron microscopy (cryo-EM) to elucidate the molecular mechanism of TraI dimer loading onto *oriT*. Our biochemical assays using forked DNA oligonucleotides reveal the specific length of the DNA bubble required for efficient TraI helicase loading. These findings provide critical insights into the conformational dynamics of TraI during plasmid transfer, advancing our understanding of the molecular machinery driving bacterial conjugation and its role in the spread of antimicrobial resistance.

KEYWORDS: *Relaxase, TraI, Bacterial conjugation, Type IV Secretion System.*

REFERENCES

[1] Ilangovan, A., Kay, C.W., Roier, S., El Mkami, H., Salvadori, E., Zechner, E.L., Zanetti, G. and Waksman, G., 2017. Cryo-EM structure of a relaxase reveals the molecular basis of DNA unwinding during bacterial conjugation. *Cell*, *169*(4), pp.708-721.

[2] Wong, J.J., Lu, J. and Glover, J.M., 2012. Relaxosome function and conjugation regulation in F-like plasmids–a structural biology perspective. Molecular microbiology, 85(4), pp.602-617.

Uncovering the Role of IMEs in Antimicrobial Resistance and Horizontal Gene Transfer

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ABSTRACT

Integrative Mobilizable Elements (IMEs) are mobile genetic elements that integrate into bacterial genomes and excise to transfer to other bacteria. Unlike Integrative Conjugative Elements (ICEs), IMEs lack their own conjugation machinery, hijacking an ICE or plasmid Type IV Secretion System (T4SS) (1). This allows IMEs to excise, circularize, and mobilize autonomously, often without disrupting the ICE's own transfer process (2). Though widespread across bacterial species, IMEs remain relatively underexplored compared to other mobile genetic elements. Many IMEs integrate into the origin of transfer (*oriT*) or open reading frames of ICEs, using the ICE as both a host and facilitator for their mobilization (3). Besides mobility, IMEs frequently harbor genes conferring antimicrobial resistance (AMR), positioning them as key players in the horizontal spread of resistance among bacterial populations. Furthermore, the interaction between IMEs and T4SSs that enables their transfer remains an area of ongoing research.

In *Streptococcus suis*, a zoonotic pathogen, IMEs have been shown to play a major role in disseminating AMR genes. In silico analyses of 102 *S. suis* strains isolated from healthy or sick pigs, humans and wild boars, revealed that IMEs exhibit impressive diversity and plasticity. While ICEs were initially thought to be the primary vectors of AMR, it has been established that IMEs in *S. suis* carry a significant portion of these genes. IME_*SNF2* and IME_*PPI* in particular frequently carry both *tet*(O) and *erm*(B) genes. Analysis of the expression of their recombination module by quantitative PCR indicated that environmental factors, such as oxidative stress and UV exposure, play a role in their activation. This indicates that these elements may respond dynamically to external stimuli, allowing them to exploit conjugation systems under specific conditions. Understanding these environmental triggering signals could provide insights into how IMEs contribute to resistance genes spread inside bacterial communities.

KEYWORDS: *Integrative Mobilizable Elements, Streptococcus suis, Antimicrobial Resistance, Horizontal Gene Transfer*

REFERENCES:

[1] Guédon G., Libante V., Coluzzi C, Payot S and Leblond-Bourget N. The Obscure World of Integrative and Mobilizable Elements, Highly Widespread Elements that Pirate Bacterial Conjugative Systems. Genes. 2017;8:337. doi: 10.3390/genes8110337.

[2] Libante V, Sarica N, Mohamad Ali A, Gapp C, Oussalah A, Guédon G, Leblond-Bourget N. and Payot S. Mobilization of IMEs Integrated in the *oriT* of ICEs Involves Their Own Relaxase Belonging to the Rep-Trans Family of Proteins. 2020. doi: 10.3390/pathogens9010022.

[3] Libante V, Nombre Y, Coluzzi C, Staub J, Guédon G, Gottschalk M, Teatero S, Fittipaldi N, Leblond-Bourget N, Payot S. Chromosomal Conjugative and Mobilizable Elements in *Streptococcus suis*: Major Actors in the Spreading of Antimicrobial Resistance and Bacteriocin Synthesis Genes. Pathogens. 2019;9:22. doi: 10.3390/pathogens9010022.

Establishing proximity biotinylation as tool to investigate Dot/Icm T4SS effectors in *Legionella pneumophila*

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ABSTRACT

Legionella pneumophila is the facultative intracellular pathogen responsible for Legionnaires' disease – a severe form of pneumonia. *L. pneumophila* utilises the Dot/Icm Type 4 Secretion System (T4SS), to deliver more than 330 effector proteins to manipulate host cells. Many exert their roles through direct interactions with host proteins, for example, to change the subcellular localisation of the target protein, i.e., recruiting it to the *Legionella*-containing vacuole, or to disrupt the formation of the canonical and/or induce completely new, non-canonical signalling complexes (1).

Identification of the primary targets of effectors, as well as the effector-modulated interactomes and information about their subcellular localisation is therefore key to dissect the infection process.

We previously developed a system for determining effector interactomes in infected cells (2); however this system has limited power to reveal the wider interaction network of the host targets and transient interactions. Recently *E. coli* BirA-derived biotin ligases, such as TurboID, that promiscuously biotinylate proteins in their proximity have become a widely used research tool to profile protein complexes (3). Fused to a protein of interest, TurboID modifies direct interactors and all proteins in a certain volume around the bait, all of which can be isolated by streptavidin pulldown and identified by MS.

To make this technique accessible and determine its potential for the characterisation Dot/Icm T4SS we generated plasmids for expression of TurboID effector fusions in *Legionella*. Using IF microscopy and Western Blot we demonstrate that TurboID::effector fusions are translocated during infection, resulting in biotinylation of proteins in defined subcellular locations inside the host cell in as little as 30 minutes. Purification and MS analysis of biotinylated proteins showed that the "proxisome" of the well-characterised effector SidM included known interaction partners, providing proof-of-concept that this will be a valuable tool to dissect effector-driven interaction networks.

KEYWORDS: *Legionella, TurboID, proximity biotinylation, T4SS effectors, infection-dependent interactions, mass-spectrometry*

REFERENCES

(1) Lockwood, Amin, Costa and Schroeder (2022). The *Legionella pneumophila* Dot/Icm type IV secretion system and its effectors. Microbiology; vol. 168, issue 5 DOI 10.1099/mic.0.001187

(2) So, Mousnier, Frankel and Schroeder (2019). Determination of In Vivo Interactomes of Dot/Icm Type IV Secretion System Effectors by Tandem Affinity Purification. Methods Mol Biol. DOI: 10.1007/978-1-4939-9048-1_19

(3) Branon, Bosch, Sanchez, et al. (2018). Efficient proximity labeling in living cells and organisms with TurboID. Nat Biotechnol 36, 880–887. https://doi.org/10.1038/nbt.4201

Elucidating assembly and function of VirB8 cell wall subunits refines the DNA translocation model in Gram-positive T4SSs

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ABSTRACT

Conjugative Type IV secretion systems (T4SSs) are membrane-spanning multiprotein complexes specialized in the transfer of mobiles genetic element between bacteria. They are considered as main drivers for the spread of antibiotic resistance genes. While T4SSs are extensively studied in Gram-negative bacteria, their architecture and mode of function in Gram-positive bacteria remain poorly understood[1]. According to the envelope structure in Gram-positive bacteria, it has been proposed that T4SSs are made by two functional complexes, the cell wall complex and the translocon[2]. To bring new insights into the biology of these bacterial nanomachines, we focused on this study on characterizing VirB8-like proteins. Our funding revealed that the VirB8-like are cell wall components where they act as trimeric subunits, adopting a conserved assembly pattern, distinct from their counterparts in Gram-negative bacteria. Additionally, we identified the interaction of VirB8-like proteins with T4SS components, in particular the predicted translocon components, highlighting the central role of VirB8-like proteins in T4SS assembly. Notably, we revealed a direct interaction between VirB8-like proteins and DNA, indicating their direct involvement in DNA transfer during conjugation. Altogether, our data allowed us to propose a model of Gram-positive T4SSs, where VirB8-like proteins, assemble a channel-like structure in the cell wall, a model distinct from what it was described in Gram-negative bacteria[3].

