

A scanning electron micrograph (SEM) showing numerous spherical, golden-yellow S. aureus bacteria. The bacteria are arranged in a vertical column on the left side of the image. In the background, there is a complex, reddish-brown structure that appears to be a cell or a biofilm. The overall background is dark with some red and blue highlights.

# Targeting *S. aureus* toxin production

- FOR IMPROVED FOOD SAFETY AND ANIMAL HEALTH

Jenny Schelin, Applied Microbiology

Lund University, Sweden



# Lund University

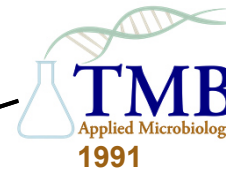
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# Faculty of Engineering – Dep. of Chemistry



**Center of Chemistry  
1964-68**



**2019-2025  
ESS – European Spallation Source**

🚲 9 min  
2,6 km

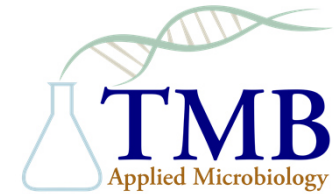


**The MAX IV Laboratory  
2016**



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# Applied Microbiology



## Microbial Physiology and Molecular Mechanisms

Industrial  
Biotech  
Biofuels

Biocatalysis

Water  
Quality

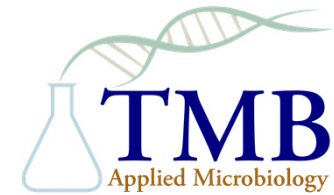
Food Safety

Probiotics

Forensic  
and  
Diagnostic  
PCR

# Food Safety Group

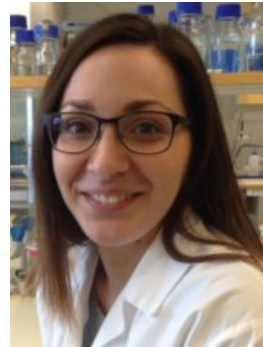
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*Peter*



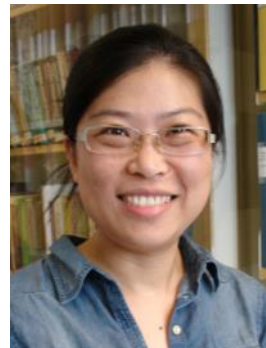
*Yusak*



*Nikoleta*



*Jenny*



*Rong*



*Nina*

Environment

Foodborne  
microbial  
virulence

Toxins



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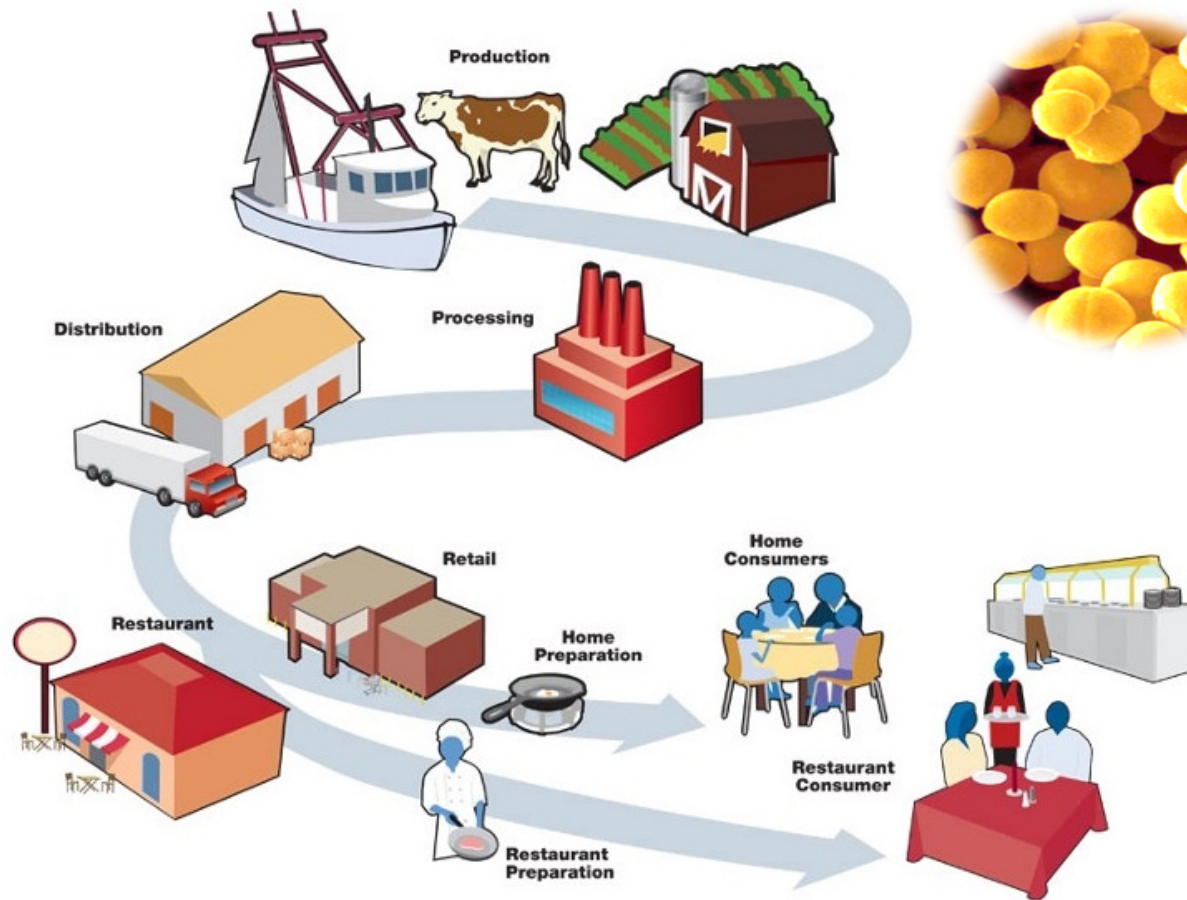
# Food safety – is this an issue?

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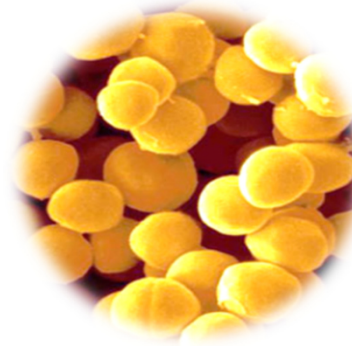
# *Staphylococcus aureus* - challenges

## Supply chain



(<http://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks/production-chain.html>)

## Biology



- Robust
- Virulence factors
- SAg toxins:
  - TSST-1 & SEs
- Mobile genetic elements
- Regulatory mechanisms
- Strain variation
- Unreported



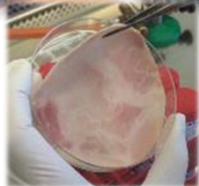
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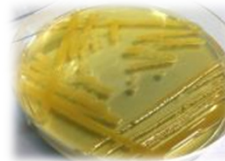
# Targeting toxin production



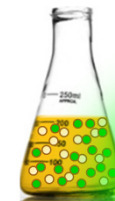
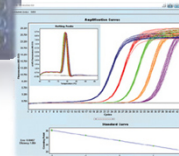
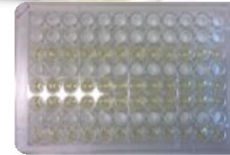
**Bovine mastitis:**  
- influence of SEC & TSST



