How to combat bacteriophages that disrupt food fermentations

Douwe van Sinderen
APC Microbiome Ireland & School of Microbiology
University College Cork, Ireland
d.vansinderen@ucc.ie

University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Microbiome Ireland
Interfacing Food & Medicine

Moved
in 1994
To Cork

Milk-to-cheese conversion using (traditional) biotechnology

Dairy fermentations using
Lactic Acid Bacteria or **LAB**
(*Lactococcus lactis/cremoris*)

Annual cheese production:

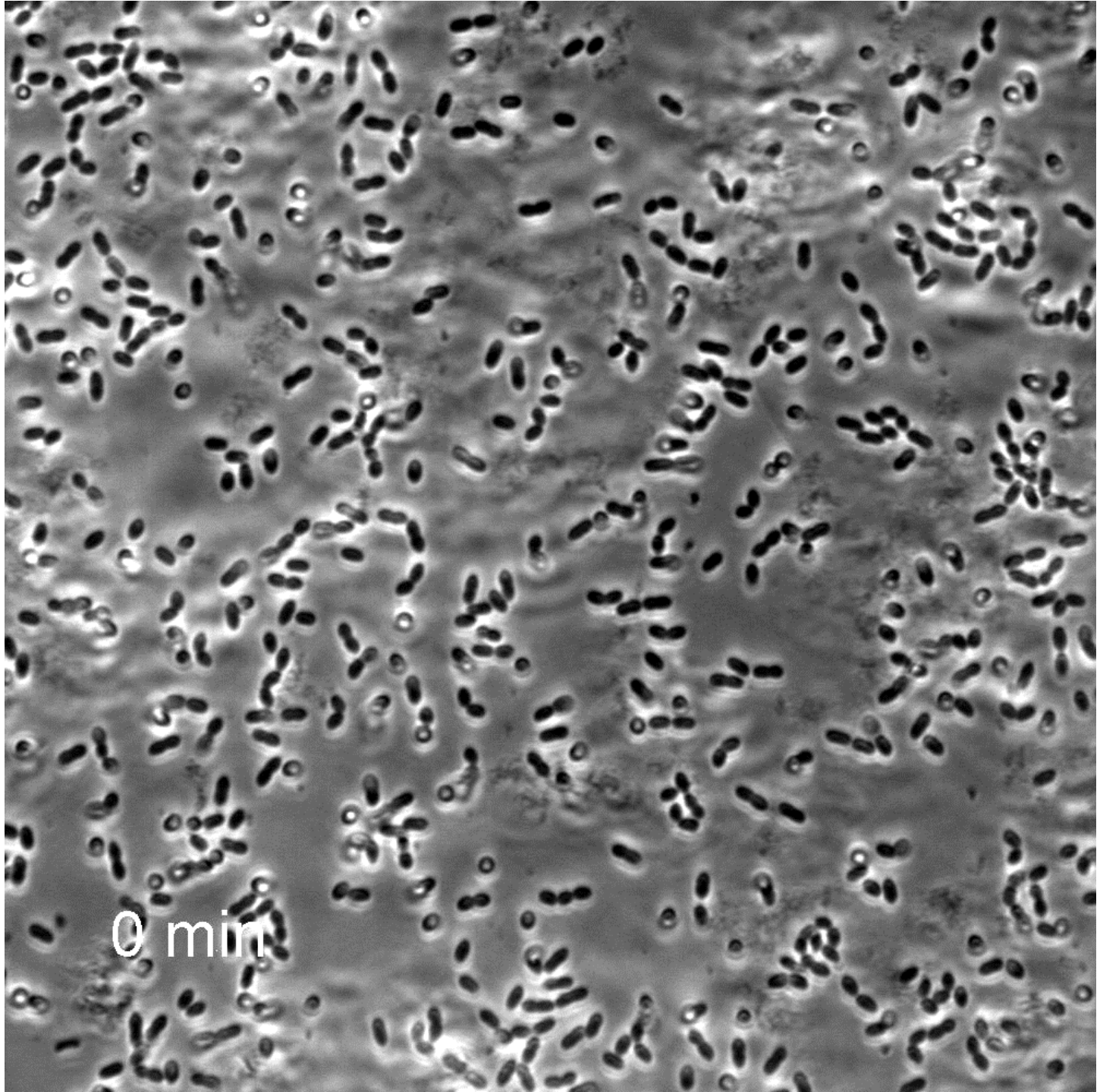
~23 million metric
Tonnes (2025 projection)

Value: 55 billion dollars

This milk conversion process involves approx. two quintillion (2,000,000,000,000,000,000) bacteria!

Ideal for phage infections, causing problems in the fermentation process

Murphy et al., 2016, Sc. Rep.



L. lactis plus bacteriophage

Courtesy:
James Murphy (UCC)
& Tâm Mignot (CNRS, Marseille)

What can we do to prevent/minimize damage caused by phage attack of starter cultures?