KEY WORDS: Conjugation, T4SS, VirB8-like, Gram-positive bacteria.

REFERENCES

[1] Bhatty, M., Laverde Gomez, J.A., Christie, P.J., 2013. The expanding bacterial type IV secretion lexicon. Res Microbiol 164, 620–639. https://doi.org/10.1016/j.resmic.2013.03.012.

[2] Grohmann, E., Christie, P.J., Waksman, G., Backert, S., 2018. Type IV secretion in Gram-negative and Gram-positive bacteria. Mol Microbiol 107, 455–471. https://doi.org/10.1111/mmi.13896

[3] Maffo-woulefack, R., Mohamad Ali, A., Laroussi, H., CAPPELE, J., Romero Saavedra, F., Ramia, N., Robbert, E., Mathiot, S., Soler, N., Roussel, Y., Fronzes, R., HUEBNER, J., Didierjean, C., Favier, F., Leblond-Bourget. N., Douzi, B. 2024. Elucidating assembly and function of VirB8 cell wall subunits refines the DNA translocation model in Gram-positive T4SSs. *In revision*.

Formation, purification, and structural analysis of protein-protein and protein-DNA complexes from the conjugative machinery of the R388 plasmid

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ABSTRACT

Bacterial conjugation is the main mechanism for horizontal gene transfer, conferring plasticity to the genome repertoire. This process is also the major instrument for the dissemination of antibiotic resistance genes. Hence, gathering primary information of the mechanism underlying this genetic transaction is of a capital interest. In bacterial conjugation, DNA transfer is mediated by the type IV secretion system (T4SS), a large macromolecular complex involved in substrate transport and pilus biogenesis. Both processes require energy usually coming from the hydrolysis of ATP catalyzed by specific ATPases. In the R388 conjugative plasmid, the relaxosome, is formed by the relaxase (TrwC) and auxiliary transfer proteins (TrwA and IHF) bound to a fragment of DNA several hundred base pairs in length, which contains the origin of transfer (oriT). Recently, the structure of the R388 T4SS has been resolved using high-resolution cryo-electron microscopy. However, many questions remain about the mechanism by which the relaxase, oriT, and accessory proteins bind to form the relaxosome and how TrwC covalently bound to the DNA is transported through the secretion channel during bacterial conjuation. In this study, we have formed and purified the TrwA-DNA and TrwB-TrwC protein complexes with the aim of resolving their structures through protein crystallography, which would help describe the steps leading to the transport of the conjugative substrate.

KEYWORDS: Bacterial *Conjugation; Antibiotic Resistance; T4SS*

REFERENCES

[1] Sugden, R., Kelly, R., & Davies, S. (2016). Combatting antimicrobial resistance globally. *Nature microbiology*, *1*(10), 16187. https://doi.org/10.1038/nmicrobiol.2016.187

[2] Macé, K., Vadakkepat, A. K., Redzej, A., Lukoyanova, N., Oomen, C., Braun, N., Ukleja, M., Lu, F., Costa, T. R. D., Orlova, E. V., Baker, D., Cong, Q., & Waksman, G. (2022). Cryo-EM structure of a type IV secretion system. *Nature*, *607*(7917), 191–196. https://doi.org/10.1038/s41586-022-04859-y

Characterization of the Xanthomonas *citri* T4SS *subunits* VirB8 and VirB6

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ABSTRACT

The type IV secretion system from the Gram-negative bacterium *Xanthomonas citri* (T4SS$_{X.citri}$) belongs to the class of minimized systems. It is an effector translocator system, specialized in the injection of toxins into other bacteria [1]. VirB8 is a key T4SS$_{X.citri}$ subunit. It is anchored in the inner membrane by a long N-terminal helix, while its globular domain interacts with other VirB8 and T4SS subunits in the periplasm. Indeed, VirB8 homologues from other bacterial species have been crystalized as dimers [2]. The VirB8 from the Xanthomonadales order differs from the canonical VirB8 observed in other species due to a long C-terminal tail. To investigate the potential role of this C-terminal extension and it is interactions with other subunits, we designed three VirB8$_{X.citri}$ constructs. Gel filtration experiments with recombinant fragments suggested that the VirB8$_{X.citri}$ C-terminal extension is critical to oligomerization; constructs lacking the C-terminal tail existed mainly as monomers. VirB6, another key T4SS subunit, is anchored in the inner membrane by two transmembrane helices. The rest of the protein lies in the periplasm and interacts with VirB5 to form a pentameric stalk structure [3]. While the exact topology of VirB6$_{X.citri}$ remains unclear, AlphaFold predictions suggested the presence of a disordered C-terminal region spanning 72 amino acids. Recombinant VirB6$_{X.citri}$, expressed in fusion either with a GFP-Histag or with a Strep-tag at the C-terminus, exhibited a monodisperse peak upon size-exclusion chromatography in the presence of DDM or LDAO. Pull-down and western-blotting assays supported the interaction of VirB6$_{X.citri}$ with VirB2$_{X.citri}$ and VirB5$_{X.citri}$. Altogether, these results provide insights on the intricate assembly and functional mechanism of the T4SS$_{X.citri}$.

KEYWORDS: *T4SS, VirB6, VirB8, Xanthomonas citri*.

References

[1] Souza, D., Oka, G., Alvarez-Martinez, C. et al. Bacterial killing via a type IV secretion system. Nat Commun 6, 6453 (2015). https://doi.org/10.1038/ncomms7453

[2] Fercher, C., Probst, I., Kohler, V., Goessweiner-Mohr, N., Arends, K., Grohmann, E., Zangger, K., Meyer, N. H., & Keller, W. (2016). VirB8-like protein TraH is crucial for DNA transfer in Enterococcus faecalis. Scientific reports, 6, 24643. https://doi.org/10.1038/srep24643

[3] Macé, K., & Waksman, G. (2024). Cryo-EM structure of a conjugative type IV secretion system suggests a molecular switch regulating pilus biogenesis. The EMBO journal, 43(15), 3287–3306. https://doi.org/10.1038/s44318-024-00135-z

Molecular interactions required for secretion of *Helicobacter pylori* CagA

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ABSTRACT

Gastric colonization with *Helicobacter pylori* strains that produce CagA and the Cag Type IV Secretion System (T4SS) confers an increased risk of stomach cancer. The Cag T4SS delivers CagA, a bacterial oncoprotein, into gastric cells. Our previous studies showed that CagF, a putative chaperone for CagA, can serve as a bait for isolating CagA and the Cag T4SS outer membrane core complex (OMCC), but the molecular interactions involved in this process remain elusive. Single-particle cryo-EM analyses of the OMCC revealed that it contains multiple copies of 5 proteins (CagY, CagX, CagT, CagM, and Cag3). To elucidate molecular interactions required for the binding of CagA to CagF and binding of the CagA-CagF complex to the Cag T4SS OMCC, we used a crosslinking mass spectrometry approach. We purified CagF and CagA from a T4SS-assembly deficient strain of *H. pylori* (Δ*cagX*), and we purified partially assembled OMCC complexes, consisting of only CagX and CagY, from a Δ*cagM* mutant. We then treated the complexes with the amine-reactive crosslinker BS3. Samples were analyzed by tandem mass spectrometry to identify sites of monolinks, intraprotein crosslinks, and interprotein crosslinks. Our analysis of the CagA-CagF complex revealed 83 interprotein crosslinks, distributed in multiple domains of CagA. Our analysis of samples from the Δ*cagM* mutant revealed 41 CagX-CagY crosslinks, 12 CagF-CagA crosslinks, and 2 CagF-CagX crosslinks. Within the cryo-EM structure of the Cag T4SS OMCC, the calculated distances between multiple crosslinked CagX/CagY lysine residues were within 30 Å. Structural models of a CagF-CagA complex were generated using AlphaFold 3; the calculated distances between multiple crosslinked CagF/CagA lysine residues in the model were within 30 Å. These results provide new insights into the molecular interactions required for secretion of CagA and suggest that CagF has important chaperone and adaptor functions in the recruitment of CagA to the Cag T4SS.

KEYWORDS: *Helicobacter pylori, Cag T4SS, gastric cancer.*

REFERENCES

[1] Frick-Cheng, A. E., Pyburn, T. M., Voss, B. J., McDonald, W. H., Ohi, M. D., & Cover, T. L. (2016). Molecular and structural analysis of the *Helicobacter pylori* cag type IV secretion system core complex. *mBio*, 7(1), 10-1128.

