13th International Symposium on Lactic Acid Bacteria

Federation of European Microbiological Societies

Koninklijke Nederlandse Vereniging voor Microbiologie

Cell wall homeostasis in lactic acid bacteria: threats and defences

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Micalis — Microbiologie de l'Alimentation au service de la Santé

INRAE — la science pour la vie, l'humain, la terre

DairySafe

IPLA CSIC

OUTLINE

1999

Antonie van Leeuwenhoek **76:** 159–184, 1999.

The biosynthesis and functionality of the cell-wall of lactic acid bacteria

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*(*Author for correspondence; E-mail: delcour@gene.ucl.ac.be)*

2014

Chapot-Chartier and Kulakauskas *Microbial Cell Factories* 2014, **13**(Suppl 1):S9
http://www.microbialcellfactories.com/content/13/S1/S9

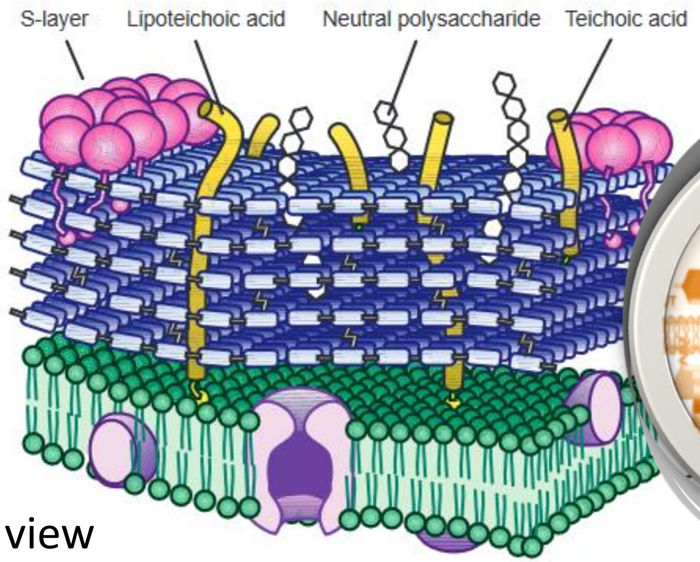
MICROBIAL CELL FACTORIES

PROCEEDINGS **Open Access**

Cell wall structure and function in lactic acid bacteria

Marie-Pierre Chapot-Chartier[1,2*], Saulius Kulakauskas[1,2]

From 11th International Symposium on Lactic Acid Bacteria



Artist´s view

OUTLINE

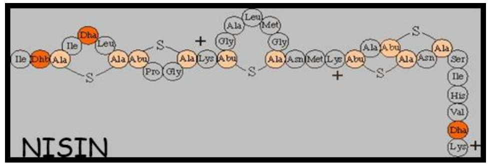
CW functions

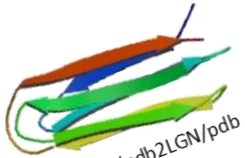
- **Essential**
  - Cell shape and cell division
- **Stress-bearing**
  - Osmotic pressure
- **Scaffold**
  - CW components
- **Sensory interface**
  - Monitoring

- **CW structural diversity**
- **CW plasticity**
  - Physiological growth
  - Under stress
- **Exploiting the CW**

THREATS

Bacteriocins





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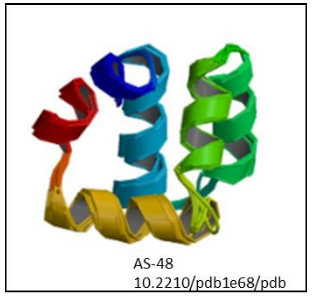
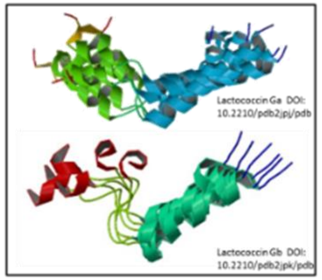
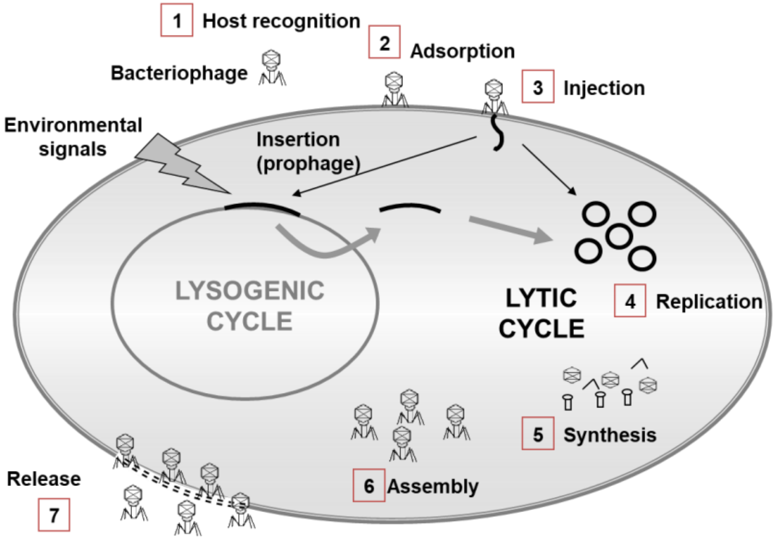
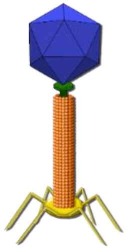




- Antimicrobial peptides
- Mode of action:
  - Pore formation (AS-48, pediocins…)
  - Inhibition CW biosynthesis (Lcn972, SalB)
  - Both (nisin, Lct3147)
  - PG hydrolysis (bacteriolysins)