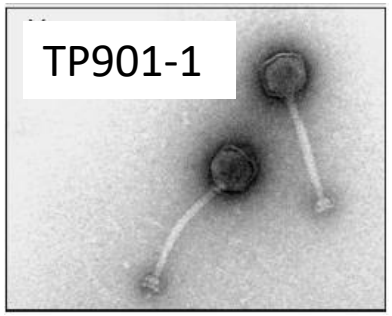
Lots of research has been done:

- A given phage will only attack a few strains of *L. lactis*
- Milk is not sterile and introduces phages into factory
- Once in the factory, impossible to eradicate

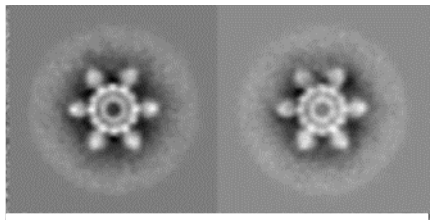
Three major questions:

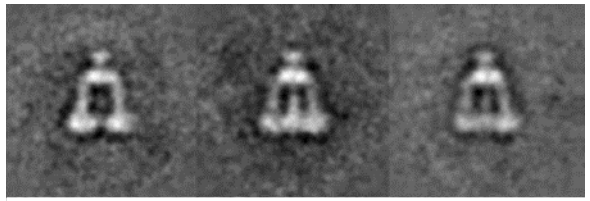
- **How** does a phage recognize a lactococcal host?
- **What** does it recognize?
- **Can** we mobilize indigenous phage immunity?