[2] Sheedlo, M. J., Chung, J. M., Sawhney, N., Durie, C. L., Cover, T. L., Ohi, M. D., & Lacy, D. B. (2020). Cryo-EM reveals species-specific components within the *Helicobacter pylori* Cag type IV secretion system core complex. *eLife*, *9*, e59495.

[3] Roberts, J. R., Tran, S. C., Frick-Cheng, A. E., Bryant, K. N., Okoye, C. D., McDonald, W. H., Cover, T.L., & Ohi, M. D. (2024). Subdomains of the *Helicobacter pylori* Cag T4SS outer membrane core complex exhibit structural independence. *Life Science Alliance*, *7*(6).

Deciphering the Secretome of *Brucella* using Non-Canonical Amino Acid Tagging (BONCAT)

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ABSTRACT

Brucellosis, a zoonotic disease, is a neglected endemic tropical illness according to the WHO primarily transmitted to humans through close contact with livestock. The etiologic agent, *Brucella spp*., is host-specific, but can infect humans as incidental hosts through the digestive or respiratory tract.

Once in the host, *Brucellae* are internalised into the *Brucella* containing vacuole (BCV). BCVs initially follow the canonical endocytic pathway, fusing with maturing endocytic vesicles, which results in an acidification of the BCV. The transient acidification triggers the non-canonical T4SS, leading to effector translocation. These effectors induce trafficking of the BCV to the ER, leading to the remodelling of the BCV to a replication-permissive state. During the intracellular lifecycle, *Brucellae* escape host recognition and interact with intracellular processes, particularly the immune response.

The major part of these *Brucella*-host interactions is believed to take place via effectors secreted into the host cells. Known effectors interact with components of the innate immune response and manipulate host membrane trafficking. Due to several challenges involving the non-canonical nature of the T4SS of *Brucella,* only a limited repertoire of effectors have been discovered. To date, mostly targeted approaches were applied for the identification and validation of effectors.
Bio-orthogonal non-canonical amino acid tagging (BONCAT) can be used as a functional, untargeted approach to identify the secretome of *Brucella* using mass spectrometry with spatiotemporal resolution.
Here we show in a first step comparable growth of the *B. microti* strain expressing the modified methionine tRNA synthethase (MetRS*) and the wild type strain, under axenic conditions and in infection. Further, azidonorleucin (Anl) was successfully incorporated by *B. microti* expressing MetRS* in broth and in infection.
Upon successful establishment of the method, BONCAT will be applied to *B. melitensis* to identify the effector repertoire in infection.

KEYWORDS: Brucella, BONCAT, metabolic labelling, mass spectrometry, infection, secretome, effector identification

Structural Characterisation of the Recipient Cell Detection Mechanism by the Type IV Secretion System

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ABSTRACT

Bacterial conjugation is a biological process through which a donor cell transfers DNA to a recipient cell. It is the primary cause of the spread of antibiotic resistance genes among bacterial populations, significantly contributing to the global antibiotic resistance crisis (1). The DNA transfer is mediated by a large molecular machinery embedded in the donor cell membranes, called the conjugative Type IV Secretion System (T4SS) (2). To establish the first contact between the two bacteria, the donor cell produces a long extracellular filament – the conjugative pilus – essential for DNA transfer (3). Despite its importance, the structure and the mechanism of the initial contact between the two bacteria during conjugation remain poorly studied. In this context, our project aims to characterize the structure of the pilus tip to the molecular mechanisms involved in the interaction with the recipient cell using several cryo-Electron Microscopy approaches.

Using the R388 plasmid, we purified pili and identified the protein localized at the pilus tip through EM labelling. We then initiated the investigation of pilus-membrane interactions via Cryo-Electron Tomography. Additionally, we characterized the function, localization, and oligomerization state of the natural conjugation inhibitor Eex, which is produced by T4SSs to prevent continuous DNA exchange between bacteria containing the same conjugative plasmid. This study provides molecular-level insights into the key step of recipient cell recognition by the pilus tip, presenting a potential target to block bacterial conjugation and limit the spread of antibiotic resistance genes.

KEYWORDS: *T4SS, Cryo-Electron Microscopy, Conjugative Pilus*

REFERENCES [3]:

[1] Partridge, S. R., Kwong, S. M., Firth, N., & Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. Clinical Microbiology Reviews, 31(4), e00088-17.

[2] Macé, Kévin, et al. « Cryo-EM Structure of a Type IV Secretion System ». Nature, vol. 607, no 7917, juillet 2022, p. 191-96. www.nature.com, https://doi.org/10.1038/s41586-022-04859-y.

[3] Goldlust, Kelly, et al. « The F Pilus Serves as a Conduit for the DNA during Conjugation between Physically Distant Bacteria ». *Proceedings of the National Academy of Sciences*, vol. 120, nº 47, novembre 2023, p. e2310842120. *DOI.org (Crossref)*, https://doi.org/10.1073/pnas.2310842120.

Prevalence and diversity of Integrative and Conjugative or Mobilizable Elements carrying antimicrobial resistance genes in *Streptococcus suis*

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ABSTRACT

Streptococcus suis, commensal of the upper respiratory tract of pigs, can lead to severe infections in post-weaning animals but also in humans after close contact with infected animals or through consumption of pork. Cases are usually sporadic but large outbreaks with fatal cases have been reported in Asia (1). This zoonotic pathogen frequently carries antibiotic resistance genes (ARGs), most of them being located on Mobile Genetic Elements (MGEs) in particular Integrative and Conjugative Elements (ICEs) and Integrative and Mobilizable Elements (IMEs) (2). The purpose of this work was to make an extensive study of the prevalence and diversity of MGEs carrying ARGs in *S. suis*. A total of 2588 genomes were clustered using dRep in order to select the best representative genomes based on assembly quality and genomic diversity. Genomes were then scaffolded using 21 reference *S. suis* genomes and annotated before searching ARGs – using ResFinder, CARD and homemade databases – and ICEs/ IMEs using ICEscreen (3). Clades were defined after alignment of core genes using PPanGGOLiN and phylogenetic tree construction. A dataset of 411 high-quality genomes (with associated metadata: country, year of isolation, host, isolation source, serotype) was created that covers the diversity of the *S. suis* species. A total of 1943 ARGs and 584 ICEs (7 families)/1069 IMEs (12 families) were detected in these genomes. Only 12 strains were devoid of ARGs. Multi-dimensional statistical analyses of the dataset revealed: (i) specific MGE co-occurrences, and (ii) statistical links between MGE and ARG contents and clade and isolation site (upper or lower respiratory tract or systemic). This study confirms the huge diversity of ICEs and IMEs carrying ARGs in *S. suis* and identifies a few avenues about the interplay between the various families of ICEs and IMEs that will be interesting to test experimentally.

KEYWORDS: *Streptococcus suis, antimicrobial resistance, Integrative Conjugative Elements, Integrative Mobilizable Elements*

REFERENCES

[1] Dutkiewicz, J., Sroka, J., Zając, V., Wasiński, B., Cisak, E., Sawczyn, A., Kloc, A. and Wójcik-Fatla, A. (2017) *Streptococcus suis*: a re-emerging pathogen associated with occupational exposure to pigs or pork products. Part I – Epidemiology, *Ann Agric Environ Med* 24(4):683-695, http://dx.doi.org/10.26444/aaem/79813

[2] Dechêne-Tempier, M., Marois-Créhan, C., Libante, V., Jouy, E., Leblond-Bourget, N. and Payot, S. (2021) Update on the Mechanisms of Antibiotic Resistance and the Mobile Resistome in the Emerging Zoonotic Pathogen *Streptococcus suis*, *Microorganisms* 9(8):1765. http://dx.doi.org/10.3390/microorganisms9081765

[3] Lao, J., Lacroix, T., Guédon, G., Coluzzi, C., Payot, S., Leblond-Bourget, N. and Chiapello, H. (2022) ICEscreen: a tool to detect Firmicute ICEs and IMEs, isolated or enclosed in composite structures. *NAR Genom Bioinform* 4(4):lqac079. http://dx.doi.org/10.1093/nargab/lqac079