***S. aureus* enterotoxin A (SEA)**  
- regulation and production



**Monitor SEA production:**  
- Detection and quantification  
→ in real time



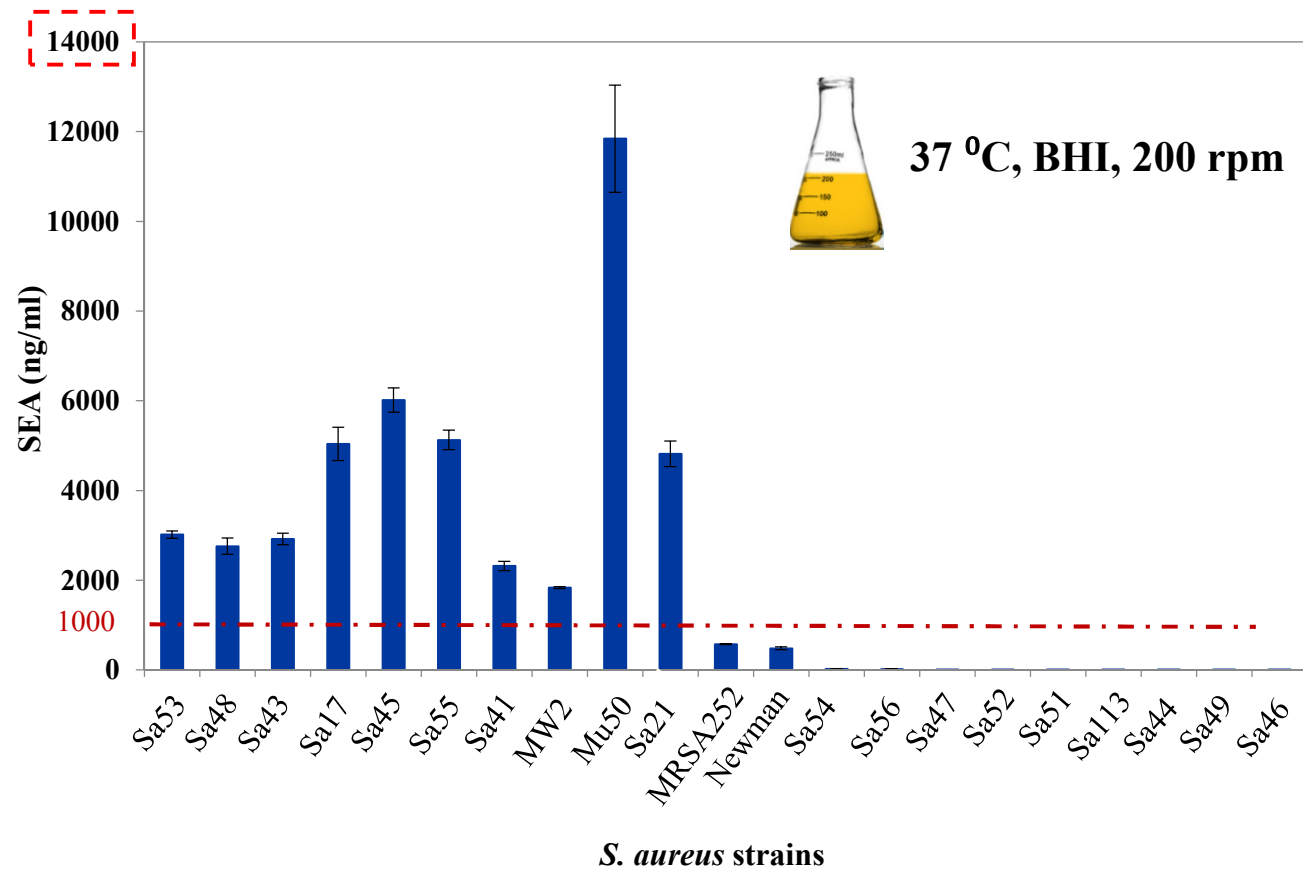
GFP



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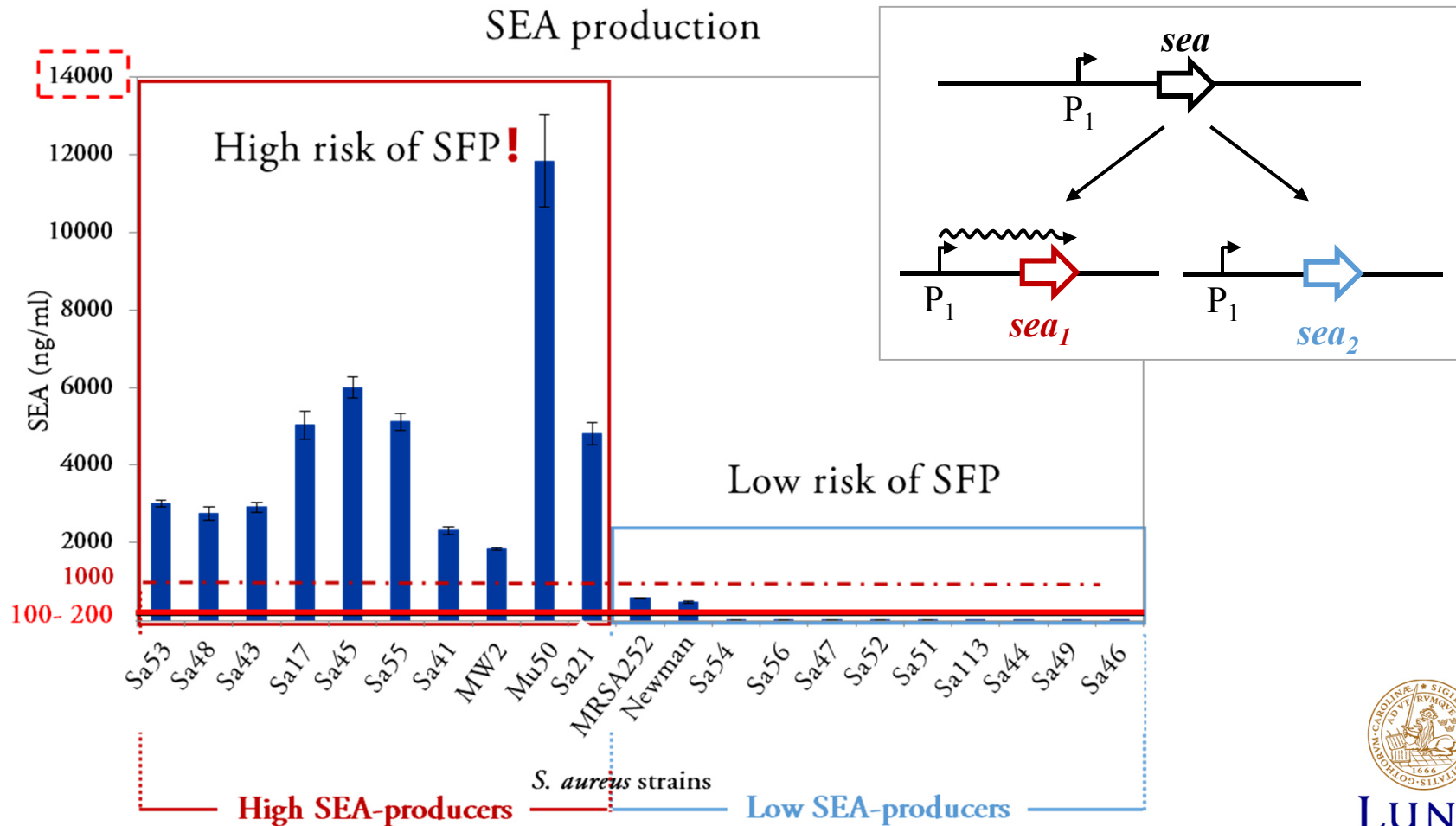
# SEA production – strain variation