Tuc2009 & TP901-1 as lactococcal model phages

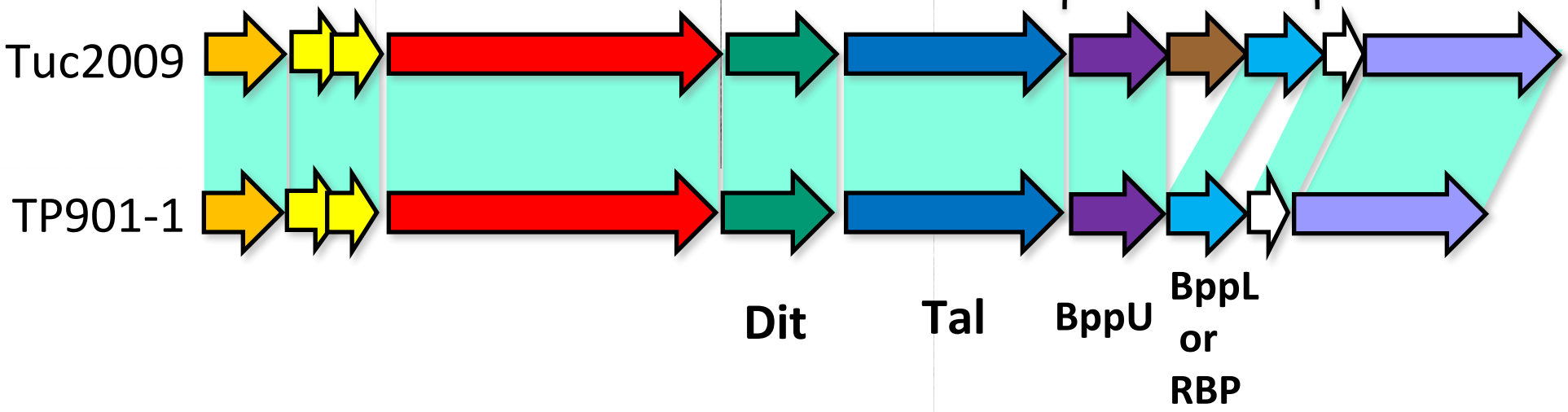


- Infect distinct strains though highly homologous





Tripods

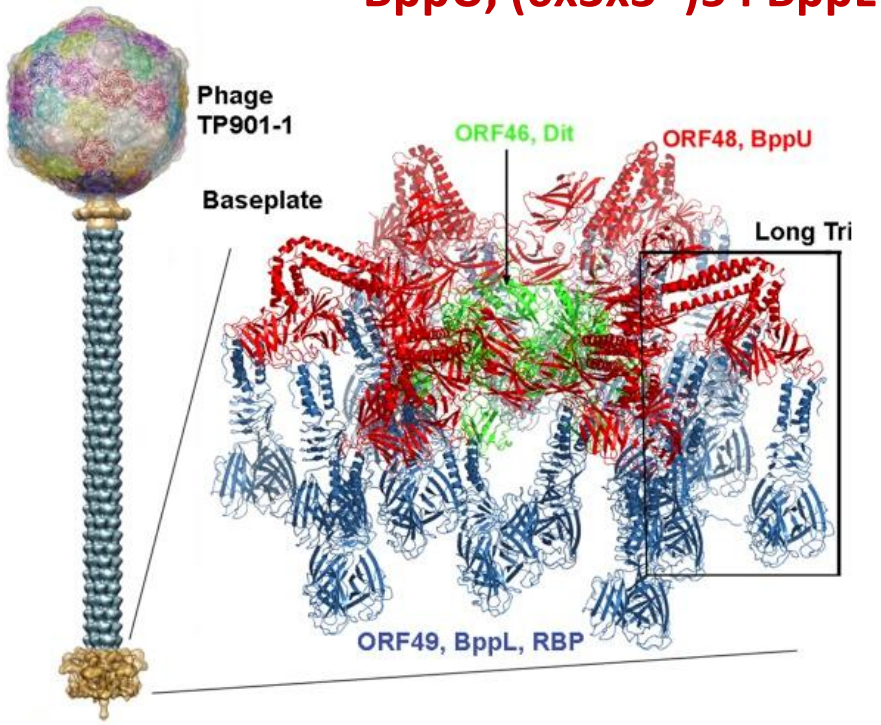


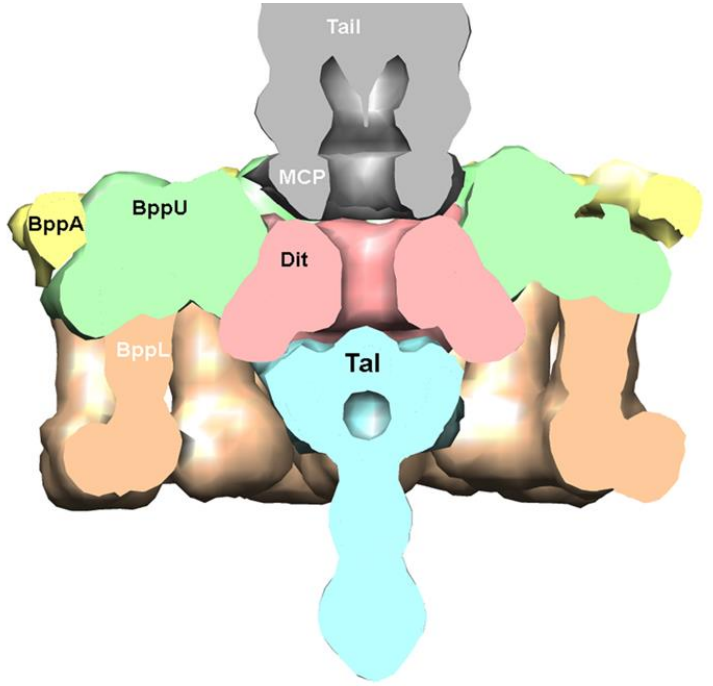
PNAS

Structure of the phage TP901-1 1.8 MDa baseplate suggests an alternative host adhesion mechanism

David Veesler[a,b,1,2], Silvia Spinelli[a,b], Jennifer Mahony[c], Julie Lichière[a,b], Stéphanie Blangy[a,b], Gérard Bricogne[d], Pierre Legrand[e], Miguel Ortiz-Lombardia[a,b], Valérie Campanacci[a,b,3], Douwe van Sinderen[c,f], and Christian Cambillau[a,b,1]

Heteromultimeric baseplate complex: 6 Dit, (6x3=)18 BppU, (6x3x3=)54 BppL (RBP), ([6x2=]12 BbbA)



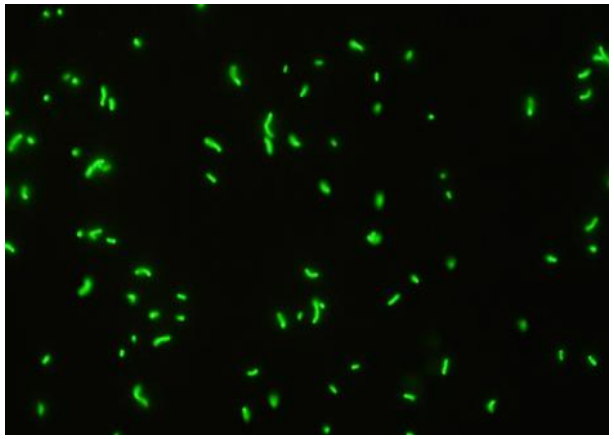


Veesler et al., PNAS, 2012; Legrand et al., mBio, 2016;
Hayes et al., Mol Microbiol, 2018; Hayes et al., Viruses, 2019