Type IV secretion system drives interbacterial competition in the plant pathogen *Xanthomonas*

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ABSTRACT

Bacteria have evolved diverse antibacterial strategies to eliminate competitors from their environment. Recently, a novel T4SS in the order *Xanthomonadales* (X-T4SS) was shown to have bacterial killing activity by injecting toxic effectors into bacteria in a contact-dependent manner [1]. This machinery exhibits structural features that distinguish it from the canonical conjugative T4SS [2]. The genus *Xanthomonas* is a large group of Gram-negative plant-associated bacteria causing diseases in more than 350 host plants worldwide. Although X-T4SSs are widely distributed among xanthomonads, knowledge about X-T4SS evolution affecting microbial ecology is limited. Here we studied X-T4SS function and evolution in *Xanthomonas* including the cereal pathogen, *Xanthomonas translucens* (*Xt*). A genomics-informed screening for X-T4SS gene clusters was conducted among all xanthomonads. Interestingly, the heterogeneous distribution of X-T4SS was mutually exclusive with the distribution of another antibacterial system, the type VI secretion system (T6SS). Using a combination of genetic and fluorescence-based methods, we demonstrate that both X-T4SS and T6SS are crucial for interbacterial competition in *Xt*. Comparative genetic and phylogenetic analyses showed that X-T4SS was likely acquired ancestrally at an early stage in *Xanthomonas* evolution, then subject to loss events in some *Xanthomonas* lineages. In addition, competition assays demonstrated that the X-T4SS was required for fitness against a cereal bacterial consortium. This work provides a framework to define the mechanisms promoting fitness in cereal phytobiomes.

KEYWORDS: *antibacterial, ecology, evolution*

REFERENCES

[1] Souza, D. P., Oka, G. U., Alvarez-Martinez, C. E., Bisson-Filho, A. W., Dunger, G., Hobeika, L., ... and Farah, C. S. (2015). Bacterial killing via a type IV secretion system. Nature Communications, 6(1), 6453.

[2] Sgro, G. G., Oka, G. U., Souza, D. P., Cenens, W., Bayer-Santos, E., Matsuyama, B. Y., ... and Farah, C. S. (2019). Bacteria-killing type IV secretion systems. Frontiers in microbiology, 10, 1078.

Structural and functional insights into OrfD, the VirB4-like component of ICESt3 from *Streptococcus thermophilus*

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ABSTRACT

Conjugative type IV secretion systems (T4SSs) are widespread bacterial nanomachines that ensure the transfer of mobile genetic elements between contacting bacteria. Although prevalent in multiple human and animal Gram-positive pathogens, their architecture and mode of function remains poorly understood. Among Gram-positive T4SSs components, VirB4-like proteins are pivotal for system assembly and function, but yet their structural organization and precise role in the conjugation process are unclear. To address this knowledge gap, we investigated OrfD, the putative VirB4-like encoded by ICE*St3*, a mobile genetic element largely found in streptococci. Structural modeling identified two distinct domains: the N-terminal domain (N-ter) and the C-terminal domain (NBD) characterized by signature motifs of AAA-ATPases. By combining multiple and complementary approaches, we showed that OrfD multimerizes mainly through its NBD, binds DNA and form a channel-like structure. This exploration provides new insights into the assembly of VirB4-like proteins in Gram-positive T4SSs and opens new perspectives on their function during the conjugative transfer.

KEYWORDS: *Bacterial conjugation, Gram-positive T4SS, VirB4-like*

Optimal secretion of effector through the Dot/Icm secretion system depends on the bipolar diguanylate cyclase Lpp0809

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ABSTRACT

The second messenger c-di-GMP has emerged as key regulator of bacterial physiology, including virulence gene expression. Recently, local interplay between specific c-di-GMP metabolizing enzymes and components of secretion systems suggested a role of c-di-GMP signaling in the molecular control of these complex machineries.

Successful *Legionella* infection requires a functional Dot/Icm type IV secretion system which translocates a large repertoire of effectors into the host cytosol. The translocation kinetics of these effectors have to be tightly coordinated to ensure their quantitative and temporal delivery upon each step of the infection process. We showed that Lpp0809, one of the 22 c-di-GMP metabolizing enzymes of *Legionella pneumophila*, is crucial for virulence both in macrophages and amoeba cells. The diguanylate cyclase activity of Lpp0809 is specifically required for bacterial survival at the early stages of infection and for adequate Dot/Icm effector secretion. Comparative transcriptomic data between the Δ*lpp0809* and the wild-type strain suggested that this protein acts at a post-transcriptional level. Localization assays showed that Lpp0809 is localized to the bacterial poles, which is consistent with a direct or indirect interaction with the polar Dot/Icm T4SS. Interestingly, this polar localization requires the c-di-GMP synthetizing domain and evolves during infectious cycle suggesting specific and transient interaction with partners at bacterial poles.

Our results strengthen the role of c-di-GMP signaling in the fine-tuned regulation of effectors translocation at the early steps of the infectious cycle of *Legionella pneumophila*.

KEYWORDS: *T4SS, diguanylate cyclase, c-di-GMP*

Effect of Emerging pollutants at environmentally relevant concentrations on the transference of antibiotic-resistant genes

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ABSTRACT

Today the rate at which new substances are being released into the environment exceeds our capacity to assess and monitor them. This gap between production and assessment poses a significant risk as the impact of these substances on biological systems is underestimated. There is a growing concern related to the presence of these contaminants in the environment and the possible selective pressure they exert on microbial communities that could enhance the transference of Antibiotic-Resistant Genes (ARGs) as a way ti cope with the stress exerted. It is widely accepted that antimicrobials such as antibiotics and disinfectants enhance horizontal gene transfer (HGT) of ARGs. New studies, point out other Emerging Pollutants (EPs) without apparent antimicrobial effect to be exerting this same effect.

The aim of this work was to establish a standard protocol to characterize the effect of Eps from different classes on the transfer of ARGs by namely conjugation. To this end, a semi-HTP platform was employed to establish a controlled system for the study of the transference of conjugative plasmid between *Escherichia coli* strains. Two representative compounds from each class (i.e., antibiotics, disinfectants, pharmaceuticals, pesticides, personal care products and industrial compounds) were selected and evaluated at concentrations ranging from 1 to 10 µg/L. In parallel, we studied the underlying molecular mechanisms to unravel their implication in the enhanced plasmid transfer under the exposure to these pollutants. In addition, we repeated the study using natural waters from two wastewater treatment plants, known as hotspots of ARGs, as inducers of conjugation to gain insight into the effect that the mixture of contaminants could exert. Interestingly, the EPs that enhanced HGT only did so at the highest concentration tested. When bacteria were exposed to water samples, the mixed effect of these contaminants at lower concentrations could enhance HGT.

KEYWORDS: *Antibiotic Resistance, Conjugation, Emerging pollutants*.

REFERENCES

[1] Cen T, Zhang X, Xie S and Li D. (2020) Preservatives accelerate the horizontal transfer of plasmid mediated antimicrobial resistance genes via differential mechanisms, *Environment International* 138:105544, https://doi.org/10.1016/j.envint.2020.105544

[2] Zhang Q, Liu X, Zhou H, Lu Y, Wu L, et al. (2022) Reduction pathway of graphene oxide affects conjugation-mediated horizontal gene transfer under environmental conditions, *Chemical Engineering Journal* 450:138301, https://doi.org/10.1016/j.cej.2022.138301

Legionella pneumophila LpDot1 interferes with host nuclear functions by altering paraspeckles