- 21 strains: food isolates and fully sequenced strains

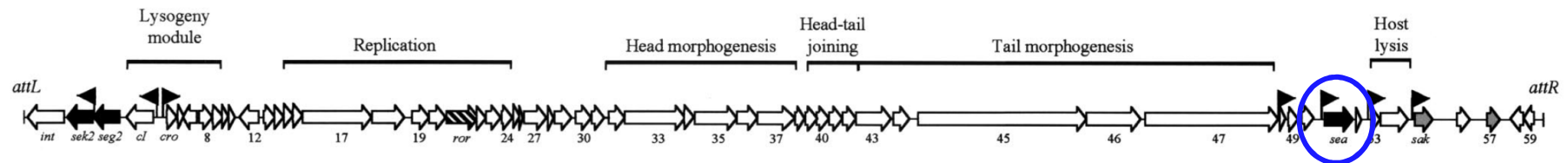


# *sea* gene – two alleles – high & low producers



# *sea* gene – temperate bacteriophage

- Genetic backbone: *Siphoviridae* bacteriophages



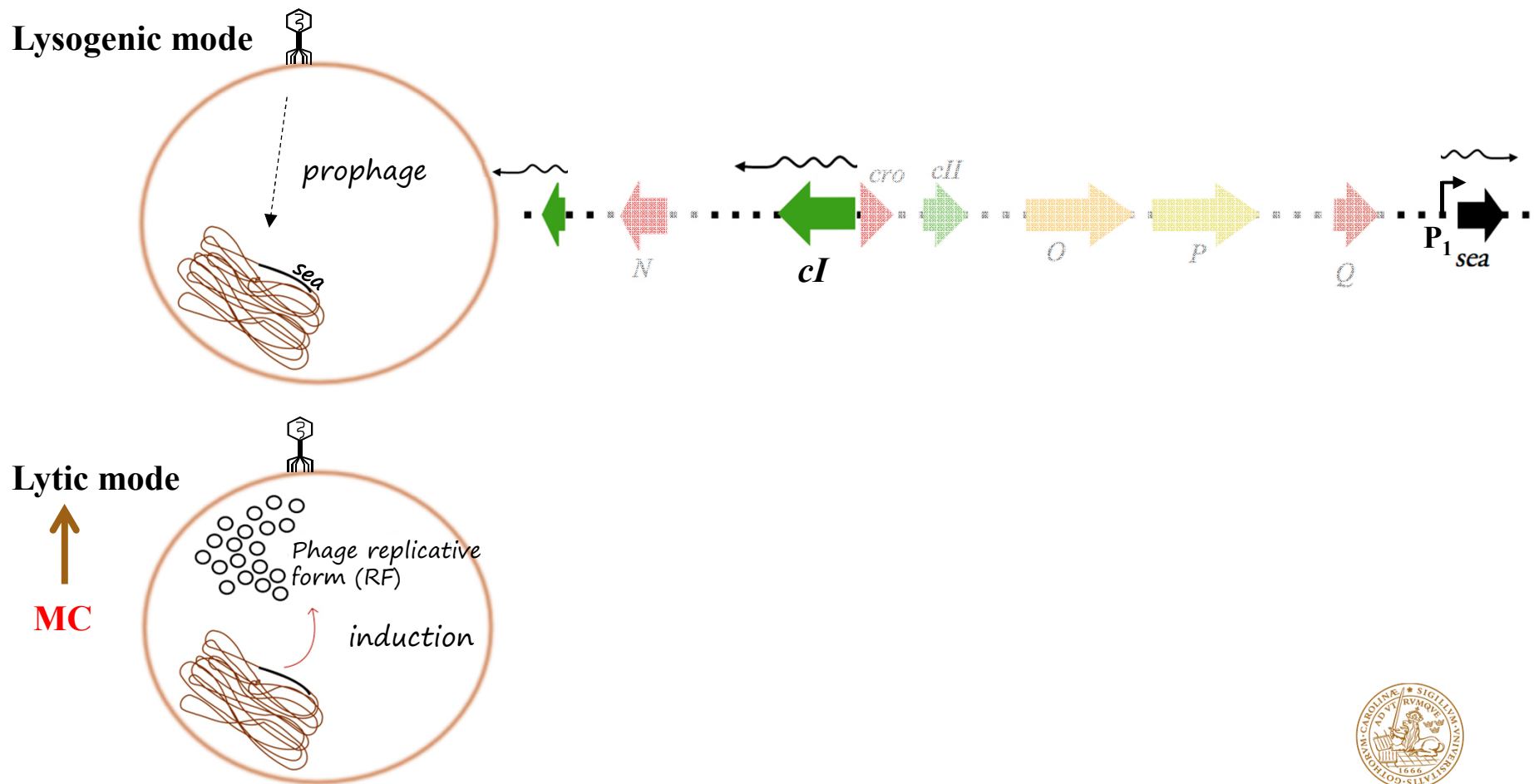
Ref: Sumbly P. & Waldor M. K. (2003)  
*J Bacteriol* (185) 6841-51

➤ Link between phage life cycle & *sea* expression?



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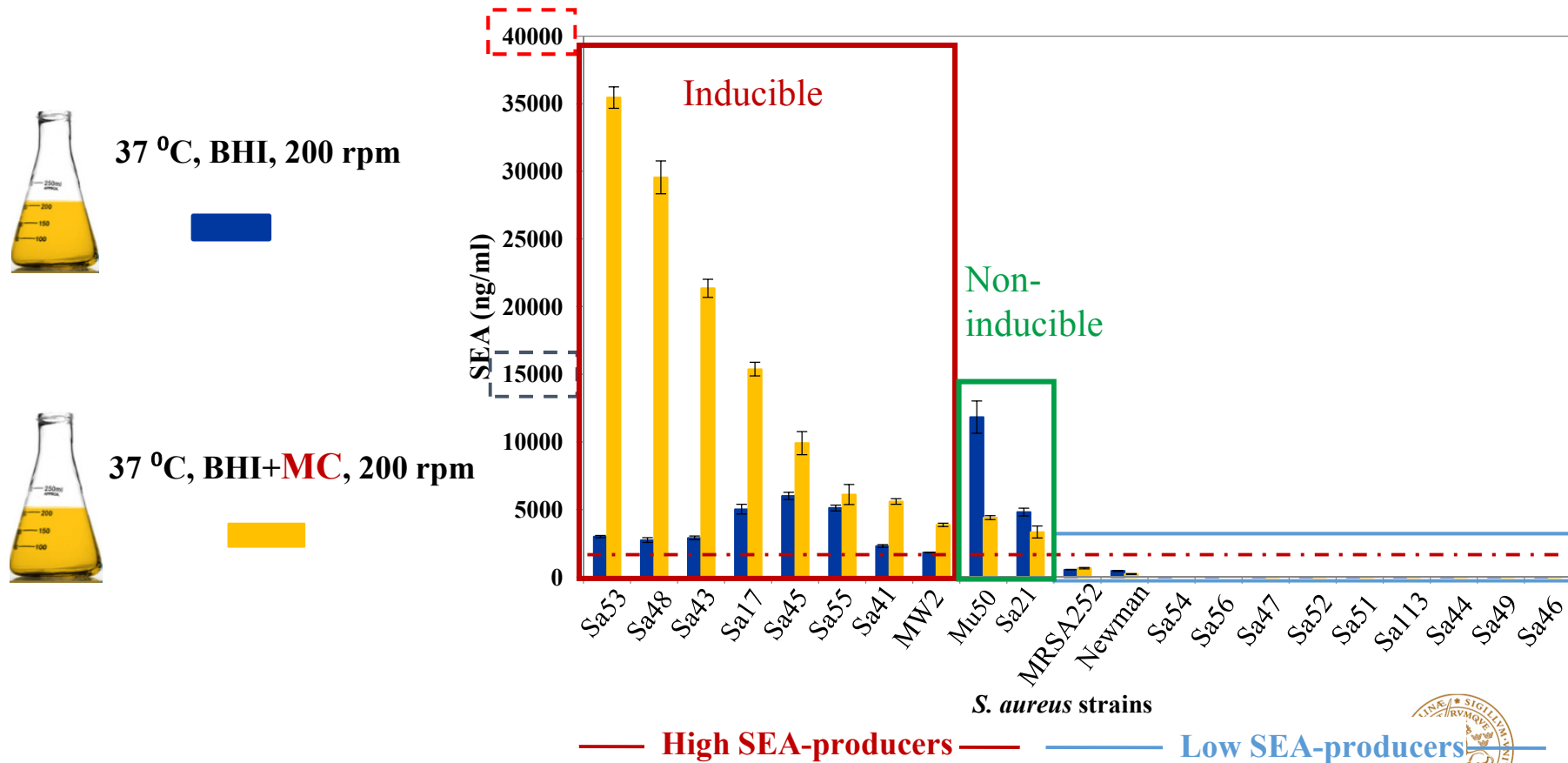
# Phage life cycle and SEA production