L. cremoris UC509.9

L. cremoris 3107

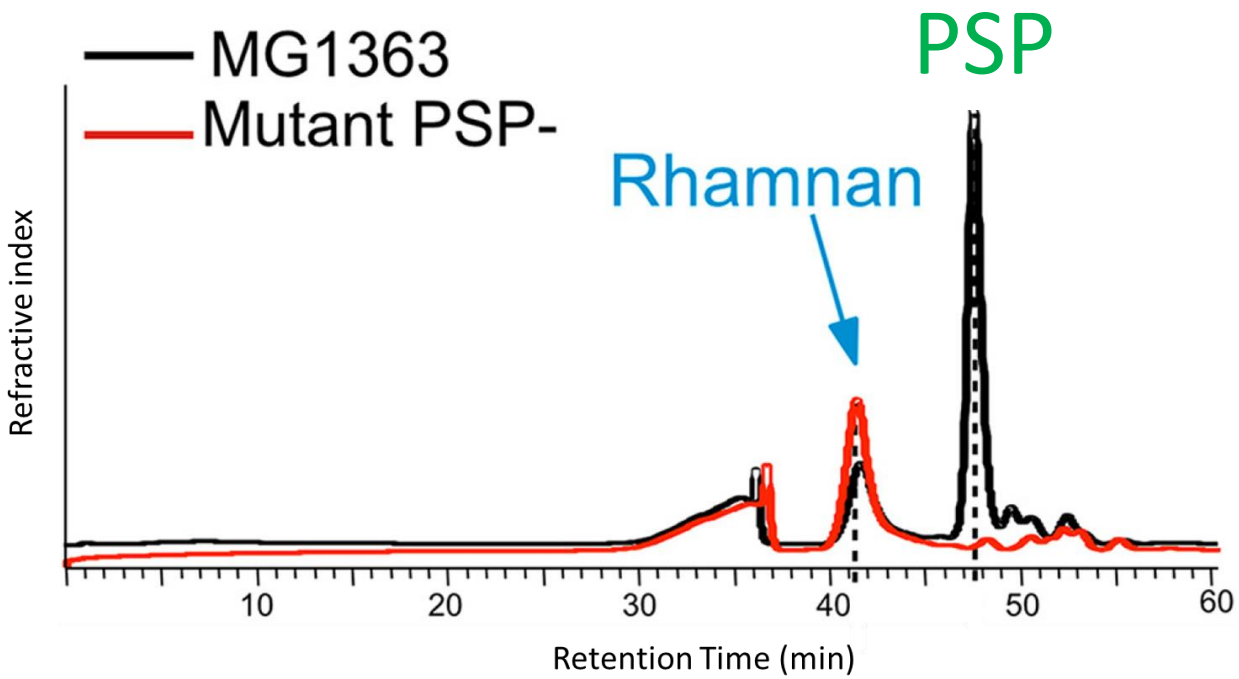
GFP-labelled
Tripods Tuc2009



Casey *et al*., 2022, mBio

What does the phage recognize on the host???

The lactococcal cell envelope contains a **c**ell **w**all **p**oly**s**accharide (CWPS):
which consists of a conserved rhamnan plus a variable pellicle or PSP

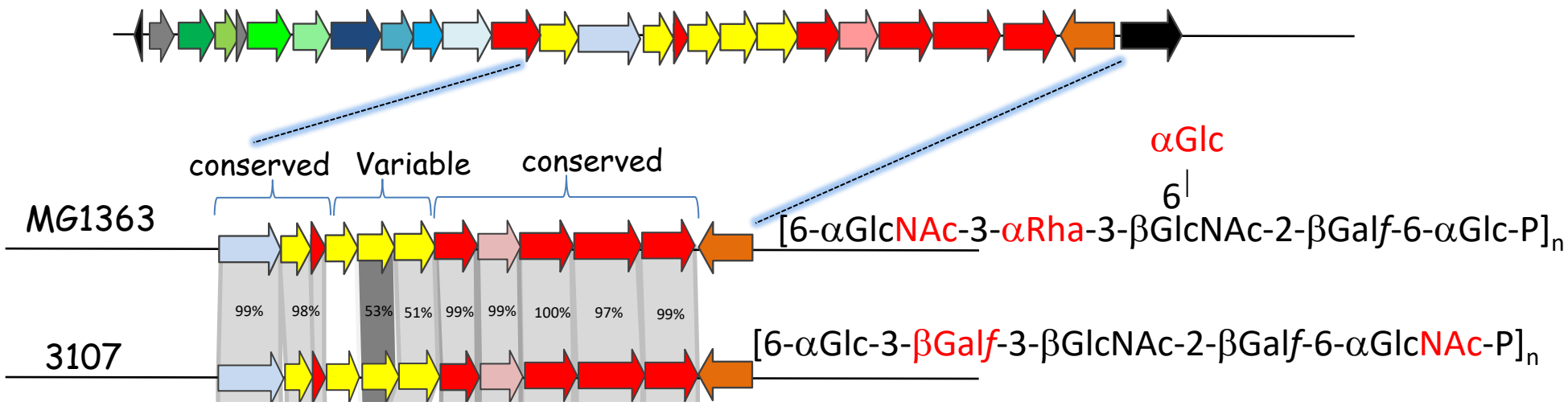


- Cell pellet
- Treat with SDS, prot/nucleases, and Hydrofluoric acid
- Purify by SEC-HPLC

Mutations in the *cwps* cluster of *L. lactis* MG1363 cause a PSP-negative phenotype and phage insensitivity

Chapot-Chartier et al., JBC 2010; Mahony et al., AEM, 2013; Sadovskaya et al., Mbio, 2017