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ABSTRACT

Understanding how pathogens manipulate the epigenetic regulation of the host to proliferate and survive may help finding new strategies to fight infectious diseases (1). One of these pathogens is *Legionella pneumophila*, a bacterium that replicates naturally in aquatic amoeba, but can also infect human cells and cause a severe pneumonia in humans, called Legionnaires' disease. Uniquely, *L. pneumophila* encodes a large repertoire of proteins encoding eukaryotic-like motifs acquired from its hosts and translocated via a specialized T4SS called Dot/Icm. Some of them target the host cell nucleus and reprogram the cellular response to the bacterial advantage (2). Here we identified and characterized a *L. pneumophila* protein, predicted to encode a lysine methyltransferase similar to human Dot1L (disruptor of telomeric silencing-1), therefore named *Lp*Dot1. In humans, Dot1L is known to catalyze the methylation of Lys79 on histone H3 (H3K79). Interestingly, in the genome of its protozoan host *Acanthamoeba castellanii*, we identified three Dot1-like proteins: two that are more similar to the human one, and a shorter version that is similar to the bacterial LpDot1, suggesting that the *lpDot1* gene was acquired through horizontal gene transfer from its protozoan host. Here we hypothesized that LpDot1 mimics the functions of its homologous proteins in eukaryotes. We solved the crystal structure of LpDot1 and determined its 3D structure at >2.4Å resolution. By using a MS/MS approach on the nuclear cell proteome, we identified specific LpDot1 targets and could show that LpDot1 is not a histone methyltransferase like in humans, but it has methyltransferase activity on nuclear non-histone proteins, predominantly RNA binding proteins that play multiple regulatory roles in the nucleus, such as subnuclear body formation, transcriptional regulation, genome stability and alternative splicing. For one of them we identified the unique site of methylation, that occurs in a structural motif important for its activities. Furthermore, we observed that *L. pneumophila* infection causes its degradation in a *Lp*Dot1-dependent manner, altering subnuclear body formation with resulting in a slight influence of LpDot1 on protein synthesis and a dramatic effect on alternative splicing on a gene involved in cell death. Altogether, these results suggest that *L. pneumophila* hijacks host nuclear functions through a unique secreted methyltransferases that modifies a RNA binding protein in the host cell to help its intracellular survival.

KEYWORDS: *Legionella pneumophila*, *epigenetics, nuclear effectors*

REFERENCES

[1] Bierne H, Pourpre R. 2020. Bacterial Factors Targeting the Nucleus: The Growing Family of Nucleomodulins. Toxins (Basel) 12:220–19.

[2] Schator D, Mondino S, Berthelet J, Silvestre CD, Assaya MB, Rusniok C, Rodrigues-Lima F, Wehenkel A, Buchrieser C, Rolando M. 2023. Legionella para-effectors target chromatin and promote bacterial replication. Nat Commun 14:2154.

Type 4 secretion system protein-protein interaction and structure prediction using PPIFold

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ABSTRACT

Bacteria utilize diverse secretion systems to interact with other organisms, with the Type IV Secretion System (T4SS) being particularly significant. T4SS is conserved across bacterial species and plays critical roles in biological processes. It facilitates DNA secretion, enabling inter-bacterial genetic exchange and the spread of antibiotic resistance genes. Additionally, T4SS delivers proteins, many of which are toxic to eukaryotic cells, contributing to bacterial pathogenicity. Advancements in artificial intelligence and structural modelling, recently celebrated with the 2024 Nobel Prize in Physics, have revolutionized protein-protein interaction (PPI) predictions. Leveraging these technologies, we developed PPIFold, an automated pipeline designed to predict large sets of PPIs. Applied to T4SS plasmids, this pipeline successfully identified novel homologous proteins, even in cases lacking sequence or structural homology. Our findings provide new insights into the T4SS protein network, enabling us to propose hypotheses regarding its assembly and functional interactions

Characterization of the ICE*Ec1* coupling protein from *Escherichia coli* ECOR31

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ABSTRACT

Antibiotic Resistance (AR) is one of the greatest threats to human health. Conjugative plasmids and Integrative Conjugative Elements (ICEs) constitute the main drivers of AR spread. Although ICEs outnumber conjugative plasmids, yet they still have been largely overlooked as vectors of AR[1]. ICEs are typically found integrated in the host chromosome and encode the machinery for their conjugation, which consists of: (*i*) proteins that form the relaxosome; (*ii*) proteins that form the Type IV Secretion System (T4SS); and (*iii*) the Type IV Coupling Protein (T4CP) that links the relaxosome with the T4SS. T4CPs are essential for conjugation, but only a few (mainly plasmid-encoded) have been exhaustively studied, such as $TrwB_{R388}$, encoded by the conjugative plasmid R388, or $MobB_{CloDF13}$, which is one of the few T4CP encoded by a mobilizable plasmid, CloDF13. A homologue of this latest T4CP, $mobB_{ICE}$, was identified within the ICE*Ec1* from *Escherichia coli* ECOR31, which carries a DNA-mobilization region related to CloDF13, but encodes a functional T4SS similar to the one present in the conjugative plasmid R6K[2]. Additionally, ICE*Ec1* contains a wide distributed High-Pathogenicity Island among *Enterobacteriaceae*. $MobB_{ICE}$ shows 31% of identity and 42% similarity with $MobB_{CloDF13}$. It was shown that $mobB_{ICE}$ is involved in the transfer of a plasmid carrying the origin of transference (*oriT*) present on ICE*Ec1*[2]. We have shown that $mobB_{ICE}$ requires $mobC_{ICE}$ to transfer its *oriT*, using the T4SS of R388. In addition, we have purified a soluble version of $MobB_{ICE}$ that seems to exist as a monomer and dimer. Currently, we are investigating its properties. Our project is focused on the characterization of an ICE T4CP, with the ultimate goal of finding ICE conjugation inhibitors to block or, at least, minimize the spread of AR.

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KEYWORDS: *T4CP, ICE,* $MobB_{ICE}$

REFERENCES

[1] Botelho J, Schulenburg H. (2021) The role of integrative and conjugative elements in antibiotic resistance evolution. *Trends in Microbiology*. Jan;29(1):8-18. doi: 10.1016/j.tim.2020.05.011.

[2] Schubert, S., Dufke, S., Sorsa, J. and Heesemann, J. (2004) A novel integrative and conjugative element (ICE) of *Escherichia coli*: the putative progenitor of the *Yersinia* high-pathogenicity island. *Molecular Microbiology*, 51: 837-848. https://doi.org/10.1046/j.1365-2958.2003.03870.x

Uncovering the Role of IMEs in Antimicrobial Resistance and Horizontal Gene Transfer

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ABSTRACT

Integrative Mobilizable Elements (IMEs) are mobile genetic elements that integrate into bacterial genomes and excise to transfer to other bacteria. Unlike Integrative Conjugative Elements (ICEs), IMEs lack their own conjugation machinery, hijacking an ICE or plasmid Type IV Secretion System (T4SS) (1). This allows IMEs to excise, circularize, and mobilize autonomously, often without disrupting the ICE's own transfer process (2). Though widespread across bacterial species, IMEs remain relatively underexplored compared to other mobile genetic elements. Many IMEs integrate into the origin of transfer (*oriT*) or open reading frames of ICEs, using the ICE as both a host and facilitator for their mobilization (3). Besides mobility, IMEs frequently harbor genes conferring antimicrobial resistance (AMR), positioning them as key players in the horizontal spread of resistance among bacterial populations. Furthermore, the interaction between IMEs and T4SSs that enables their transfer remains an area of ongoing research.

In *Streptococcus suis*, a zoonotic pathogen, IMEs have been shown to play a major role in disseminating AMR genes. In silico analyses of 102 *S. suis* strains isolated from healthy or sick pigs, humans and wild boars, revealed that IMEs exhibit impressive diversity and plasticity. While ICEs were initially thought to be the primary vectors of AMR, it has been established that IMEs in *S. suis* carry a significant portion of these genes. IME_*SNF2* and IME_*PPI* in particular frequently carry both *tet*(O) and *erm*(B) genes. Analysis of the expression of their recombination module by quantitative PCR indicated that environmental factors, such as oxidative stress and UV exposure, play a role in their activation. Notably, we also observed the mobilization of the IME_*guaA* through an ICE of the Tn*1549* family in *Streptococcus suis*, further emphasizing the role of IMEs in the spread of AMR across different bacterial populations.

KEYWORDS: *Integrative Mobilizable Elements, Streptococcus suis, Antimicrobial Resistance, Horizontal Gene Transfer*

REFERENCES:

[1] Guédon G., Libante V., Coluzzi C, Payot S and Leblond-Bourget N. The Obscure World of Integrative and Mobilizable Elements, Highly Widespread Elements that Pirate Bacterial Conjugative Systems. Genes. 2017;8:337. doi: 10.3390/genes8110337.