- **MC** = Mitomycin C: Phage inducing agent



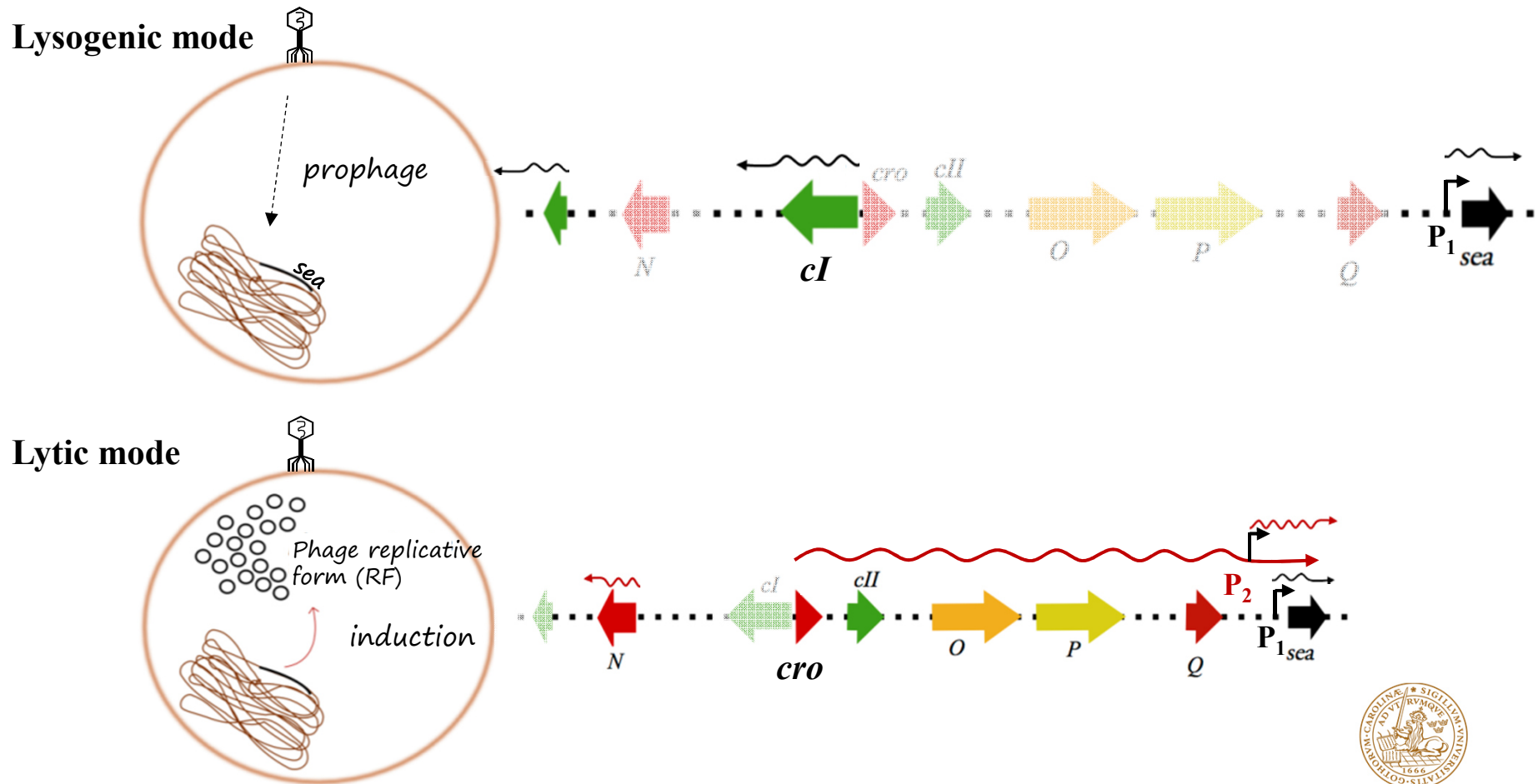
# Phage induction – SEA production



- **MC** = Mitomycin C: Phage inducing agent



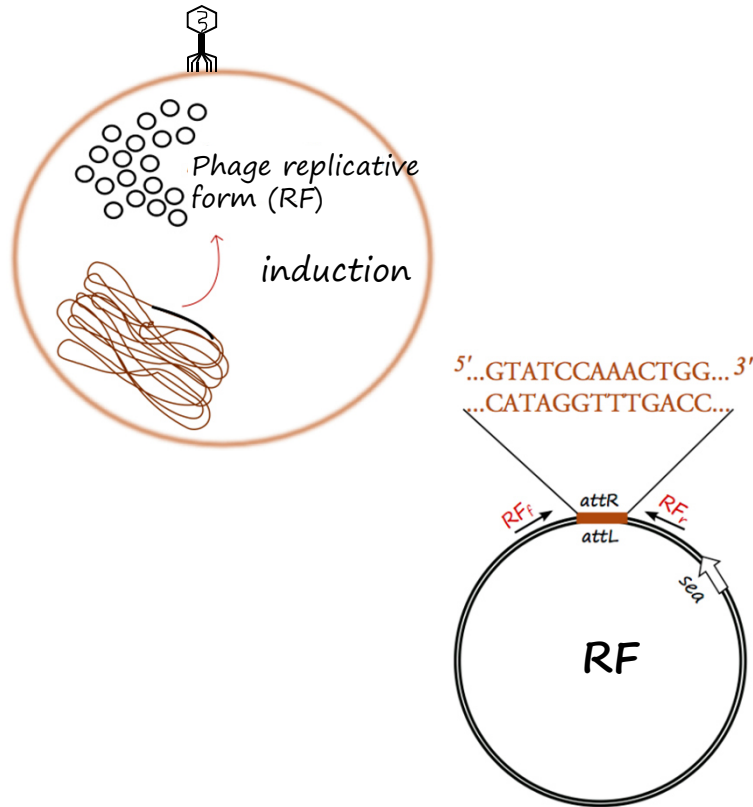
# Phage life cycle and SEA production



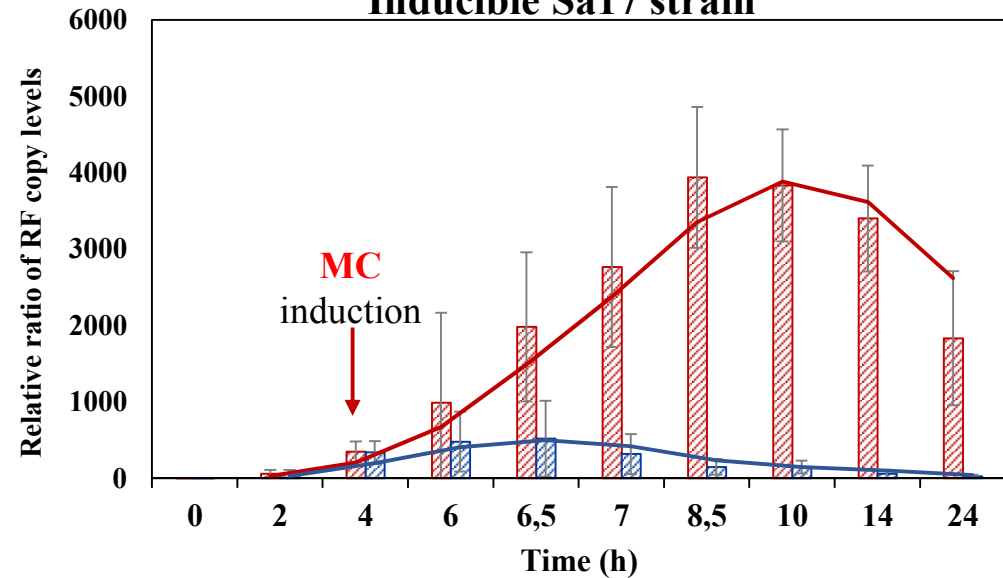
✓ Phage-activated *sea* mRNA from  $P_2$  & more SEA