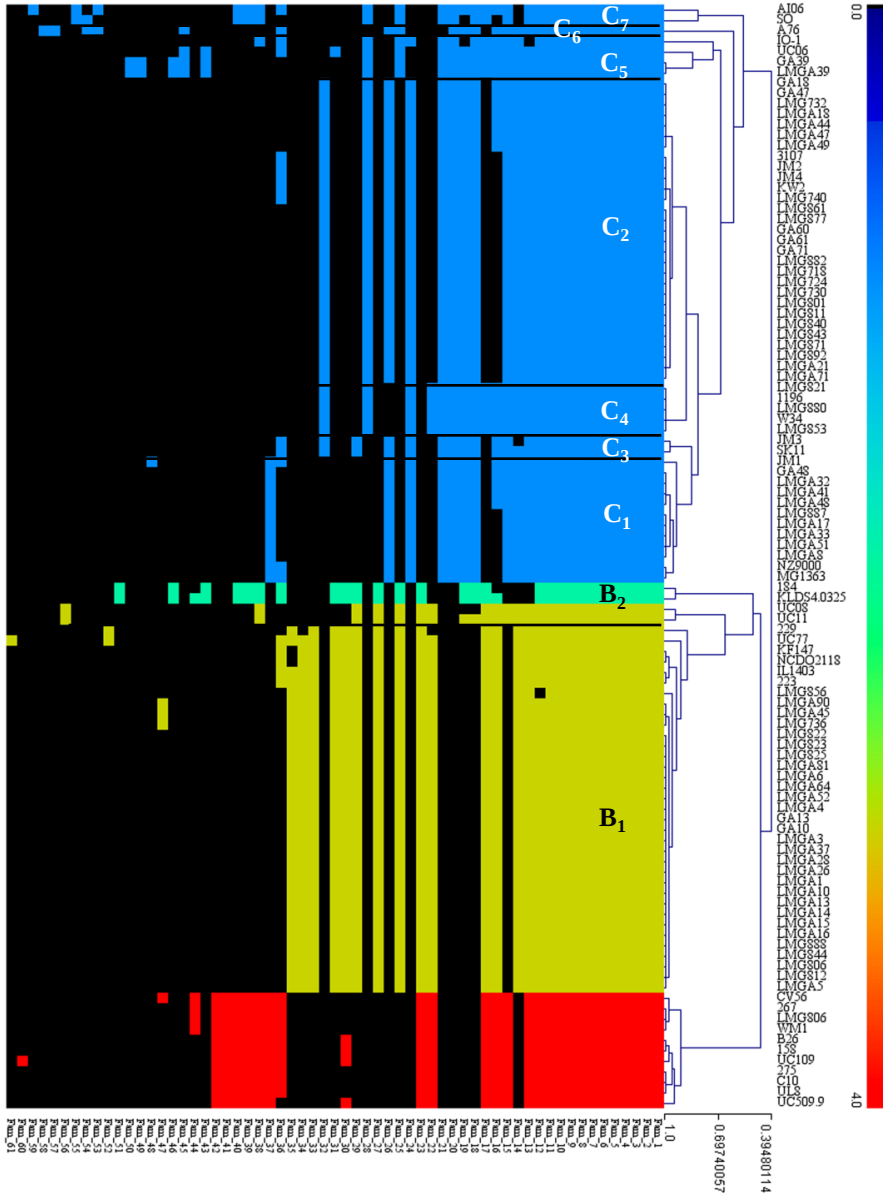
Genetic diversity among *cwps* cluster and PSP structure



Gene swapping of the variable region leads to PSP inversion (NMR surface scanning) and to host range swapping of TP901-1