Bacteriophages

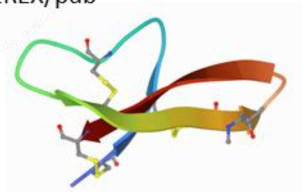




- Viruses of bacteria
- Mode of action:
  - Receptor
  - Endolysins

Host factors

10.2210/pdb1REX/pdb



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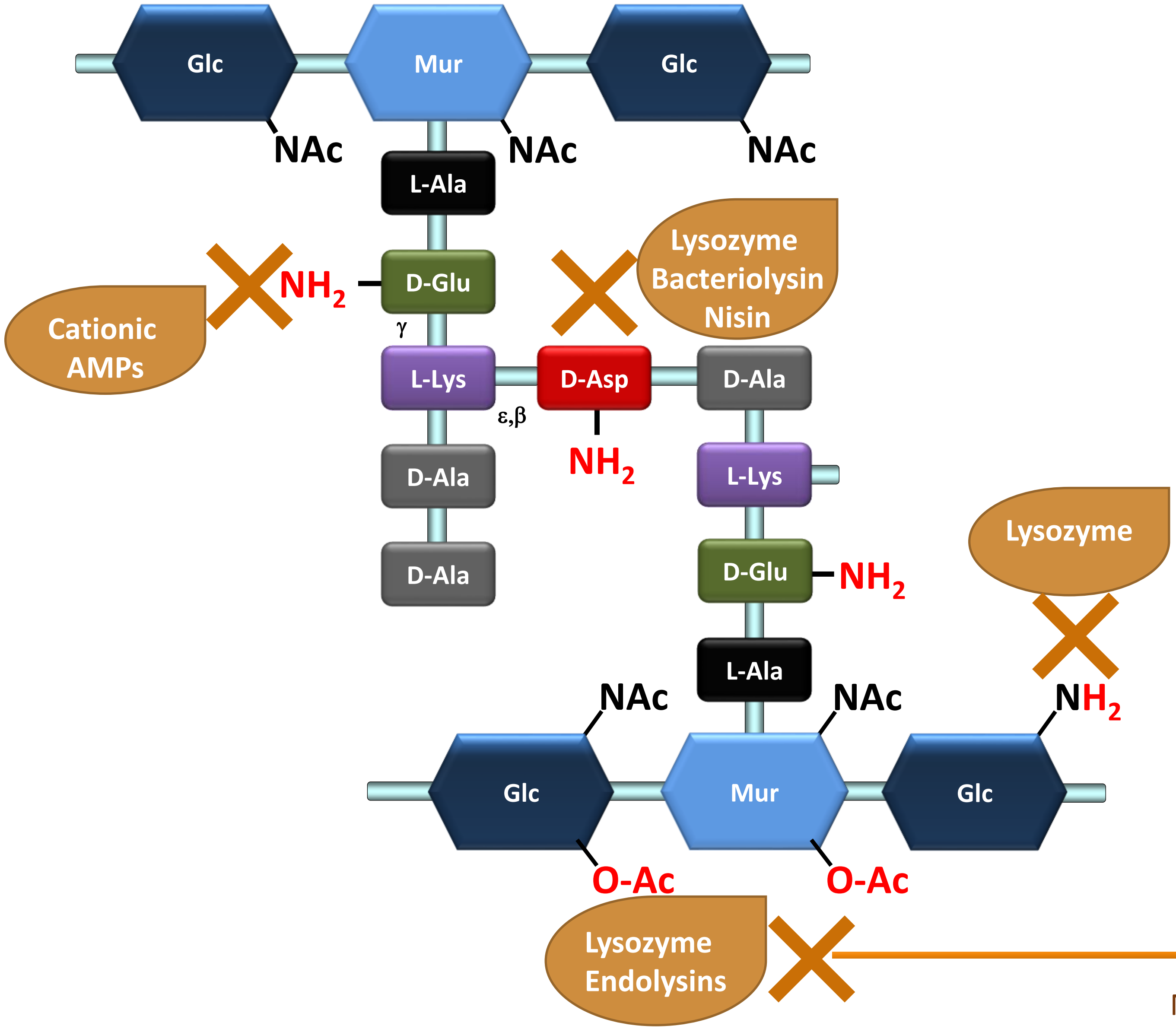
- Lysozyme
  - PG hydrolase
- AMPs:
  - Lipid II

CW structural diversity: peptidoglycan



- **PG chemotype**:
  - Di-amino acid aa3: L-Lys, mDAP, L-Orn
  - D-Ala → D-Lac
- **Cross-linking**
  - Direct
  - Interpeptide bridge
- **Other modifications**
  - Amidation (D-Glu, mDAP, D-Asp)
  - *N*-deacetylation
  - *O*-acetylation

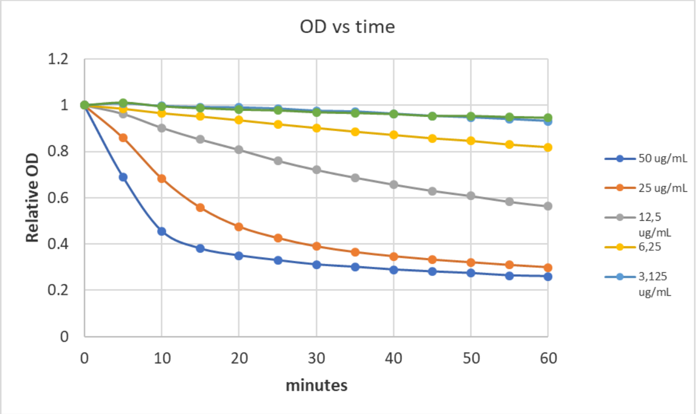
Modified from Chapot-Chartier & Kulakauskas, 2014. Microbial Cell Factories