[2] Libante V, Sarica N, Mohamad Ali A, Gapp C, Oussalah A, Guédon G, Leblond-Bourget N. and Payot S. Mobilization of IMEs Integrated in the *oriT* of ICEs Involves Their Own Relaxase Belonging to the Rep-Trans Family of Proteins. 2020. doi: 10.3390/pathogens9010022.

[3] Libante V, Nombre Y, Coluzzi C, Staub J, Guédon G, Gottschalk M, Teatero S, Fittipaldi N, Leblond-Bourget N, Payot S. Chromosomal Conjugative and Mobilizable Elements in *Streptococcus suis*: Major Actors in the Spreading of Antimicrobial Resistance and Bacteriocin Synthesis Genes. Pathogens. 2019;9:22. doi: 10.3390/pathogens9010022.

Characterization of the dynamics of a Type IV Secretion System (T4SS) substrate and interaction with ATPase domains

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ABSTRACT

Xanthomonas citri is a phytopathogen that causes serious diseases in citrus plants. We showed previously that *X. citri* is equipped with a T4SS, which is used to inject toxins into competing bacteria of different species [1]. XacT4SS consists of twelve subunits, which form a channel crossing the inner and outer membranes of *X. citri*. Three ATPases (VirD4, VirB4 and VirB11) are involved in toxin substrate recruiting and translocation [2]. All XacT4SS effectors (X-Tfes) share a small (11 kDa) C-terminal domain so-called XVIPCD, which interacts with the VirD4 all-alpha domain (VirD4-AAD) (18 kDa) [3]. XVIPCD binding to VirD4-AAD is the first step in the effectors translocation mechanism and is essential for the XacT4SS bactericidal activity [1]. Previously, we used NMR spectroscopy to solve the three-dimensional structure of the XVIPCD from the XAC2609 X-Tfe [3]. Here, we investigated the interaction between XAC2609 XVIPCD and the VirD4- AAD using NMR titrations. The intrinsic dynamics of the isolated XVIPCD and VirD4-AAD domains was characterized by ^{15}N CPMG R2 relaxation dispersion experiments. We found that XVIPCD is highly flexible, while AAD behaves as a rigid protein. Quantitative analysis of XVIPCD relaxation dispersion data indicated that it visits a low populated unfolded state in equilibrium with the native state. We observed that XVIPCD unfolds at low pH (pH = 5) but becomes structured at higher pHs. Due to the high molecular weight of X-Tfes and the low diameter of XacT4SS hypothetical channel, X-Tfes are probably transported in an unfolded state. However, once in the recipient cells, X-Tfes should refold to promote cytotoxicity. These hypotheses are consistent with the biophysical behavior of the XAC2609 XVIPCD domain.

KEYWORDS: *Xanthomonas citr*i, *VirD4, NMR spectroscopy*.

References

[1] D.P. Souza, G.U. Oka, C.E. Alvarez-Martinez, A.W. Bisson-Filho, G. Dunger, L. Hobeika, N.S. Cavalcante, M.C. Alegria, L.R.S. Barbosa, R.K. Salinas, C.R. Guzzo, C.S. Farah, Bacterial killing via a type IV secretion system, Nat Commun 6 (2015) 6453. https://doi.org/10.1038/ncomms7453.

[2] G.G. Sgro, G.U. Oka, D.P. Souza, W. Cenens, E. Bayer-Santos, B.Y. Matsuyama, N.F. Bueno, T.R. dos Santos, C.E. Alvarez-Martinez, R.K. Salinas, C.S. Farah, Bacteria-Killing Type IV Secretion Systems, Front. Microbiol. 10 (2019) 1078. https://doi.org/10.3389/fmicb.2019.01078.

[3] G.U. Oka, D.P. Souza, W. Cenens, B.Y. Matsuyama, M.V.C. Cardoso, L.C. Oliveira, F. da Silva Lima, I.M. Cuccovia, C.R. Guzzo, R.K. Salinas, C.S. Farah, Structural basis for effector recognition by an antibacterial type IV secretion system, Proc Natl Acad Sci USA 119 (2022) e2112529119. https://doi.org/10.1073/pnas.2112529119.

Structure of the type IV secretion system encoded by the *E.coli* F plasmid

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ABSTRACT

The conjugative Type 4 Secretion System (T4SS) encoded by the F plasmid in *E. coli* plays a pivotal role in the spread of Anti-Microbial Resistance (AMR). Genes encoded in the Tra gene cluster of the F plasmid provide the structural components of this multimeric complex, enabling horizontal gene transfer (HGT) by facilitating DNA transfer between donor and recipient bacterial cells. Despite the discovery of F plasmid-mediated conjugation 78 years ago, the complete structural details of the F plasmid's T4SS remain unresolved (1,2,3). This study aims to elucidate the high-resolution structure of a fully-assembled F-type T4SS through an integrative approach, combining single-particle cryo-electron microscopy (SP cryo-EM), cross-linking mass spectrometry (CL-MS), and AlphaFold modeling. Initial work successfully purified a complex that includes not only the previously characterized outer membrane components but also the elusive inner membrane and pilus-forming elements, as confirmed by mass spectrometry. This achievement represents significant progress toward overcoming long-standing challenges in isolating the intact T4SS complex. Additionally, interaction models of key subunits were generated using AlphaFold-3, providing valuable insights into the molecular interactions governing the system's function. This integrated approach will allow for the generation of a comprehensive model of the T4SS architecture, which is critical for understanding its role in AMR transmission. Ultimately, this work advances the biological understanding of F plasmid-mediated AMR and offers a structural framework for designing inhibitors to prevent the dissemination of resistance genes. The results lay the groundwork for future research aimed at inhibiting HGT, addressing the global health challenge posed by AMR.

KEYWORDS: *F plasmid, cryo-EM, F T4SS Structure*.

REFERENCES

[1] Lederberg, j., Tatum, E. Gene Recombination in Escherichia Coli. Nature 158, 558 (1946). https://doi.org/10.1038/158558a0

[2] Amin, H., Ilangovan, A. & Costa, T.R.D. Architecture of the outer-membrane core complex from a conjugative type IV secretion system. Nat Commun 12, 6834 (2021). https://doi.org/10.1038/s41467-021-27178-8

[3] Liu, X., Khara, P., Baker, M.L. et al. Structure of a type IV secretion system core complex encoded by multi-drug resistance F plasmids. Nat Commun 13, 379 (2022). https://doi.org/10.1038/s41467-022-28058-5

Disruption of the nucleoli and translation by a *Legionella* Dot/Icm T4SS effector

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ABSTRACT

Upon host cell contact, the facultative intracellular bacterial pathogen *Legionella pneumophila* translocates more than 300 effector proteins through the Dot/Icm type IV secretion system. These effector proteins manipulate numerous cellular processes to the benefit of the bacteria, enabling the evasion of phagolysosomal degradation and the generation of the replication-permissive *Legionella*-containing vacuole.

Using bioinformatics we identified a family of peptidase C58-like T4SS effectors encoded in a wide variety of *Legionella* species. Functional analysis of the most conserved member of the family revealed that it is a protease that cleaves itself and, upon ectopic expression, has profound effects on the physiology of eukaryotic cells, inducing changes to ER structure, Golgi apparatus and nuclear morphology, without causing cell membrane permeability or lysis.

Substrate trapping experiments with the inactive enzyme in combination with mass spectrometry yielded a large number of interactors involved in ribosome biogenesis and function. Subsequent *in vitro* and *in cellulo* analysis showed that the active protease compromised translation directly but in cells in addition triggered a complete loss of the nucleoli and accumulation of nucleolar proteins in the cytoplasm.

During infection, the effector did not induce as profound effects on host cell physiology as upon ectopic expression; likely because *L. pneumophila* 130b seems to control its expression tightly and delivers only small amounts of the effector. Nevertheless, reduced replication of the *L. pneumophila* strain lacking the effector in macrophages and mice showed that it has a significant, non-redundant role for the manipulation of host cells by the bacteria.