Ainsworth et al., mBio, 2014
Mahony et al., AEM, 2015

L. Lactis genome sequencing reveals four cwps genotypes



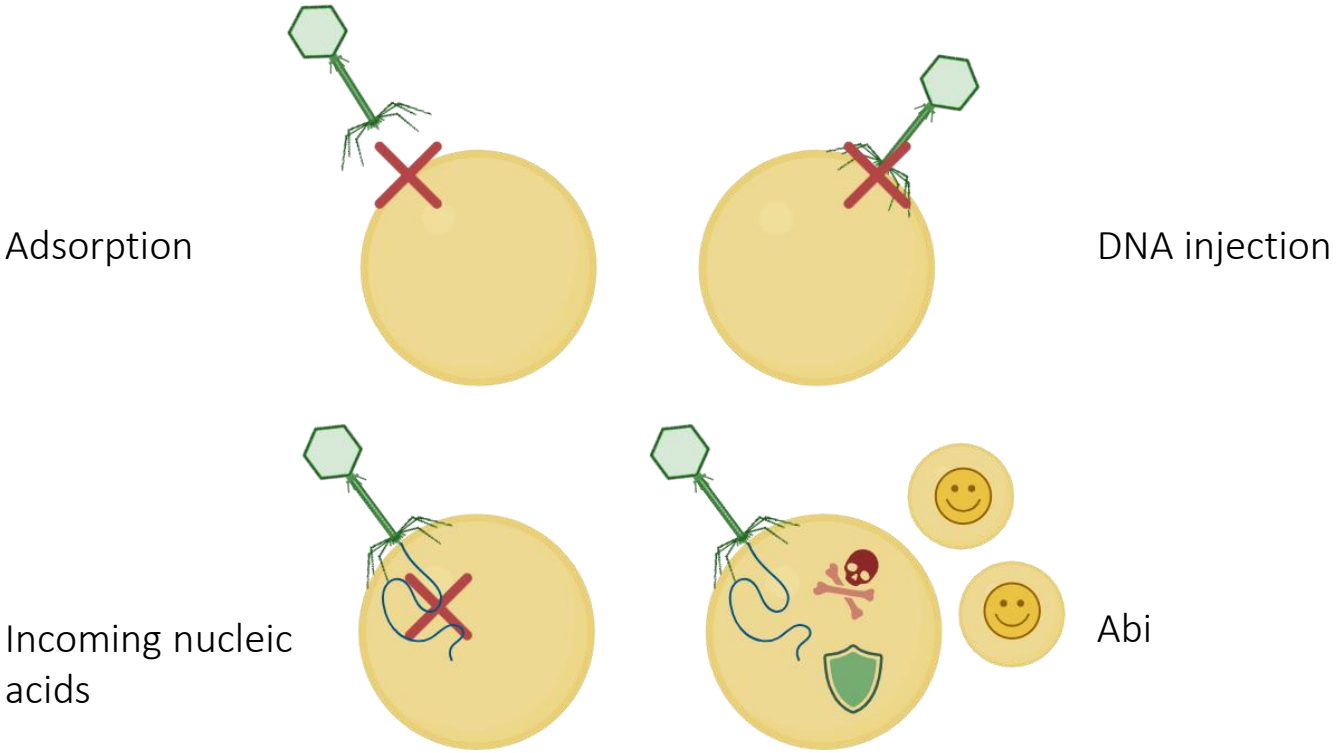
Structures of representatives of each *cwps* genotype have been determined

RBP variation is correlated to the CWPS type of corresponding host (rational starter culture design)

Mahony et al., Mol Microbiol, 2020; Parlindungan et al., Int J Food Microbiol. 2024

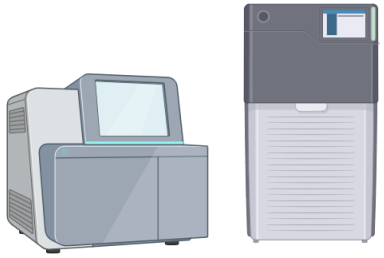
Bacterial immunity

- Multiple and diverse bacterial phage defence systems
- Abortive infection (Abi) - one of the most common system in *Lactococcus*

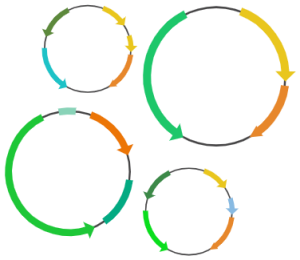


Identification of novel antiphage systems

Genomes of **53** lactococcal **strains sequenced**



321 plasmids extracted & annotated

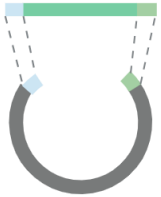


Plasmid genes grouped into 1020 families

Putative phage defence systems selection criteria

Selection of **198 candidate defence systems**

Cloned in **pNZ44/pPTPi**



Phage testing

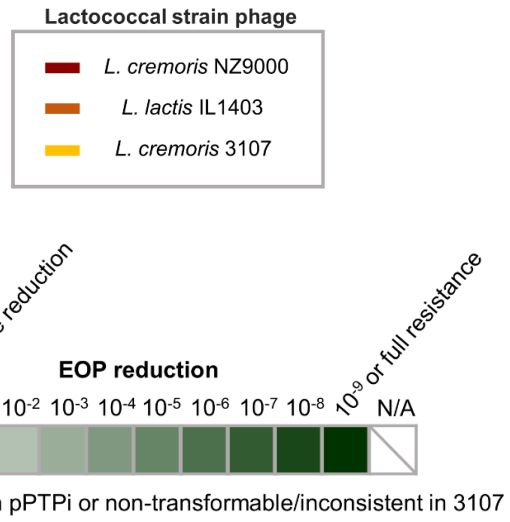
Nucleic Acids Research, 2024, **52**, 9760–9776
https://doi.org/10.1093/nar/gkae671
Advance access publication date: 9 August 2024
Molecular Biology