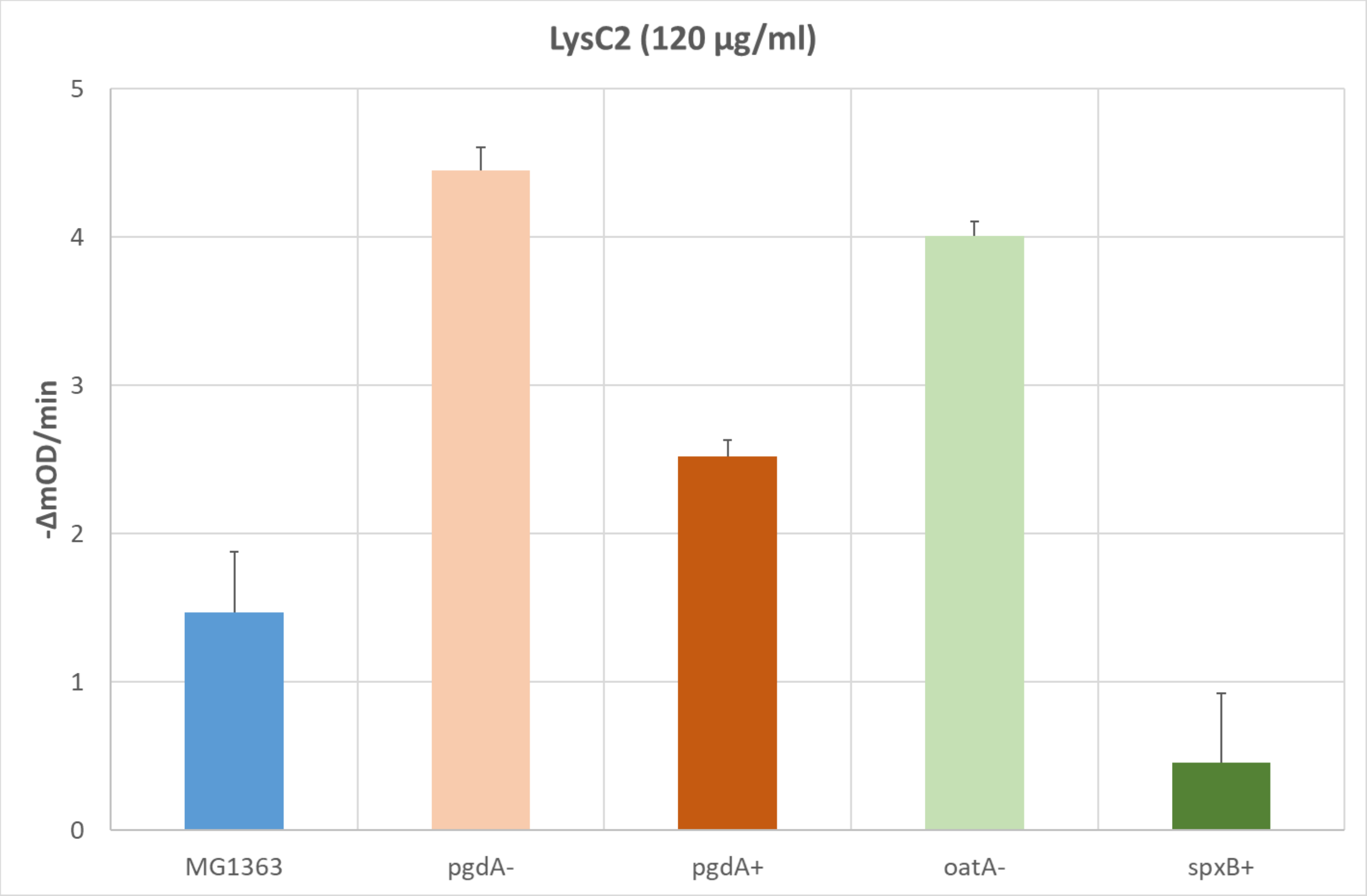
CW structural diversity: peptidoglycan

❖ *In vitro* activity of phage endolysins

Turbidity reduction assays

↓





pgdA: *N*-acetylglucosamine deacetylase
oatA: MurNAc *O*-acetyltransferase

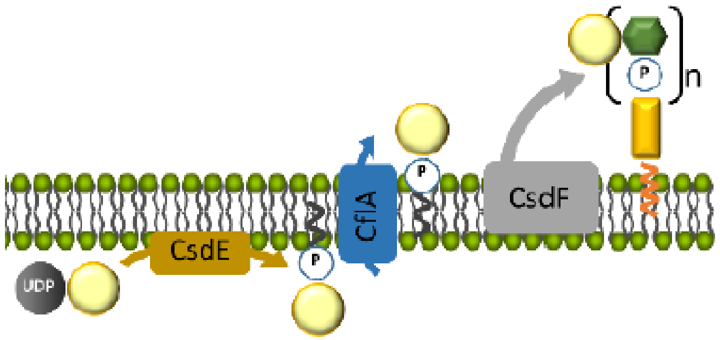
N-deacetylation

O-acetylation

Escobedo et al. 2019. Viruses; Pérez de Pipaon et al, unpublished

CW structural diversity: teichoic acids

- D-ala esterification LTA, *dltABCD*
  - Bacteriocins and AMPs resistance
  - *L. delbrueckii* phage LL-H
- LTA galactosylation (*L. lactis)*
  (three component glycosylation system)
  - *csdEF + cflA*