KEYWORDS: *Legionella, Dot/Icm effector, functional characterization, protease, translation, nucleoli*

REFERENCES

[1] Lockwood, Amin, Costa and Schroeder (2022). The *Legionella pneumophila* Dot/Icm type IV secretion system and its effectors. Microbiology; vol. 168, issue 5 DOI 10.1099/mic.0.001187

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Elucidating the function and constituents of an anomalous T4SS in Rickettsia

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ABSTRACT

There has been a recent global increase in cases of tick-borne diseases, many of which are caused by members of the *Rickettsia* genus. These obligate intracellular bacterial pathogens encode an anomalous VirB/D4 T4SS system, which has undergone significant duplications and deletions of certain subunits. Most notably, *Rickettsia spp* encode five diverse paralogs of the inner membrane channel protein VirB6, each with unique N- and/or C-terminal extensions. The T4SS genes are also scattered throughout the rickettsial chromosome making it hard to predict if additional components are part of this system. This subunit diversity suggests that the rickettsial T4SS is not assembled like other well-studied systems. Using *R. parkeri* as a model, we focused on the five rickettsial VirB6 paralogs to probe subunit interaction and function through the bacterium's infectious lifecycle. To understand the role of VirB6 paralogs during infection, we isolated an *R. parkeri* transposon mutant strain expressing a truncated form of the VirB6 paralog RvhB6e (*rvhB6e*::Tn). We found that the *rvhB6e*::Tn strain is defective in host cell invasion, but has no other lifecycle stage defects nor globally impaired effector secretion. We next used co-immunoprecipitation and mass spectrometry to identify RvhB6e interacting partners. These data showed that RvhB6e interacts with the other four RvhB6 proteins and a novel Rickettsiaceae-specific protein, which we called RviA. Interestingly, RviA contains a domain with structural homology to the tip region of the outer membrane conjugation system protein TraN. Structural modeling suggests that RviA interacts with the extended N-terminal sequence of RvhB6e, highlighting the potential importance of these unique extended regions, although the localization and function of RviA are currently unknown. Thus, our study of the rickettsial T4SS has allowed us to uncover new, non-canonical T4SS components and improves our understanding of how this anomalous T4SS functions during the rickettsial lifecycle.

KEYWORDS:

Rickettsia, Pathogenesis, Host-Pathogen, Non-canonical

REFERENCES

[1] Gillespie, J.J. *et al* (2016) The Rickettsia type IV secretion system: unrealized complexity mired by gene family expansion, *Pathog Dis.* 74(6): ftw058. doi: 10.1093/femspd/ftw058

[2] Lamason, R.L., Kafai, N.M. and, Welch, M.D. (2018) A streamlined method for transposon mutagenesis of *Rickettsia parkeri* yields numerous mutations that impact infection. PLoS ONE 13(5): e0197012. https://doi.org/10.1371/journal.pone.0197012

Investigating the molecular basis of effector delivery through the bacterial Type IV Secretion System

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ABSTRACT

Bacterial Type IV secretions systems (T4SSs) are large macromolecular complexes that transport proteins across bacterial membranes and enable horizontal gene transfer. These two functions of T4SSs make them clinically relevant as several bacterial pathogens employ a T4SS during the infection. Furthermore, T4SS-mediated horizontal gene transfer plays a role in the proliferation of antibiotic resistance genes. The T4SSs are heavily studied due to their clinical importance; however, it is currently unknown how T4SSs translocate effector proteins or DNA in any organism.

Here we explored a memetic system to induce T4SS effector protein secretion in the model organism Legionella pneumophilia, a clinically relevant intracellular bacterial parasite. The novelty in this employed method is that it induces T4SS effector protein secretion without bacterial internalisation into the host cytoplasm, which has been a significant impediment to studying the T4SS in its active state. The actively secreting T4SSs were analysed using a combination of in situ Cryo-Electron Tomography and Sub-Tomogram averaging.

The in situ T4SS structures from our memetic systems show several large-scale structural rearrangements during active secretion. The most striking of these changes occur in the outer membrane core complex (OMCC), which undergoes a 45o shift downwards toward the inner membrane. The downward movement of the OMCC is coupled to protein density alteration at the central interface of the OMCC and the outer membrane. This structural insight provides one of the first insights into a mechanistic understanding of structural reorganisation necessary for effector protein secretion through the Legionella pneumophilia T4SS.

KEYWORDS: *Cryo-ET; Subtomogram; Legionella*

Revealing membrane insertion mechanisms of *Legionella's* integral membrane effector proteins in host cells

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ABSTRACT

During infection, *Legionella pneumophila* translocates more than 300 effector proteins into host cells using the specialized Dot/Icm type IV secretion system (T4SS)[1]. Many effector proteins contain a hydrophobic transmembrane domain (TMD) to fulfill their function in host cell membranes[2]. However, the mechanisms T4-secreted TMD effectors (TMEs) use to target and insert into the correct membranes of eukaryotic hosts remain to be elucidated.

To understand the relevance of host cell proteins involved in membrane insertion, the interacting proteome of bacterial TMEs was assessed. The intracellular environment of four *Legionella* TMEs, differing in hydrophobicity and position of their TMD, was characterized using TurboID-mediated proximity-dependent biotinylation[3] and subsequent mass spectrometry. Proteomic analysis revealed that T4-secreted TMEs interact with host proteins that are involved in the biogenesis of membrane protein at the endoplasmic reticulum (ER). Interestingly, TMEs with their TMD located towards the C-terminus exhibit interaction with several proteins involved in signal recognition particle-dependent ER targeting and post-translational Sec61-mediated ER insertion. Moreover, all TMEs were found to be associated with proteins of the ER-Golgi, and endosomal vesicle trafficking pathway.

To further investigate intracellular trafficking of TMEs within the host cell, the subcellular localization of TMEs was analyzed by fluorescence microscopy. T4-secreted TMEs were predominantly observed in the membrane of the *Legionella*-containing vacuole and the surrounding ER, suggesting that secreted TMEs are targeted and inserted into the ER at early time points of infection. TMEs expressed in host cells also co-localized with the ER. To further validate the mechanisms of TME membrane integration and trafficking, specific inhibitors targeting Sec61-mediated membrane insertion or secretory pathways will be employed.

In conclusion, these preliminary observations suggest that T4-secreted TMEs, depending on their TMD position, utilize eukaryotic membrane protein biogenesis pathways to insert into the ER membrane and further traffic to their designated compartment within the host.

KEYWORDS: *Legionella pneumophila, T4SS, Transmembrane effector proteins, Membrane protein biogenesis, Endoplasmic reticulum, Secretory pathway*

REFERENCES

[1] Hilbi, H., Nagai, H., Kubori, T. & Roy, C. R. Subversion of Host Membrane Dynamics by the Legionella Dot/Icm Type IV Secretion System. in *Type IV Secretion in Gram-Negative and Gram-Positive Bacteria* (eds. Backert, S. & Grohmann, E.) vol. 413 221–242 (Springer International Publishing, 2017). https://doi.org/10.1007/978-3-319-75241-9_9

[2] Krampen, L. *et al.* Revealing the mechanisms of membrane protein export by virulence-associated bacterial secretion systems. *Nat Commun* 9, 3467 (2018). https://doi.org/10.1038/s41467-018-05969-w

[3] Branon, T. C. *et al.* Efficient proximity labeling in living cells and organisms with TurboID. *Nat Biotechnol* 36, 880–887 (2018). https://doi.org/10.1038/nbt.4201

NMR solution structure of OrfM from ICE*St3* of *Streptococcus thermophilus*

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ABSTRACT

Integrated and conjugative elements (ICEs) are mobile genomic elements that are responsible for horizontal gene transfer by conjugation [1], which is a way for bacteria to disseminate resistance to antibiotics. Understanding the mechanism of conjugation may therefore provide clues to tackle this major public health problem.

ICEs are found both in Gram-positive and Gram-negative bacteria. Conjugation mainly uses the type IV secretion system (T4SS), a multiprotein membrane complex, which has been extensively studied in Gram-negative bacteria, but only scarcely in Gram-positive ones. ICEs of the superfamily of Tn*916* from *Enterococcus faecalis* are widespread in Gram-positive bacteria [2] and include ICE*Bs1* from *Bacillus subtilis* and ICE*St3* from *Streptococcus thermophilus.* Our study focuses on OrfM and OrfL proteins encoded by ICE*St3*, which are the homologs of the Tn*916* HelP protein, that has been proved to be involved in conjugation [3].