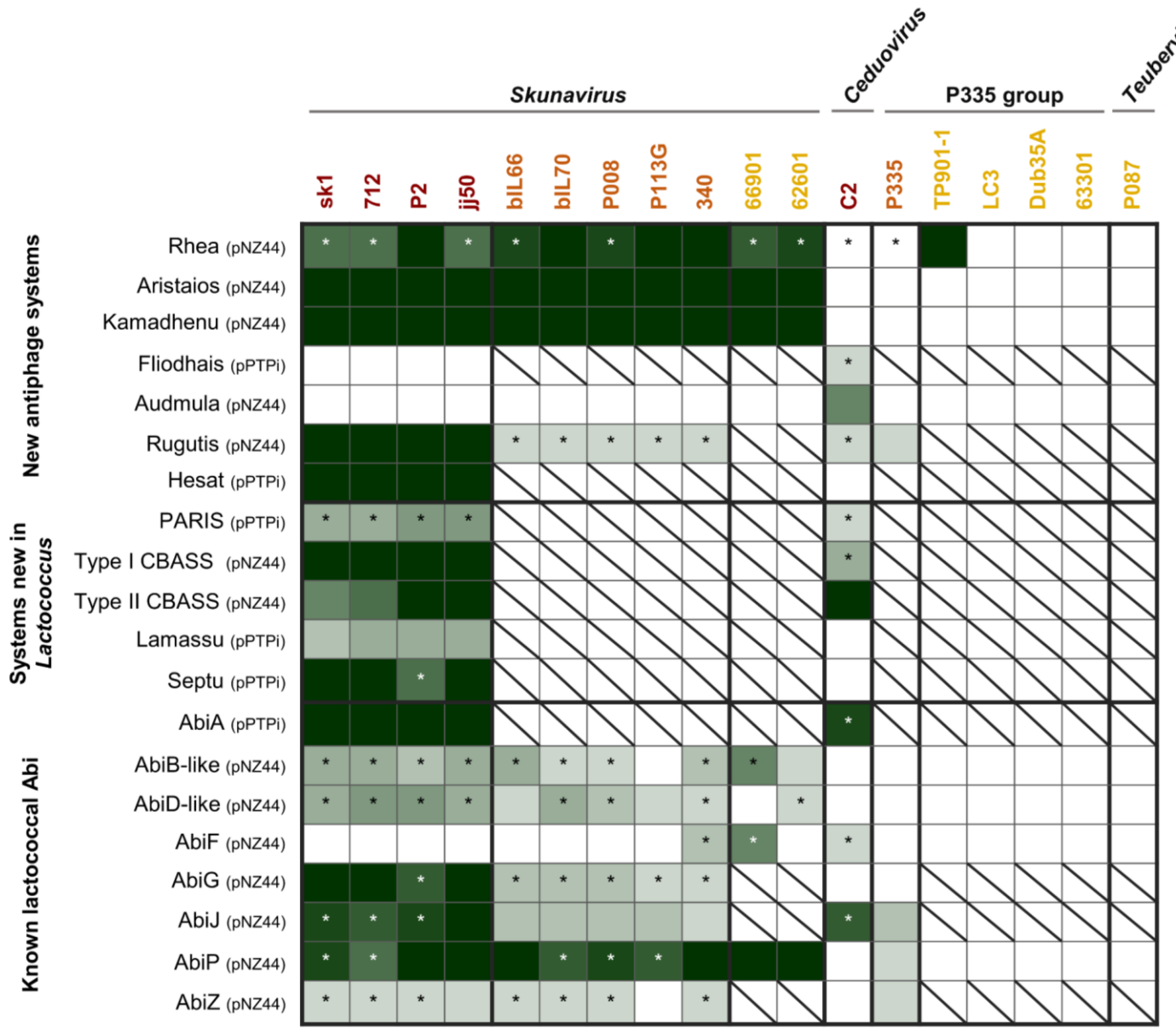
Discovery of antiphage systems in the lactococcal plasmidome

Andriana Grafakou[1,†], Cas Mosterd[1,†], Matthias H. Beck[1], Philip Kelleher[1], Brian McDonnell[1], Paul P. de Waal[2], Irma M.H. van Rijswijck[2], Noël N.M.E. van Peij[2], Christian Cambillau[1,3], Jennifer Mahony[1] and Douwe van Sinderen[1,*]

Antiphage activity spectrum

- 20 functional antiphage systems identified
- All except two effective against most frequently encountered phages
- Many systems on conjugative plasmids





Conclusions & perspectives

- We know how phage recognizes host
- We can predict phage sensitivity of a strain
- Rational starter culture design

- Many new anti-phage systems identified (also in S. thermophilus (Kelleher et al., NAR, 2024)
- Allows ‘construction’ of phage immunity into lactococcal strains
- Problem solved!?

Collaborators

Microbiome Institute
Interfacing Food & Medicine
http://apc.ucc.ie

Christian Cambillau, Silvia Spinelli, Renaud Vincentelli & Tam Mignot, CNRS, Marseille, France
Irina Sadovskaya, Boulogne-sur-Mer, France
Evguenii Vinogradov, NRCC, Ottawa, Canada
Stefan Oscarson, UCD, Ireland
Pierre Legrand, St Aubin, France
Marie-Pierre Chapot Chartier & Saulius Kulakauskas, INRA, Jouy-en-Josas, France
Yann Guerardel, University of Lille, France