Modified from Theodorou et al.
J. Biol. Chem. (2020) 295:5519

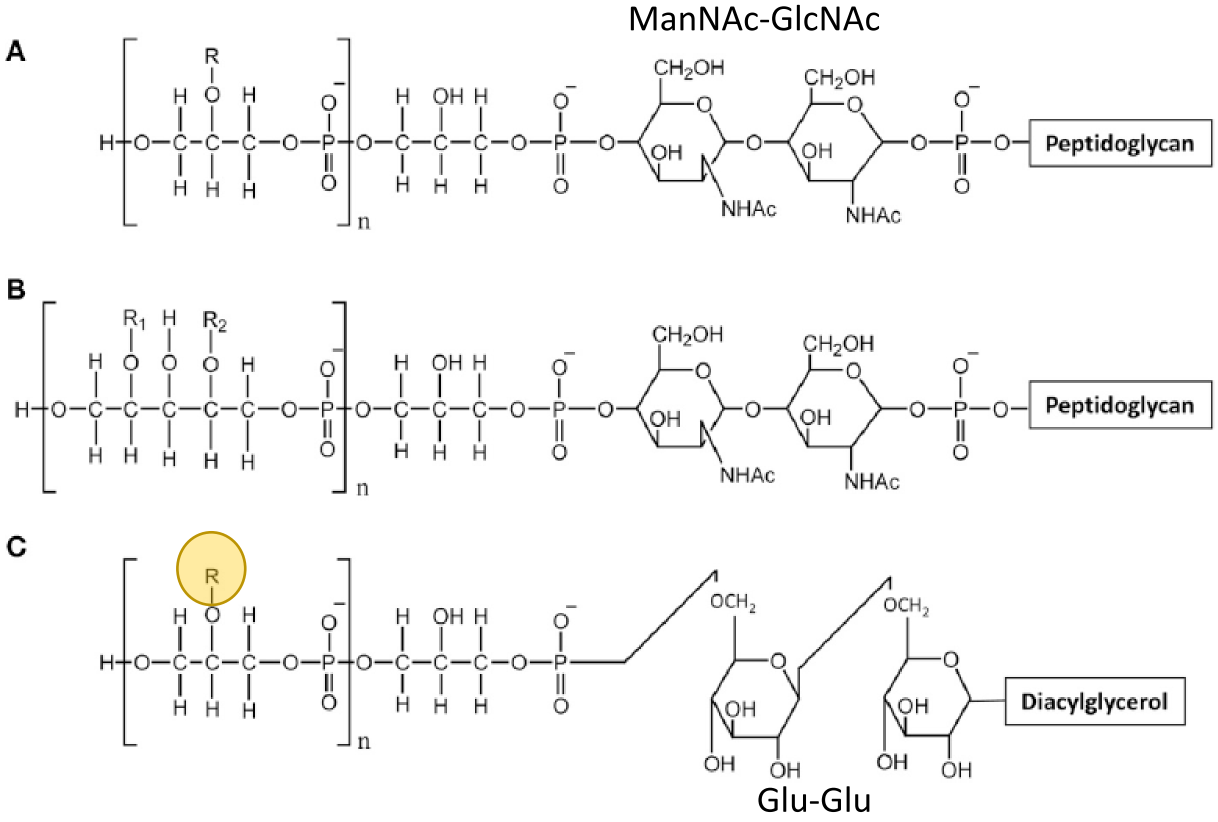
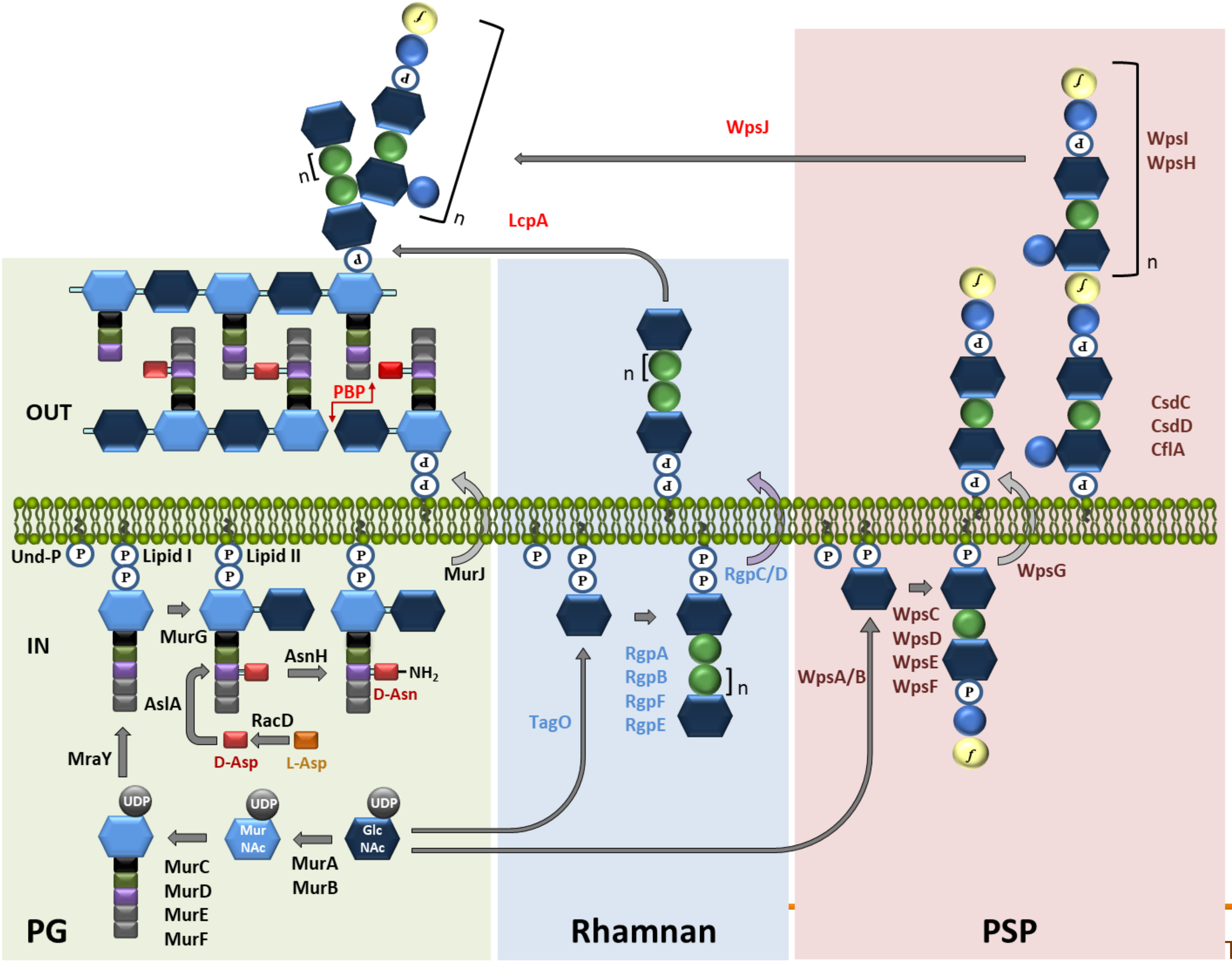


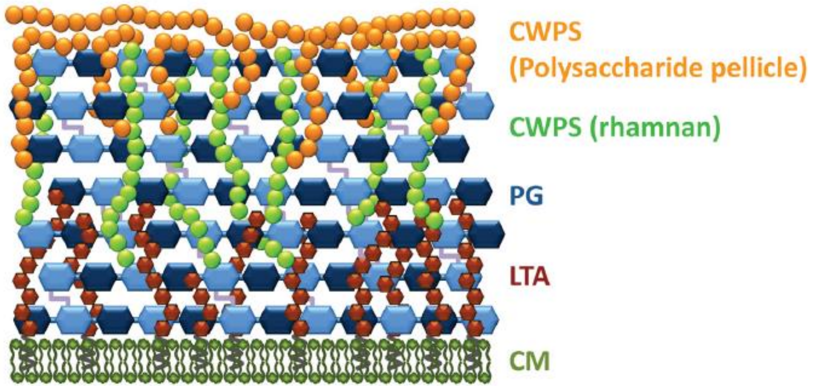
FIGURE 3 | Structure of teichoic acids. (A) WTA with poly-glycerol-phosphate chains; **(B)** WTA with poly-ribitol-phosphate chain; **(C)** LTA with poly-glycerol-phosphate chains. R, R1, R2 indicate potential substituent groups of polyols chains (e.g., D-Ala, Glc, Gal, GlcNAc).

