

# MICROBIAL STRESS 2020

16-18 NOVEMBER |



## Exploiting the lactococcal cell envelope stress response for proficient dairy starters

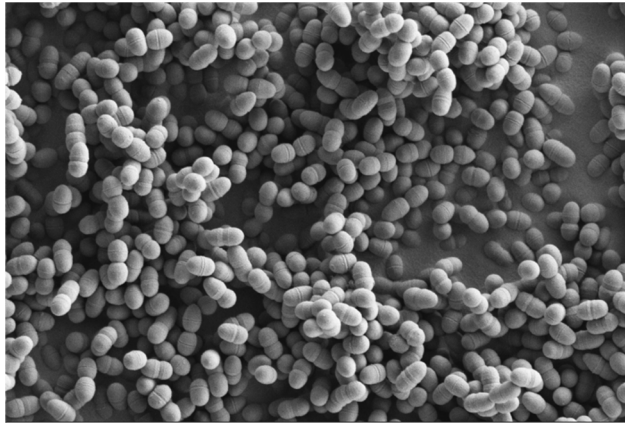
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# INTRODUCTION



Dairy starter



## STRESSFUL CONDITIONS

- Biomass production
- Low pH
- NaCl
- Antimicrobials
- ...

*Lactococcus lactis*



Cell factory

How can we improve  
strain robustness?





## HYPOTHESIS:

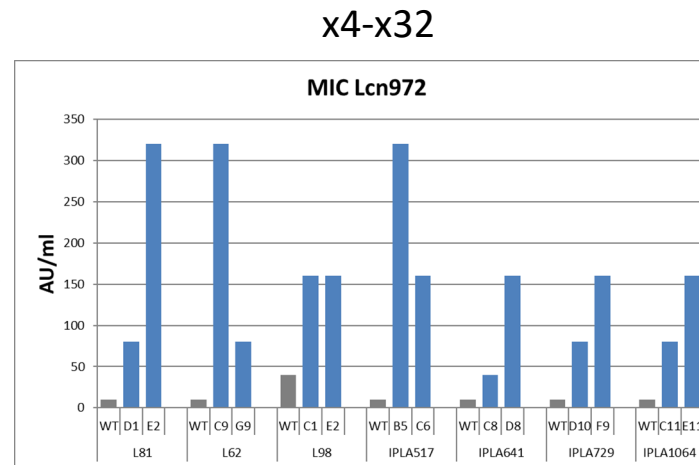
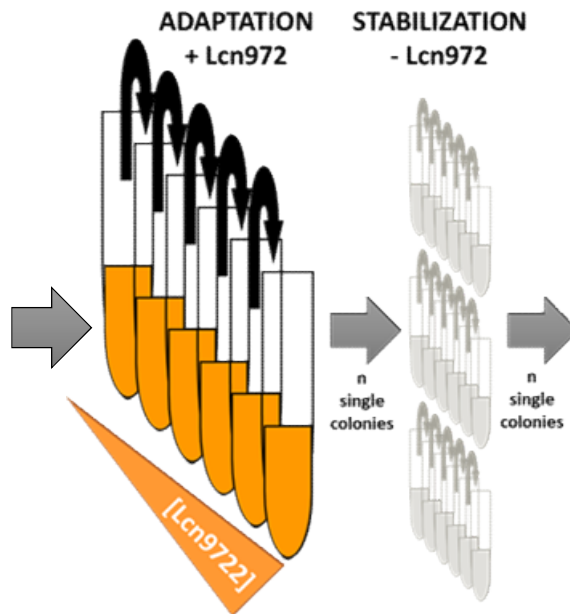
Mutations of technological interest might be selected and fixed by triggering the **cell envelope stress response** for an extended period

## METHODS

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 commercial starters
- 4 field isolates



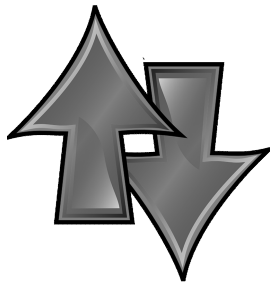
### Lcn972R (n=16)