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Andriana Grafakou

Cas Mosterd

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Mona Alqarni
Eoghan Casey
Barry Collins
Francois Douillard
Robin Eijlander
Andrea Erazo Garzon
Sofia Ruiz-Cruz
Brian McDonnell
Stephen Hayes
John Kenny
James Murphy
Nicolas Richet
Jos Seegers
Stephen Stockdale
Ilias Theodorou
Angela Makumi
Philip Kelleher

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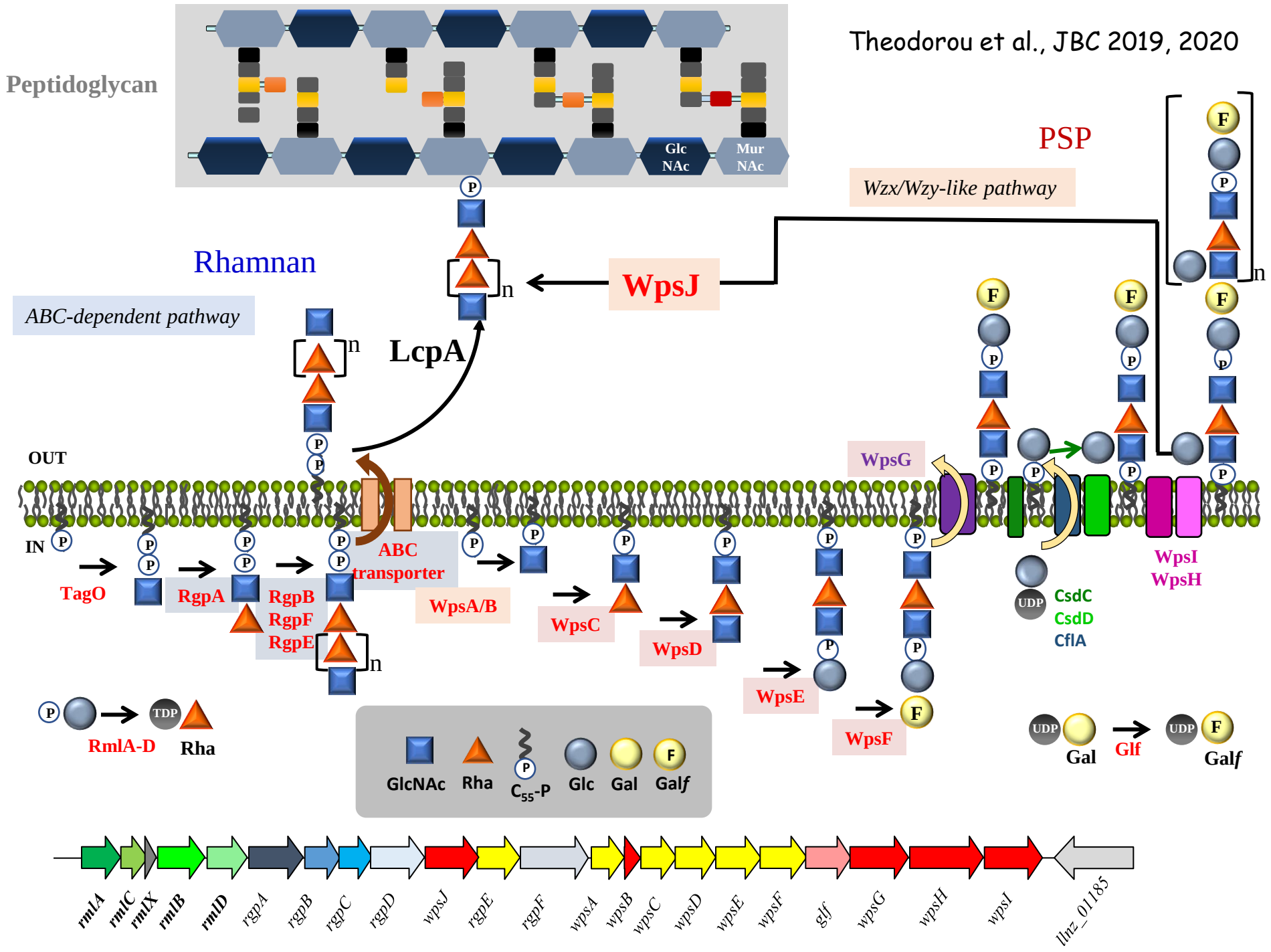
Theodorou et al., JBC 2019, 2020
Peptidoglycan
Glc NAc
Mur NAc
PSP
Wzx/Wzy-like pathway
Rhamnan
ABC-dependent pathway
WpsJ
LcpA
OUT
IN
TagO
RgpA
RgpB
RgpF
RgpE
ABC transporter
WpsA/B
WpsC
WpsD
WpsE
WpsF
WpsG
WpsI
WpsH
CsdC
CsdD
CflA
RmlA-D
Rha
Gal
Glf
Galf
GlcNAc
Glc
C55-P
rmlA rmlC rmlX rmlB rmlD rgpA rgpB rgpC rgpD wpsJ rgpE rgpF wpsA wpsB wpsC wpsD wpsE wpsF glf wpsG wpsH wpsI llnz_01185