Taken from Chapot-Chartier, 2014. Microbial Cell Factories

CW structural diversity: CWPS



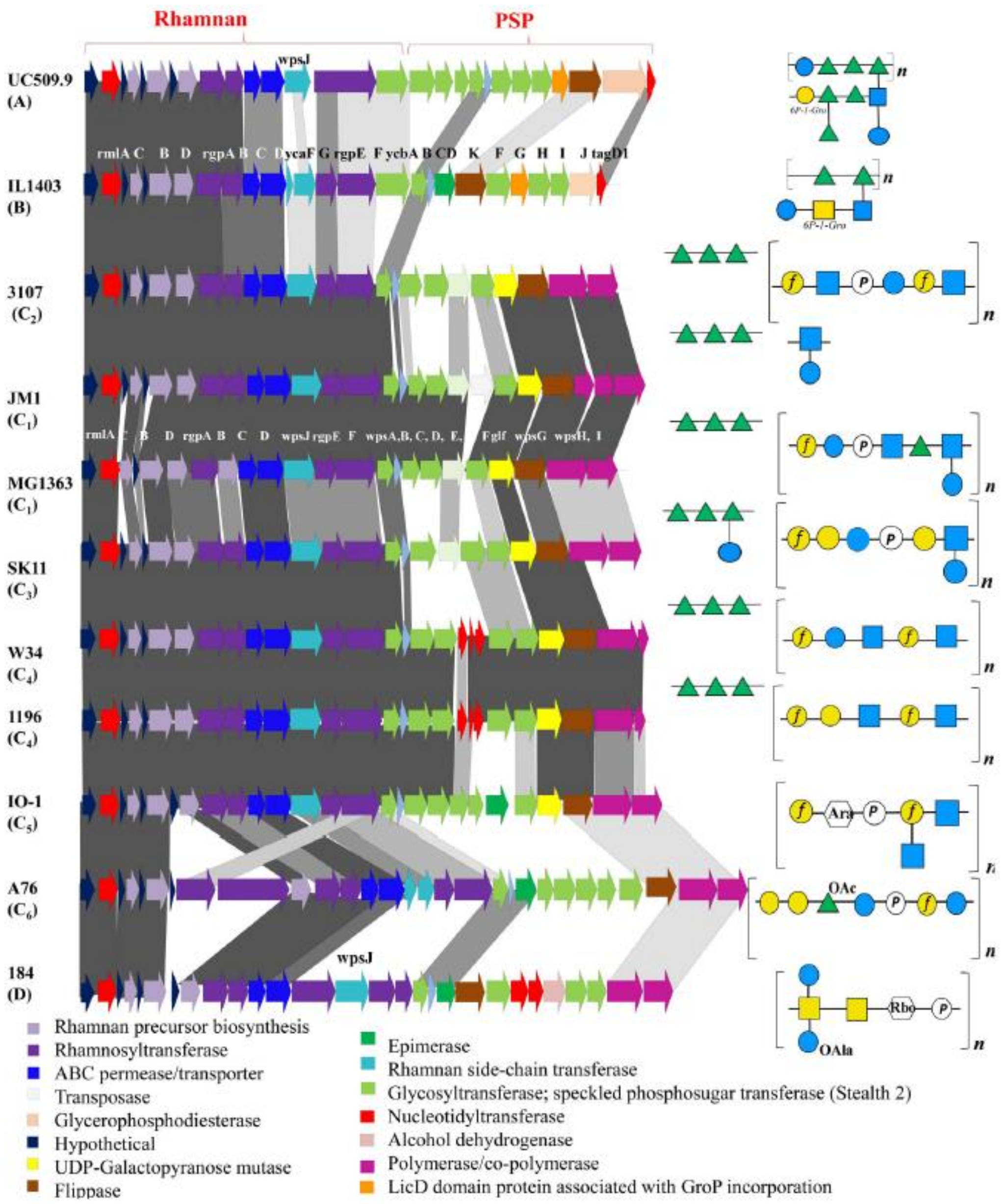
- *L. lactis*: rhamnan and PSP
- Dual chain assembly pathway



Sadovskaya et al. 2017. mBio 8:e01303-17
Theodorou et al. 2019. J. Biol. Chem. 294:17612
Theodorou et al. 2020. J. Biol. Chem. 295:5519