- Phenotypic tests
- Draft genomes

# RESULTS



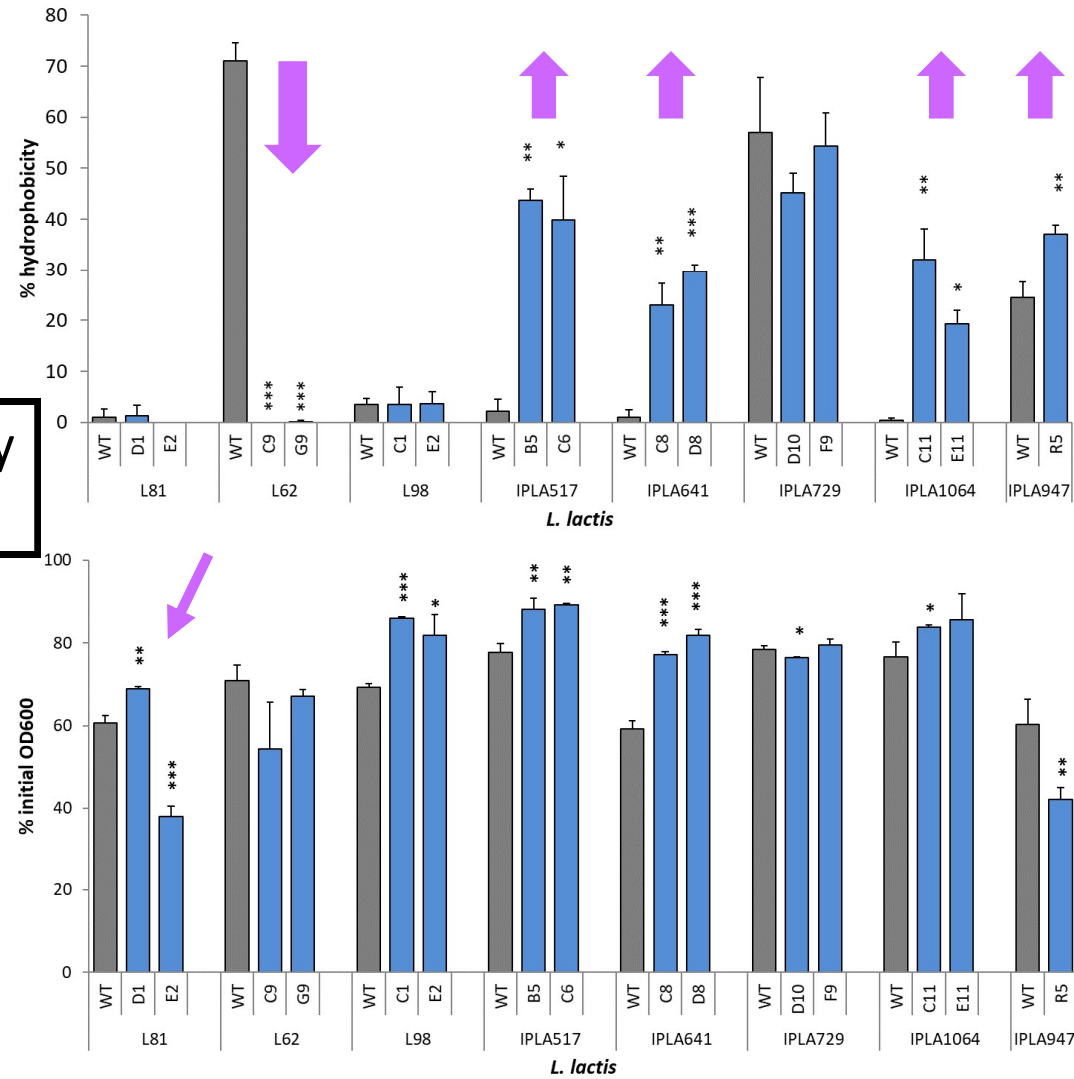
- Growth rate
- Milk acidification
- Nisin production