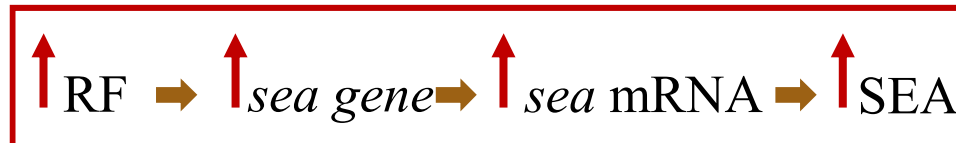
# RF copies & *sea* gene dose effect



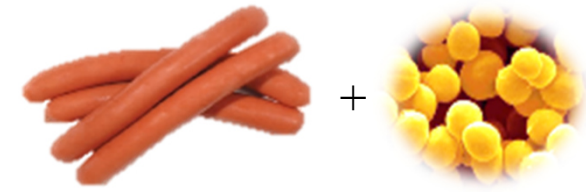
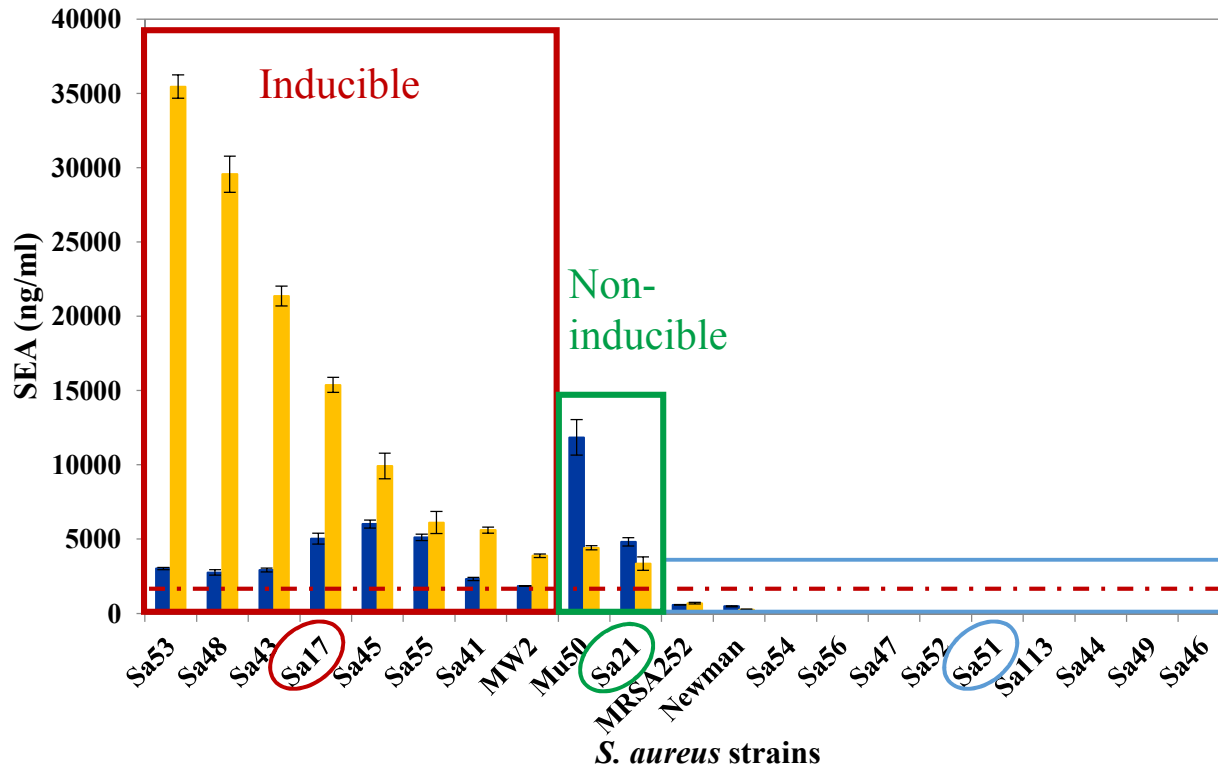
Phage replicative form copies  
Inducible Sa17 strain



MC induction RF  
Control RF



# What happens in food?



- Sodium acetate (E262)
- Sodium nitrate (E250)

Sa17  
Sa21  
Sa51



Incubation 14 days  
at 15 °C

— High SEA-producers — — Low SEA-producers —

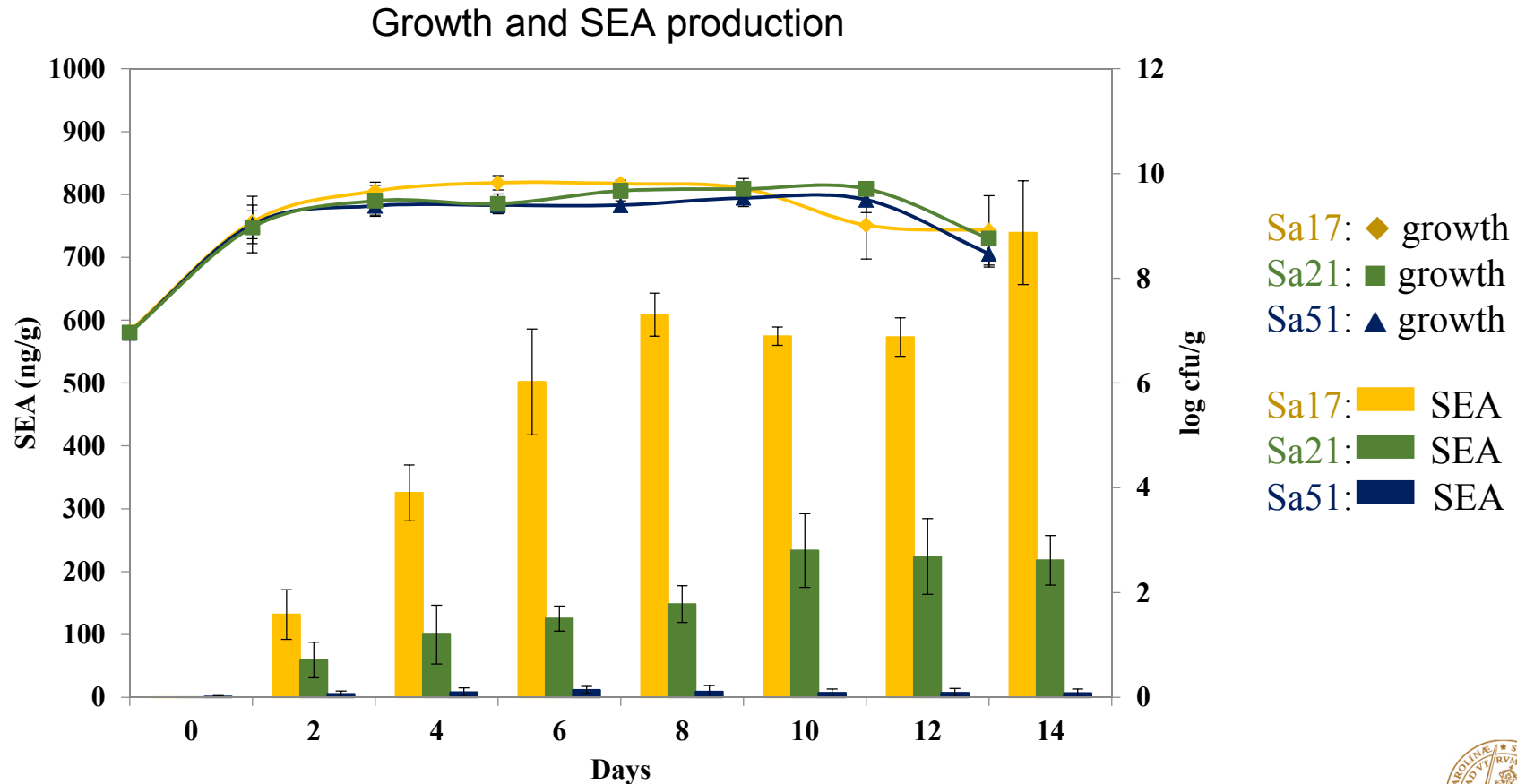
➤ What about SEA production?