Taken from Martínez et al, 2020. FEMS Microbiol Rev. 44:538

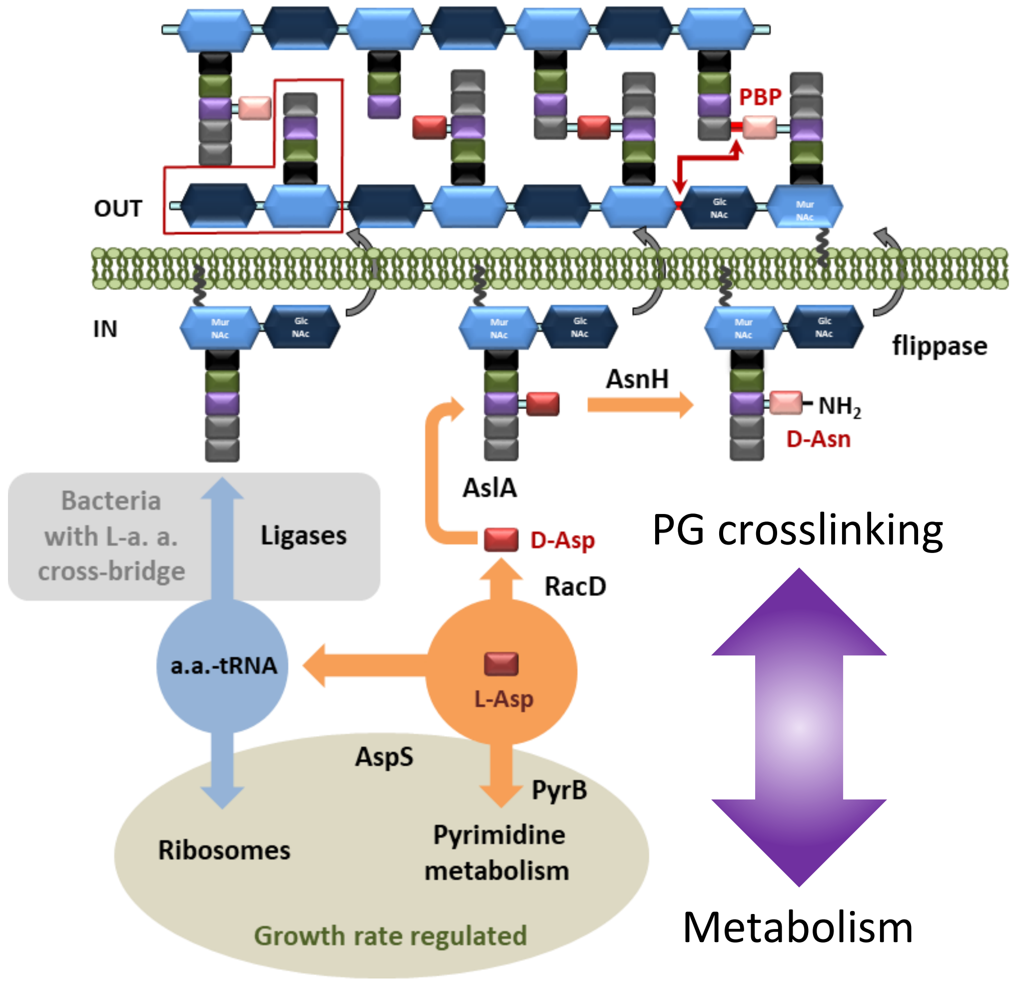
CW structural diversity: CWPS



- ❖ Phage susceptibility
- ❖ Phage-host interactions
- ❖ PCR typing tools

Taken from Mahony et al. 2020 Molecular Microbiology. 114:582

CW Plasticity

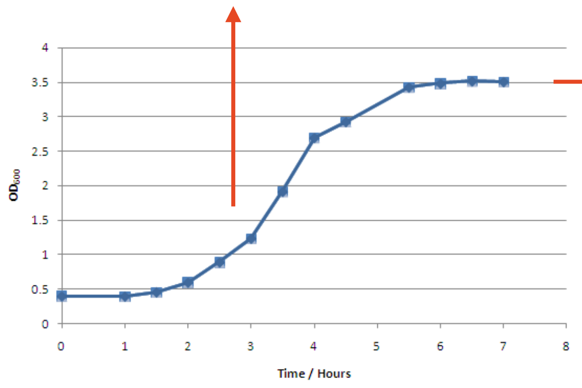


❖ PyrB: aspartate carbamoyltransferase

L. lactis ΔpyrB

- Lysozyme R
- Thicker PG (more rigid cells)
- Higher crosslinking

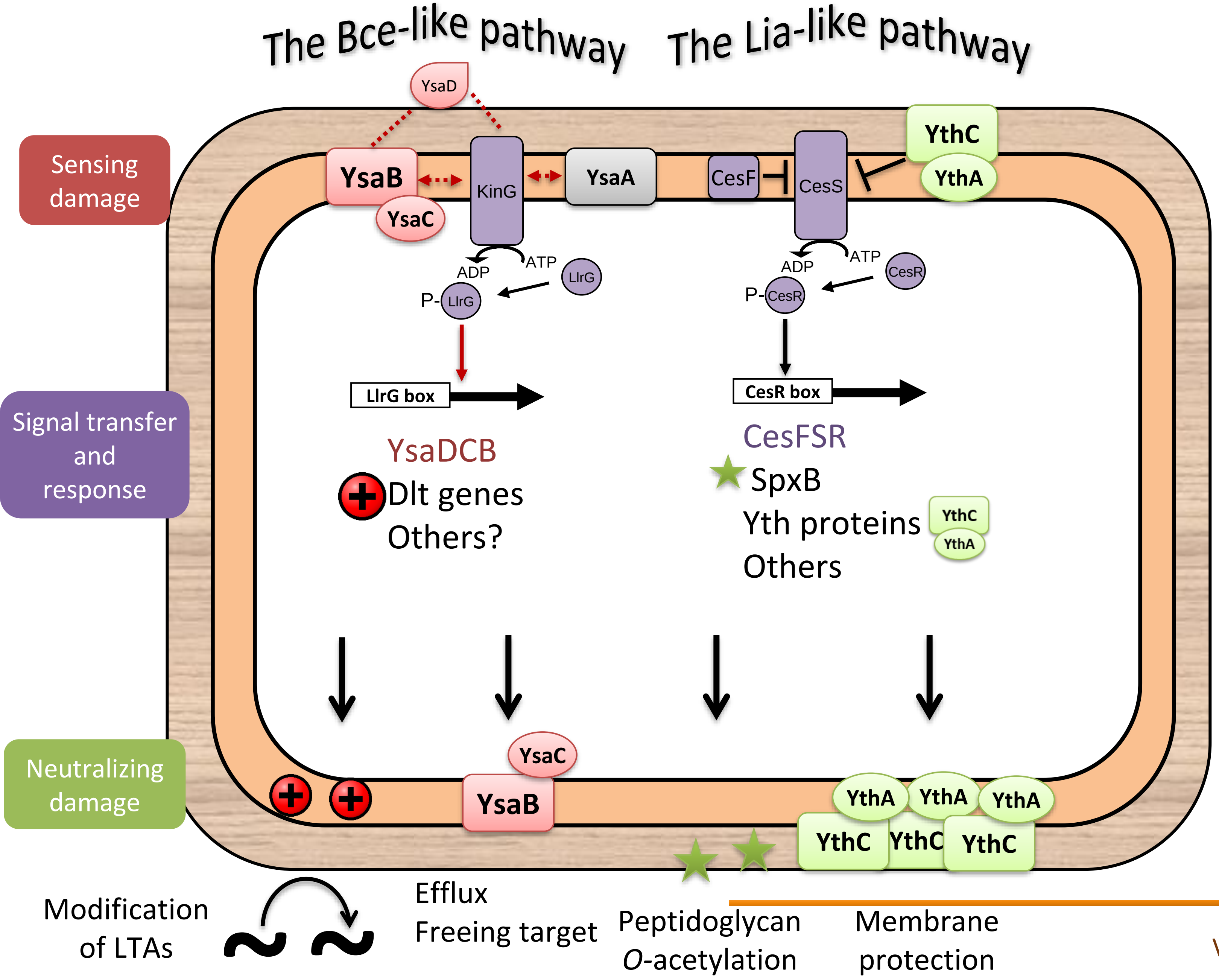
Exp. growth
↓↓ PG crosslinking



Stationary phase
↑↑ PG crosslinking

Modified from Solopova et al. 2016. JBC 291:11323.

CW plasticity: the Cell Envelope Stress (CES) response



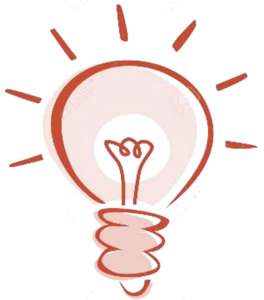
L. lactis

- **CesFSR**
  - Intramembrane-sensing HK
  - CesF negative regulator
  - SpxB →OatA→PG O-acetylation
  - Yth proteins (Psp-like)

- **TCS-G**
  - Intramembrane sensing HK
  - ABC-transporter (YsaCB)
  - Accessory proteins (YsaD, YsaA)
  - MG1363 *ysaB*‾

Veiga et al, 2007; Martínez et al., 2007; Campelo et al. 2020.