- Hydrophobicity
- Autolysis



- Plasmid loss:
  - Lactose (1/15)
  - Bacteriophage resistance (3/15)
- Increased sensitivity:
  - 50 °C, 30 min: 7/11
  - pH 2: no change (n=7)
  - NaCl: 7/15

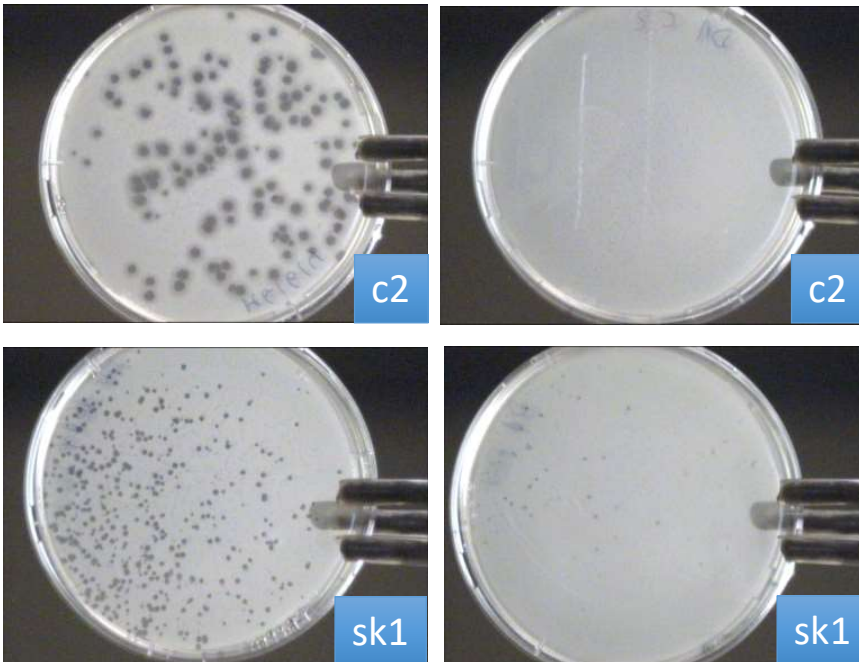


# RESULTS

## BACTERIOPHAGE RESISTANCE

*L. lactis* MG1614

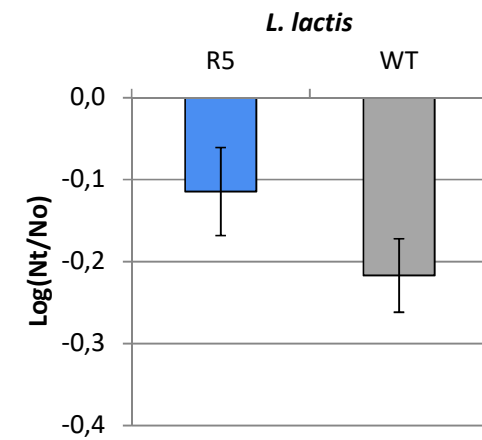
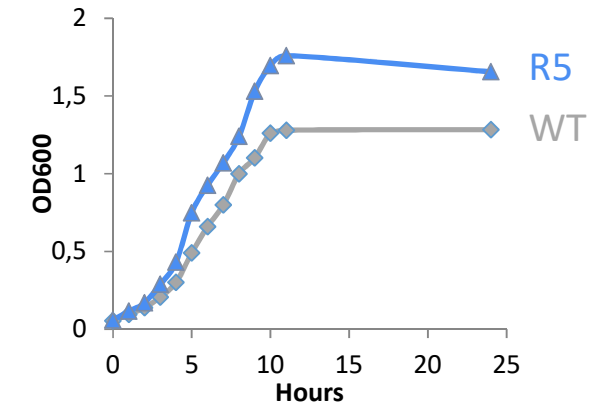
Lcn972R