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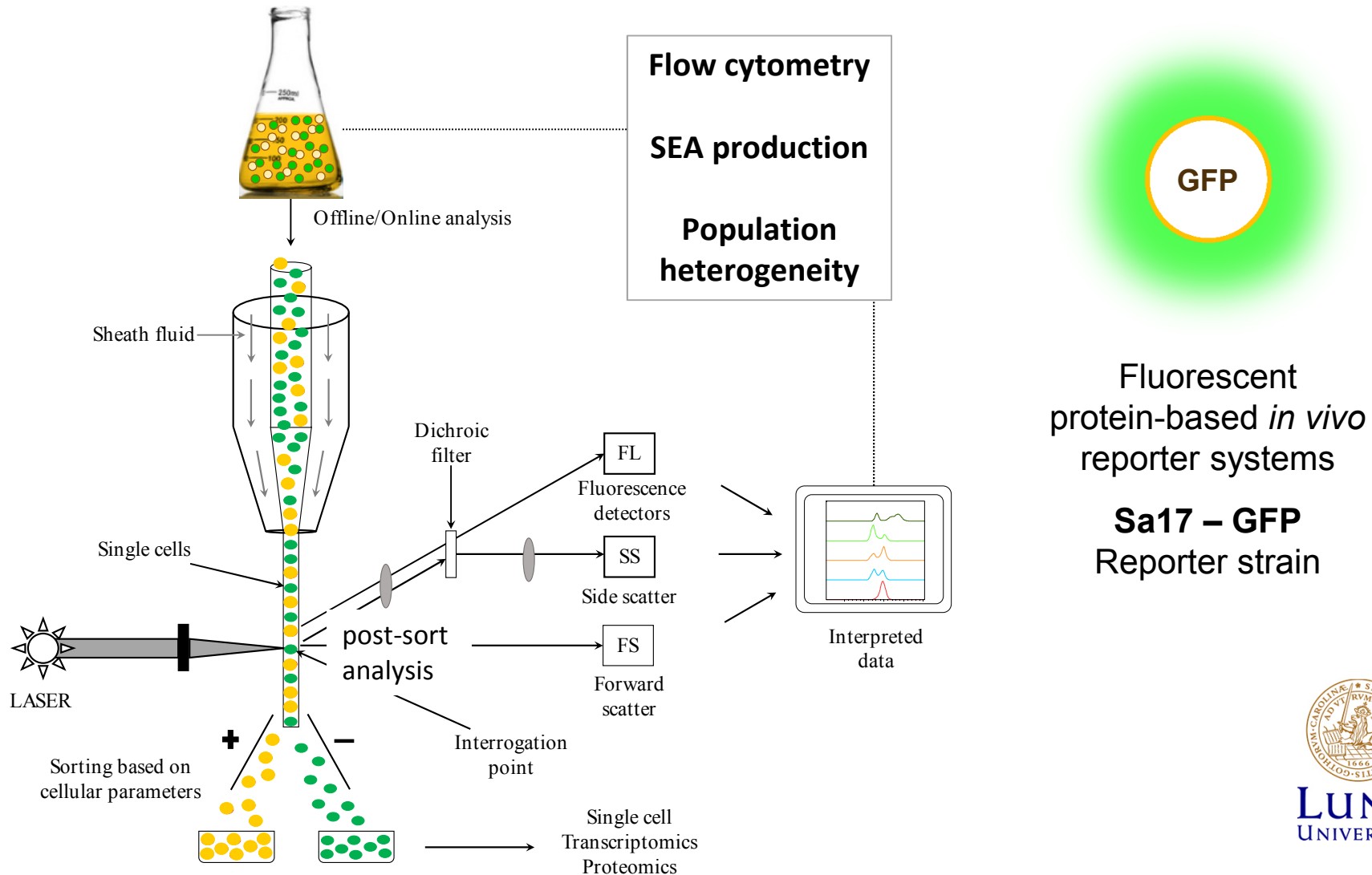
# SEA production in sausage



✓ Pattern as under **MC** induced lab conditions



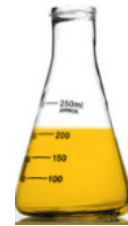
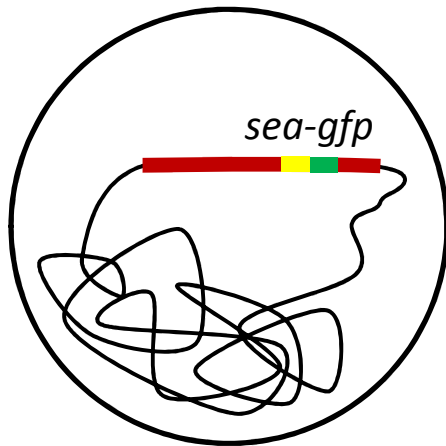
# Monitor SEA production in real-time



# Sa17- GFP SEA reporter strain

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*S. aureus* Sa17 - GFP

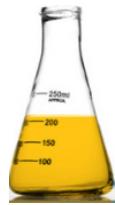


37 °C, BHI, 200 rpm

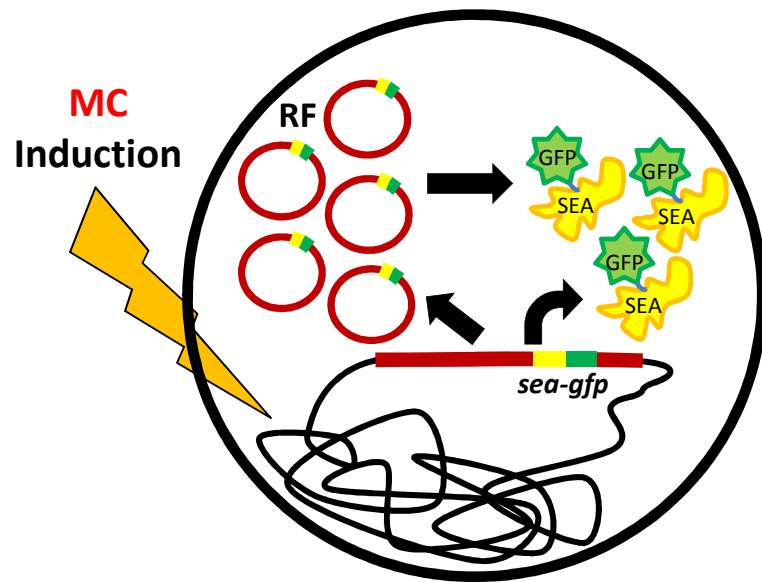
Strains	Mean $\mu$ max	Mean max OD <sub>620</sub>
Sa17 (n=3)	1.34 ± 0.05	17.95 ± 1.72
Sa17-GFP (n=8)	1.32 ± 0.08	17.53 ± 1.53