We obtained an experimental 3D structure of OrfM by Nuclear Magnetic Resonance (NMR), revealing an oligonucleotide/oligosaccharide-binding fold (OB-fold). NMR ^{15}N relaxation experiments showed that OrfM is mainly monomeric in solution and contains several flexible regions. The tight complex, that OrfM has been shown to form in the presence of OrfL, was modelled using AlphaFold3 and exhibited an association mode similar to that of some dimeric OB-fold proteins.

KEYWORDS: *conjugation, ICE, relaxosome, Gram-positive bacteria, OB fold, NMR structure, dynamics*

REFERENCES

[1] Johnson,C.M. and Grossman,A.D. (2015) Integrative and Conjugative Elements (ICEs): What They Do and How They Work. *Annu. Rev. Genet*., 49, 577–601.

[2] Ambroset,C., Coluzzi,C., Guédon,G., Devignes,M.-D., Loux,V., Lacroix,T., Payot,S. and Leblond-Bourget,N. (2016) New insights into the classification and integration specificity of Streptococcus Integrative Conjugative Elements through extensive genome exploration. *Front. Microbiol*., 6, e1483.

[3] Thomas,J., Lee,C.A. and Grossman,A.D. (2013) A Conserved Helicase Processivity Factor Is Needed for Conjugation and Replication of an Integrative and Conjugative Element. *PLoS Genet*, 9, e1003198.

Visualizing the structure and dynamics of the horizontal gene transfer during bacterial conjugation

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ABSTRACT

Bacterial conjugation is a sophisticated process that facilitates horizontal gene transfer between bacterial cells and is also responsible for the proliferation of antibiotic resistance genes among the bacterial population[1]. Conjugation is a process of unidirectional transfer of single-stranded DNA from donor cells to recipient cells via a contact-dependent (tight transfer) manner or through the pilus lumen in physically distant cells[2,3]. The conjugational transfer of DNA is mediated by the Type IV Secretion System (T4SS), one of the most versatile and elaborate secretion systems in bacterial cell envelopes[1]. T4SS assembles highly dynamic filamentous structures (T4SS pilus) that undergo cycles of extension and retraction. The T4SS pilus plays a major role in bringing the donor and recipient cells together to form a stable mating junction, and it is also shown to serve as a conduit for DNA transfer in physically distant cells[3]. The biochemical, molecular, and structural framework of conjugation remains poorly understood. Here, we use genetics, fluorescence microscopy, and cryo-electron tomography (cryo-ET) to investigate the molecular and structural basis of conjugation. Our study revealed the molecular anatomy of conjugating cells. This study provides key insights into the T4SS-dependent conjugation process, thus advancing our understanding of bacterial horizontal gene transfer and its role in spreading antibiotic resistance.

KEYWORDS : *Bacterial conjugation, Cryo-ET, T4SS.*

REFERENCE:

1. Costa, T.R.D., et al. Structural and functional diversity of type IV secretion systems. Nat Rev Microbiol 22, 170–185 (2024).
2. Couturier, A. et al. Real-time visualisation of the intracellular dynamics of conjugative plasmid transfer. Nat Commun 14, 294 (2023).
3. Goldlust, K. et al. The F pilus serves as a conduit for the DNA during conjugation between physically distant bacteria. PNAS,120(47) (2023).

Cryo-EM structure of the F plasmid relaxosome provides a molecular basis for DNA recruitment and processing in bacterial conjugation

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ABSTRACT

Bacterial conjugation is the unidirectional transport of genetic materials from a donor to a recipient cell. It is mediated in the donor cell by three major complexes: a DNA-processing machinery called the relaxosome, a double-membrane spanning transfer machinery termed type 4 secretion system (T4SS), and an extracellular appendage termed pilus. While the structures of the T4SS and pilus are known, that of the relaxosome is not. Here, we describe the cryo-EM structure of the fully assembled relaxosome encoded by the F plasmid in two different states corresponding to various functional steps along the DNA processing reaction. Altogether, the structural and molecular biology results we will present shed unprecedented light on a complex essential in the spread of antibiotic resistance genes among bacterial populations.

KEYWORDS: *Conjugation, Structural Biology, Relaxosome, Protein-protein interactions, Protein-DNA interactions*

Structural Studies of X-Tfe Recognition by VirD4 Coupling Protein of a Type IV Secretion System

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ABSTRACT

Xanthomonas citri and other species of the order Xanthomonadales carry a special Type IV Secretion system (X-T4SS) involved in antagonistic encounters between bacterial species (bacterial warfare). The toxins (X-Tfes) secreted by the X-T4SS of X. citri are diverse in size, architecture and function. However, they all carry a domain called XVIPCD by which they interact with the All Alpha Domain (AAD) from the Type IV coupling protein (T4CP) VirD4 for secretion. The structural details of the interaction between AAD-XVIPCD and following steps of the secretion of X-Tfes are still unknown. To solve the AAD-XVIPCD complex structure, we expressed and purified the VirD4AAD and the XVIPCD domain from X-Tfe XAC2609 (X-TfeXAC2609XVIPCD) and verified its 1:1 stoichiometry by SEC-MALS and SDS-PAGE. With the purified complex, sparse matrix crystallization trials were performed at the RoboLab Facility at the LNBio-CNPEM facility in Campinas, Brazil (two concentrations of each protein and 6 crystallization kits). Crystals with different morphology and size were found in at least 27 different conditions. Some crystals were grown in the presence of NaI and NaBr that could be useful for subsequent SAD or MAD phasing, if necessary. With these crystals, we hope to determine the high-resolution structure of the VirD4AAD- X-TfeXAC2609XVIPCD complex. The structure will reveal details of the first step in substrate selection by the X-T4SS and point towards experimentally testable hypotheses regarding subsequent steps of effector secretion.

KEYWORDS: *Xanthomonas citri, VirD4, XVIPCD, X-T4SS*

TurboID as a tool to decipher the role of TraC/VirB5 in bacterial conjugation

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ABSTRACT

Type IV secretion systems (T4SSs) are complex nanomachines that span the entire cell wall of Gram-positive and Gram-negative bacteria. Upon donor–target cell contact, T4SSs are able to act as protein translocators but also mediate DNA transfer in a process called conjugation. For this purpose, they elaborate conjugative pili or surface adhesins to promote attachment with recipient bacteria [1]. Among the diverse T4SSs, the one derived from the pKM101 conjugative plasmid is of great interest as it represents a system minimized enough to accomplish the interchange of genetic material. The pKM101-derived pilus is composed of a major subunit, TraM/VirB2, and a minor subunit, TraC/VirB5. The latter is positioned at the tip of the pilus after its polymerization, and it is hypothesized to act as an adhesin stablishing cell-cell interactions during conjugation [2]. However, little is known about specific receptors on the recipient cell.

To unravel the role of TraC/VirB5 during conjugation, we use proximity labeling (PL) as a new approach to study the interaction characteristics of proteins in living cells. PL has emerged as a powerful tool as it captures weak and transient protein-protein interactions that can be further analyzed using mass spectrometry [3]. PL is catalyzed by enzymes such as TurboID, a promiscuous ligase derived from BirA of *E. coli* that covalently tag proximal proteins by adding biotin to their lysine residues. By expressing recombinantly TraC/VirB5 fused with TurboID, we can screen for potential receptors on the surface of recipient cells. Moreover, we pursue the aim of implementing *in vivo* PL during conjugation by directly expressing TraC/VirB5-TurboID fusions from donor cells. The results will not only provide insights into the specific function of pKM101-encoded TraC/VirB5, but will also improve our current understanding of the conjugation process in minimized T4SSs.

KEYWORDS: *TurboID, conjugation, T4SS, pKM101, biotinylation*

REFERENCES

[1] T. R. D. Costa, J. B. Patkowski, K. Macé, P. J. Christie, and G. Waksman, 'Structural and functional diversity of type IV secretion systems.', *Nat Rev Microbiol*, Oct. 2023, doi: 10.1038/s41579-023-00974-3.

[2] K. Macé *et al.*, 'Cryo-EM structure of a type IV secretion system.', *Nature*, vol. 607, no. 7917, pp. 191–196, Jul. 2022, doi: 10.1038/s41586-022-04859-y.

[3] T. C. Branon *et al.*, 'Efficient proximity labeling in living cells and organisms with TurboID.', *Nat Biotechnol*, vol. 36, no. 9, pp. 880–887, Oct. 2018, doi: 10.1038/nbt.4201.

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