“Exploiting the CW”

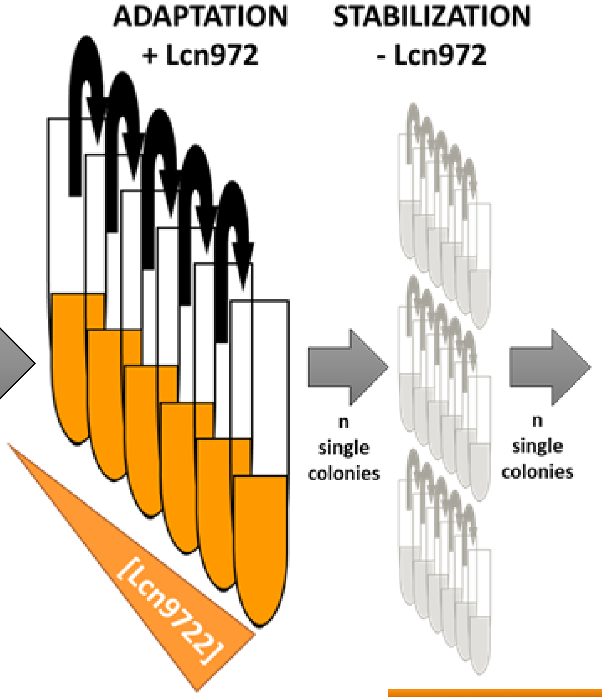


Mutations of technological interest in *L. lactis* might be selected and fixed by triggering the CES response for an extended period

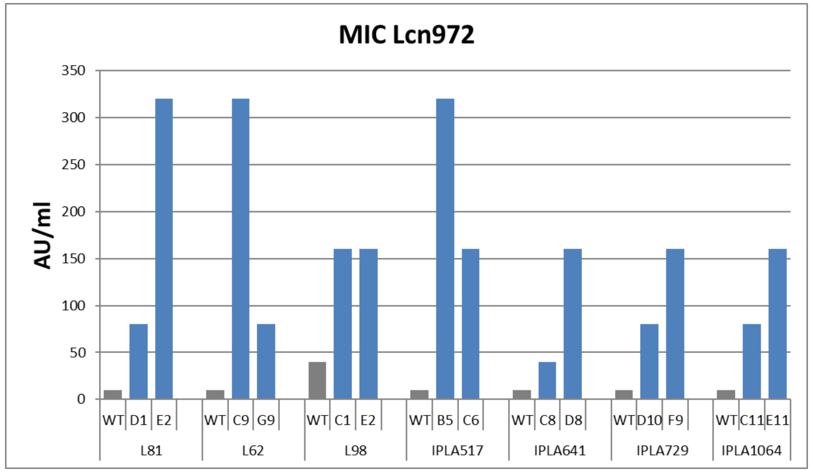
Apply **adaptive evolution** under cell envelope stress (AE-CES), using as stressor the bacteriocin **Lcn972** that inhibits cell wall biosynthesis in *Lactococcus*

***L. lactis* strains (n=9)**

- 1 lab strain (MG1614)
- 4 comercial starters
- 4 field isolates



x4-x32



Lcn972R (n=16)

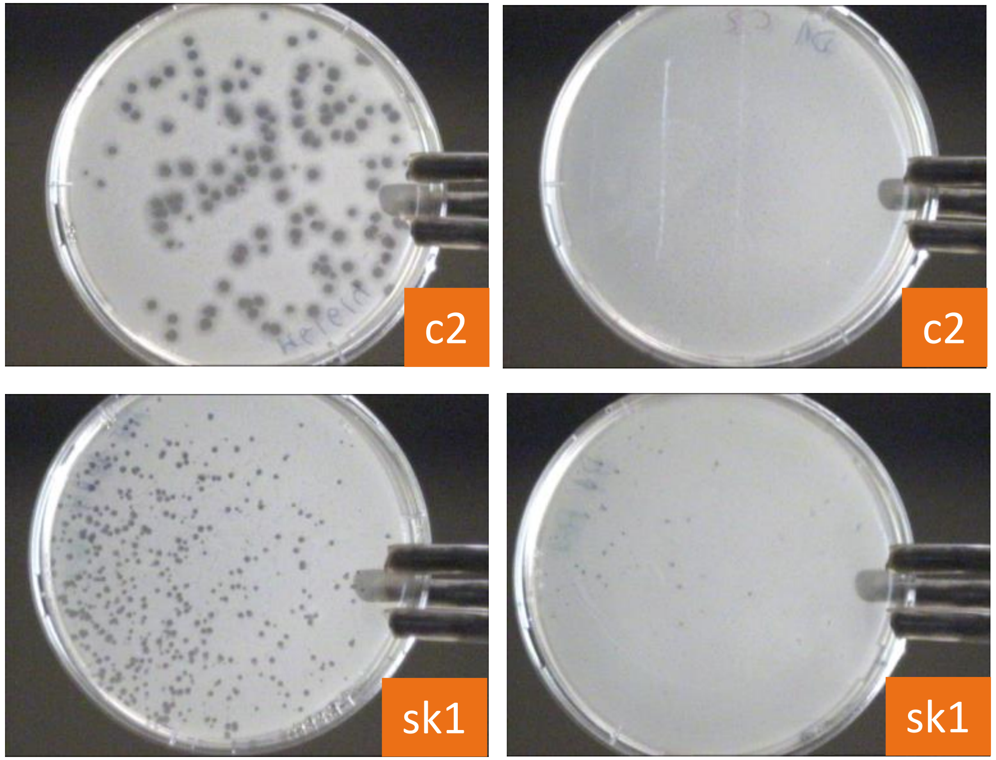
- Phenotypic tests
- Draft genomes

López-González et al. 2018. Frontiers in Microbiology 9:2654

“Exploiting the CW”