Aerobic growth

Survival  $H_2O_2$  5 mM

## OXIDATIVE STRESS

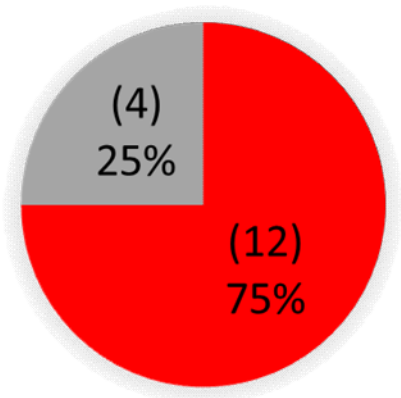




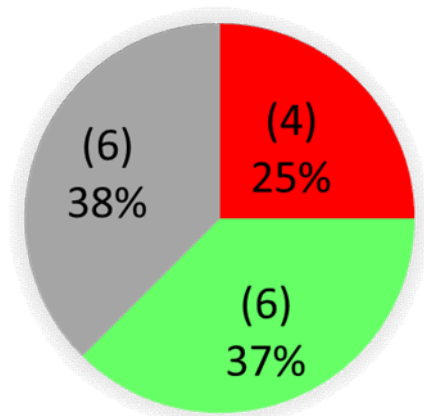
# RESULTS

## CROSS-RESISTANCE CELL WALL ANTIMICROBIALS

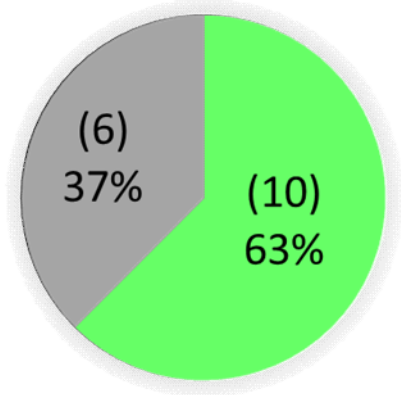
### Lysozyme



### Bacitracin

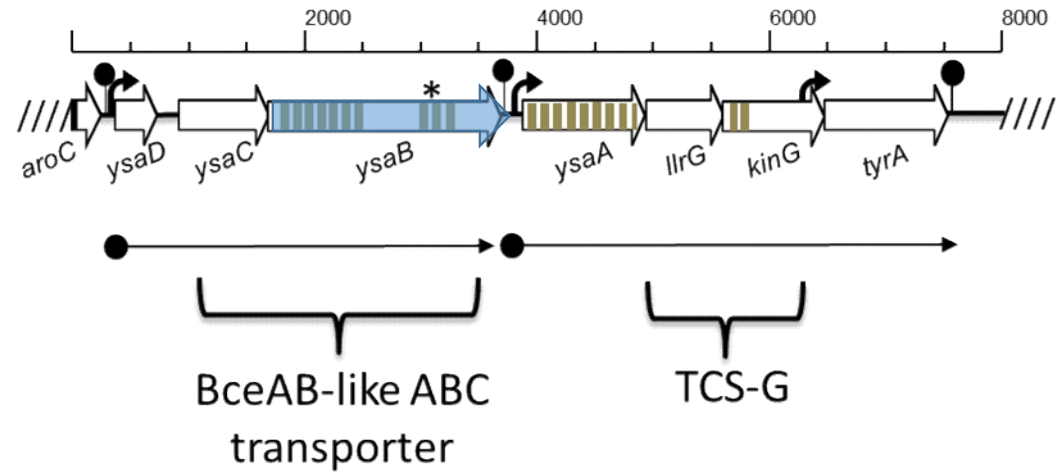


### PenG



Same  
Resistant  
Sensitive

n=16



Mutations in the permease YsaB

F<sub>577</sub>V ; I<sub>594</sub>F



Constitutive expression of the transporter

# CONCLUSIONS

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1. Adaptive evolution under cell envelope stress is a feasible tool for strain improvement programs:

- Stable mutations
- Compatible with dairy technological traits
- Diversification

2. Exposure to the bacteriocin Lcn972 selects for constitutively expressed efflux pumps that interfere with antibiotic activity

- Roces et al. 2012. PMID: 22504807
- López-González et al. 2018. PMID: 30029618
- López-González et al. 2018. PMID: 30455679
- Campelo et al. 2020. PMID: 32903467



thanks



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