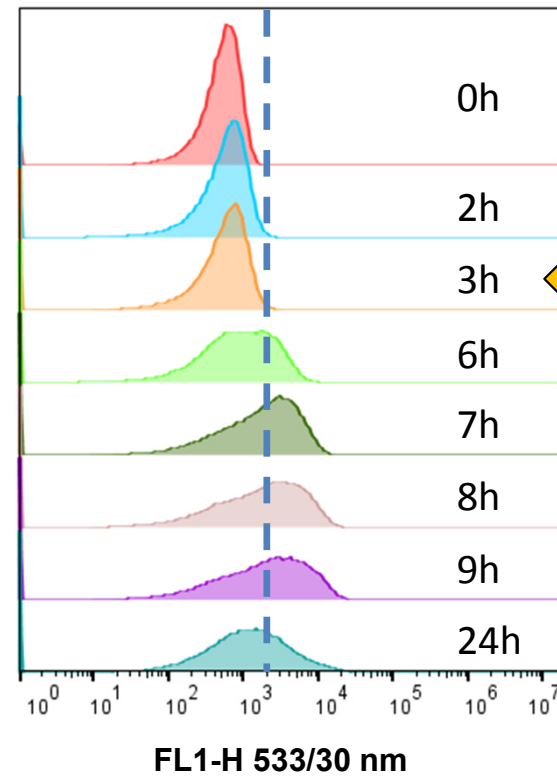
# Sa17- GFP production in real-time



37 °C, BHI + MC, 200 rpm



Fluorescence histogram  
Sa17-GFP population

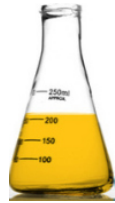


MC  
5µg/ml

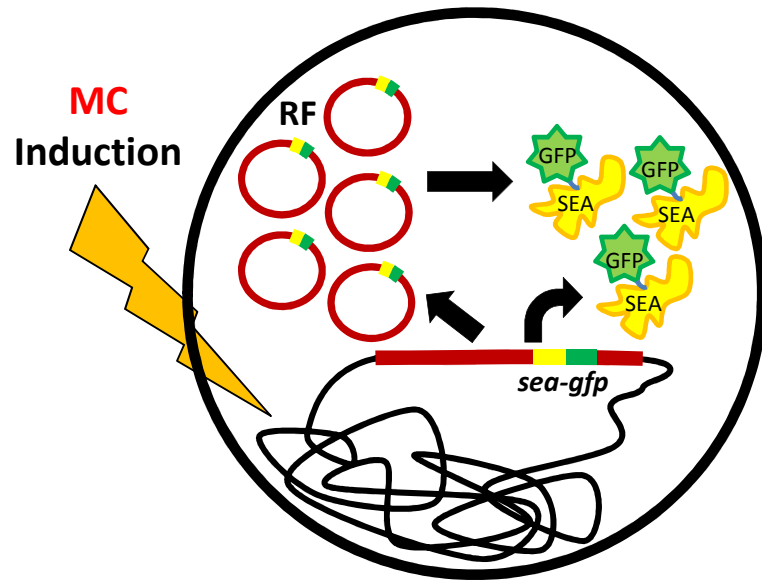


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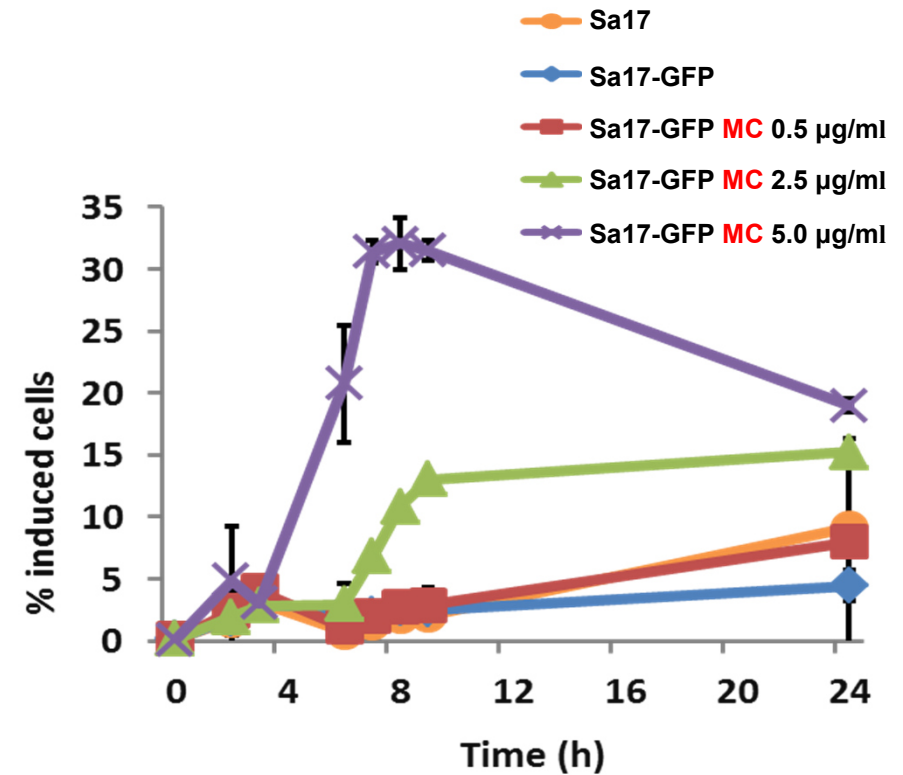
# Population heterogeneity



37 °C, BHI + MC, 200 rpm



Proportion (%) induced cells  
in a population



# Bovine mastitis – SEC and TSST

Original Analysis of Microarray data						
Pulsotype	Strain	Tst-1	A	B	C	D
e	MAS 660	1591	0,5		243	0,4
g	MAS 408				250	
j	MAS 262				409	
k	MAS 449	403			250	
o	MAS 409	1117			109	
q	MAS 024	11		1,27	174	0,4
q	MAS 602	974			160	
u	MAS 106	391		0,87	250	0,5
u	MAS 162	362			250	0,4
u	MAS 166				96	
u	MAS 209	440			250	0,6
u	MAS 247	564			211	
u	MAS 417				264	
v	MAS 015	1959		1,83	170	
v	MAS 023	2130	0,78	1,49	232	0,5
v	MAS 124	2485	0,46	1,5	212	
w	MAS 406	2396		1,5	219	0,3

## Wt strains:

- MAS 106 PT U
- MAS 602 PT Q
- MAS 660 PT E

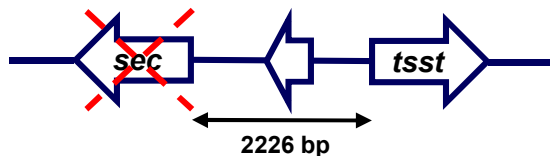


$\Delta sec$  &  $\Delta tst-1$   
deletion mutants

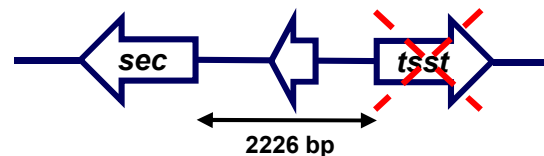
Infection model  
bovine mammary  
epithelial cells

Proteomics

## Deletion mutants:



- ✓ MAS 106 $\Delta sec$
- ✓ MAS 602 $\Delta sec$
- ✓ MAS 660 $\Delta sec$



- ✓ MAS 106 $\Delta tst-1$
- ✓ MAS 602 $\Delta tst-1$

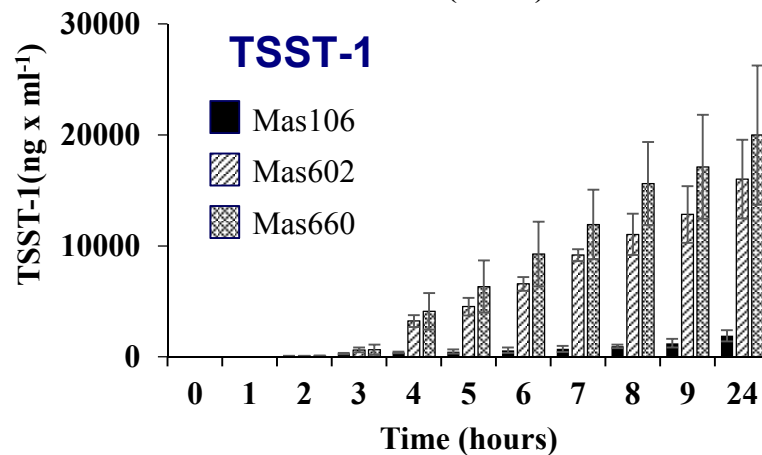
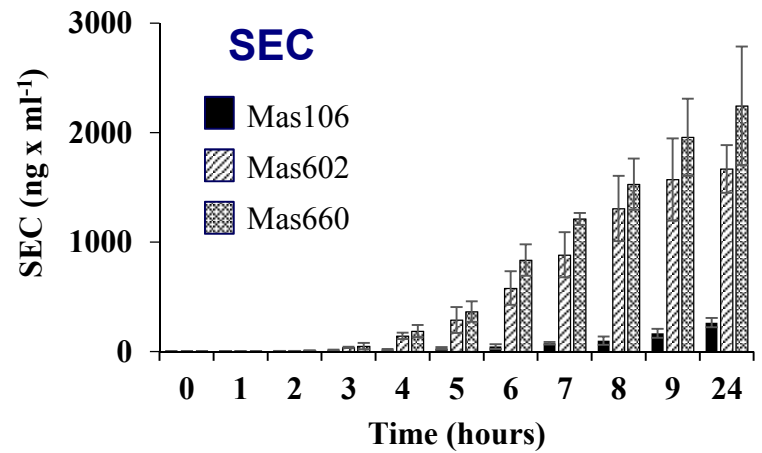