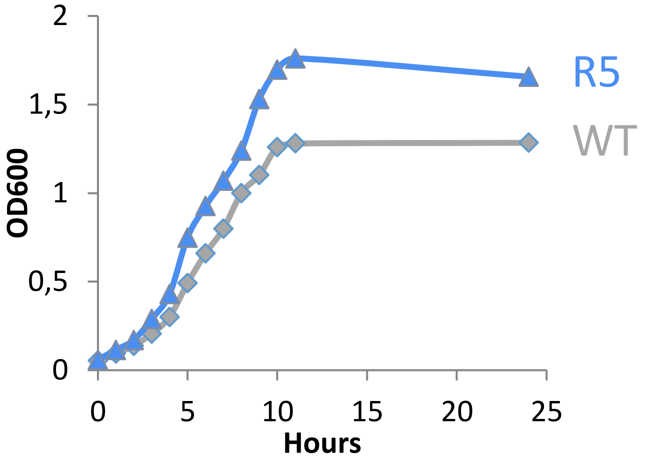
BACTERIOPHAGE RESISTANCE

L. lactis MG1614 | Lcn972R

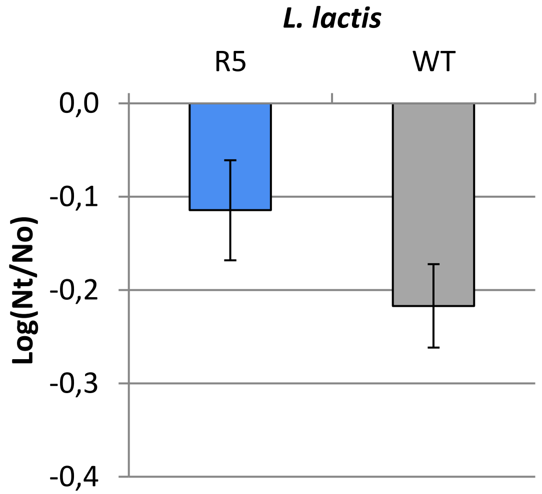


OXIDATIVE STRESS

Aerobic growth



Survival H_2O_2 5 mM



Roces et al. (2012) Appl Environ Microbiol. 78: 4157

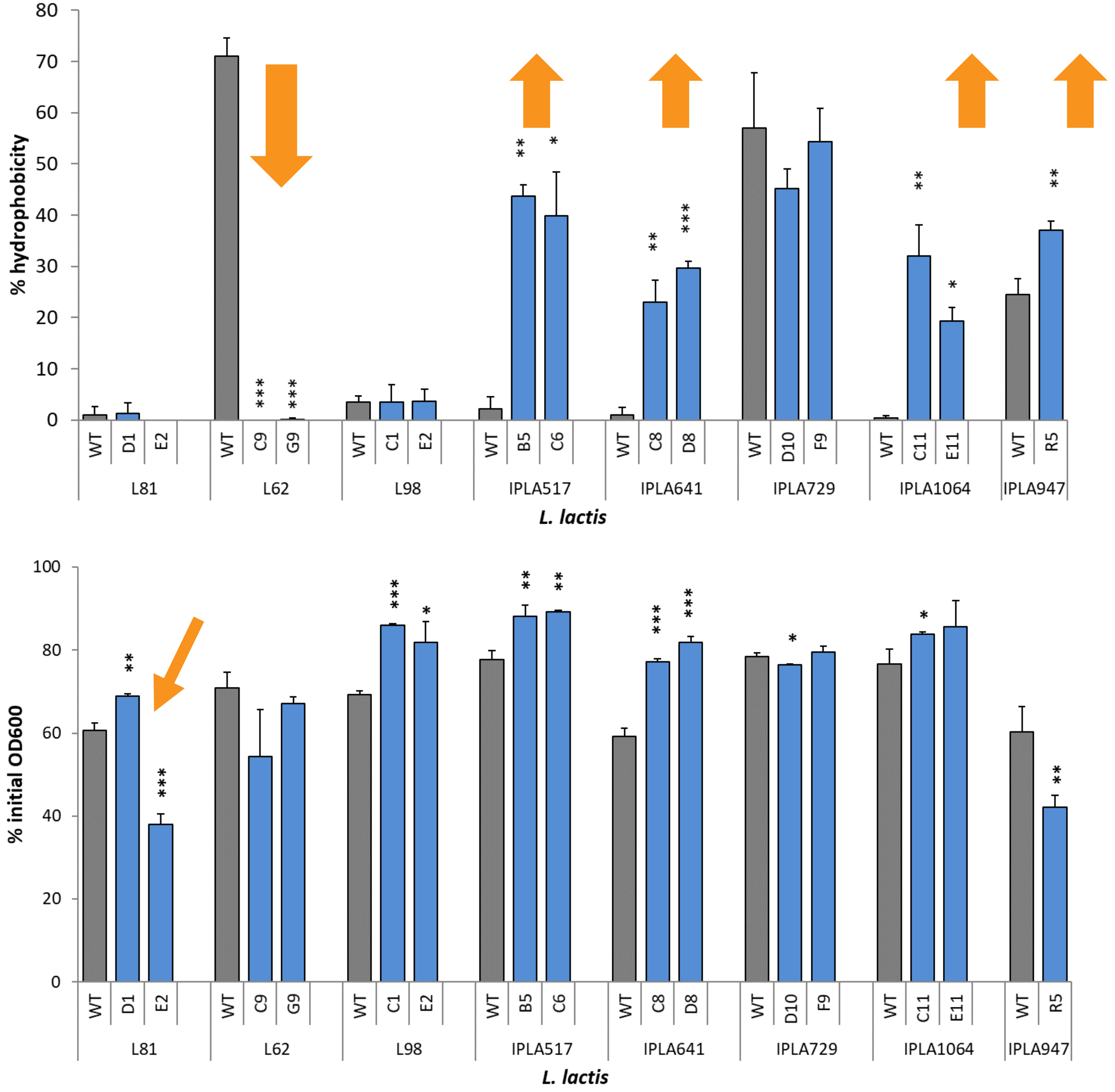
López-González et al. (2018) BMC Microbiol. 18:76

“Exploiting the CW”

- Growth rate
- Milk acidification
- Nisin production

- Hydrophobicity
- Autolysis

- Plasmid loss:
  - Lactose (1/15)
  - Bacteriophage resistance (3/15)
- Increased sensitivity:
  - 50 °C, 30 min: 7/11
  - NaCl: 7/15



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"Exploiting the CW"

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Strategies to counteract CES

AE-CES

– Feasible strategy to introduce **diversity** within strain collections

Filling the gaps

TOPIC	PROGRESS	FUTURE
CW physiology	▶ Nucleotide pools and PG plasticity	Alternative mechanisms for different PG types. Link to central metabolism, recycling
	▶ PG hydrolases in cell division	Imaging, spatial distribution, protein assemblies
CW components	▶ CWPS diversity	Role of decorations. Glycosyl transferase activities
Stress response	▶ TCSs characterized	Activation, sensing, regulatory networks
Health-promoting activities	▶ Muropeptides and host protection	In depth knowledge LAB-host molecular dialogue: motifs sensed by the host
	▶ TA modifications and impact on host physiology	
	▶ Membrane vesicles	Biogenesis, LAB-host cross-talk
	▶ Bacteriocin variants	Extended uses, antibiotic alternatives
Novel applications	▶ Bacteriophage receptors	Bacteriophage-resistant strains
	▶ LAB as cell factories	Improving product yields
	▶ Evolution under CES	CES-resistant strains, tradeoffs
	▶ Bioremediation	Defining interaction of pollutants with CW components

Taken from Martínez et al. 2020. FEMS Microbiol Rev


Micalis

INRAE
la science pour la vie, l'humain, la terre

DairySafe

Ipla CSIC

- Past and present members
- All collaborators
- Funding agencies

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IDI/2018-000119

Agencia Estatal de Investigación

Unión Europea
Fondo Europeo de Desarrollo Regional

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