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# Strain characterization

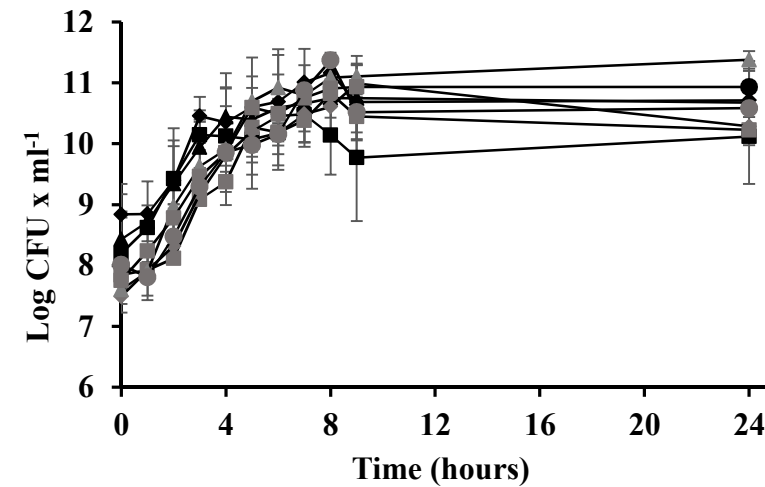
## Wt strains

→ different SEC & TSST production

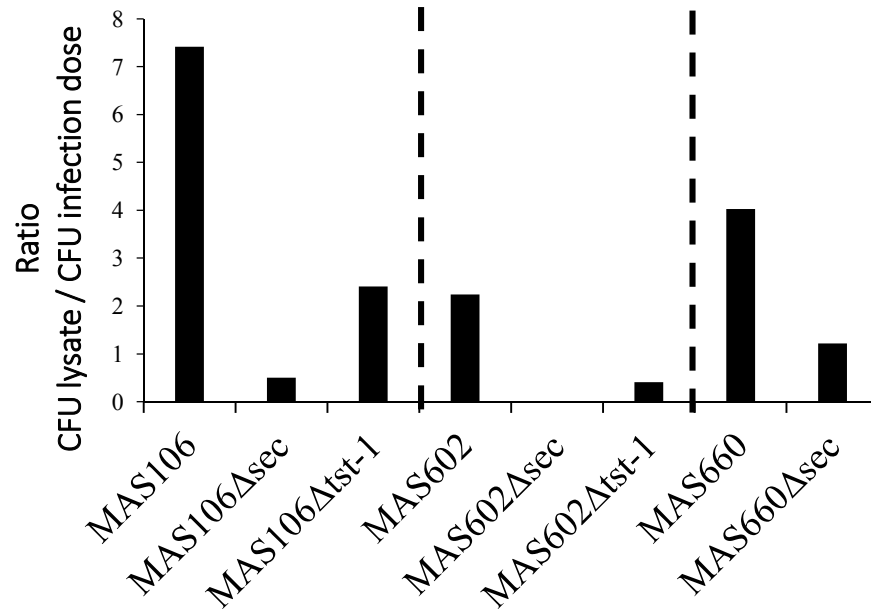
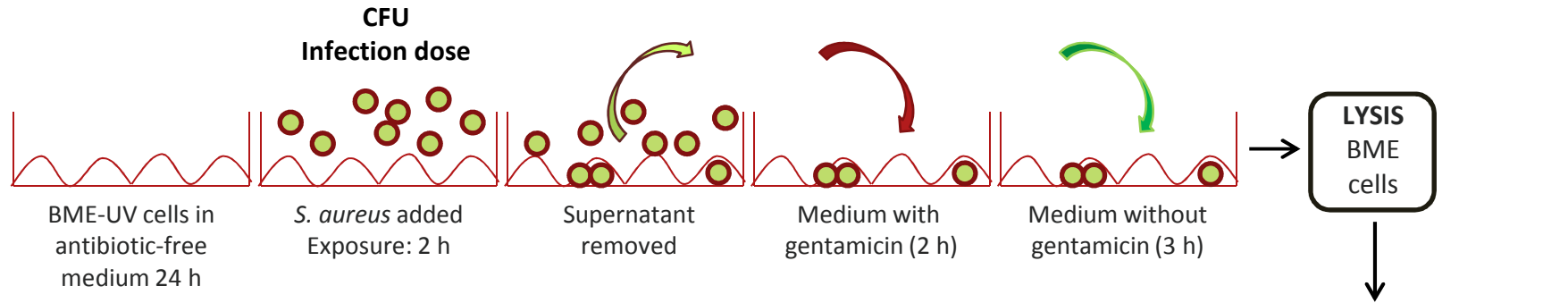


## Wt & mutants

→ similar growth



# Infection BME cells – $\Delta sec$ & $\Delta tst-1$

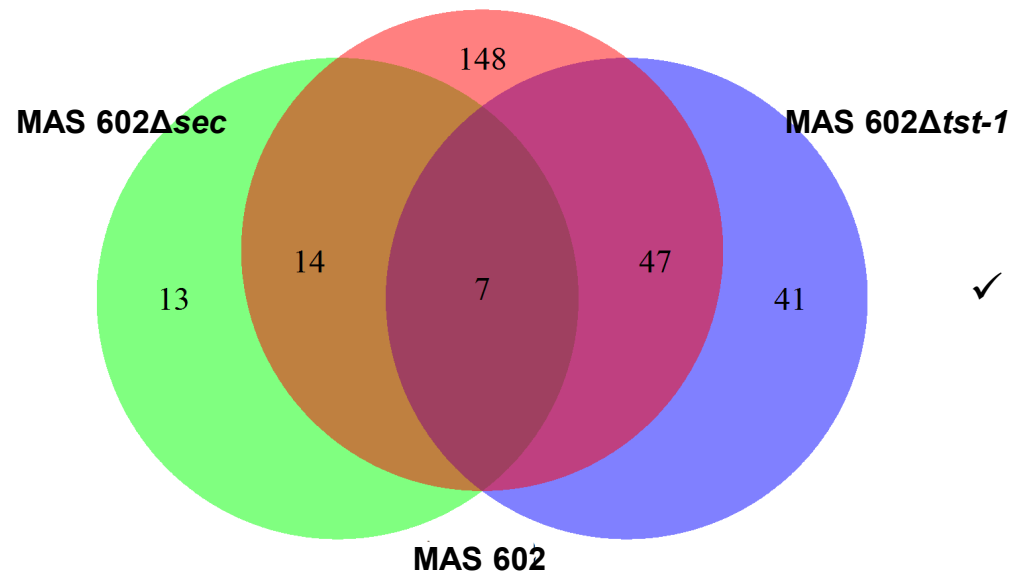
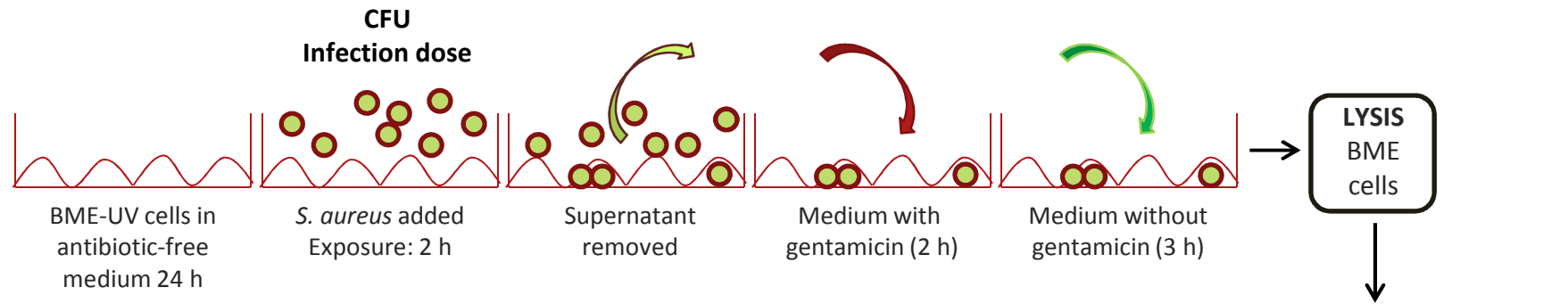


✓ Infection capability **lower** in deletion mutants



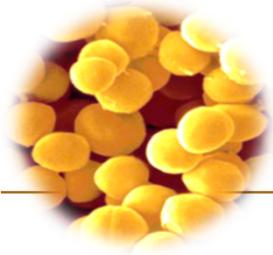


# Proteomics BME cells – MAS 602 $\Delta sec$ & $\Delta tst-1$



✓ Up- or down regulated proteins in BME-cells





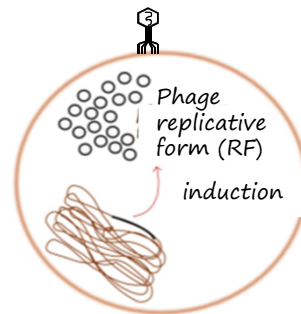
# Targeting *S. aureus* toxin production

- FOR IMPROVED FOOD SAFETY AND ANIMAL HEALTH



**Bovine mastitis:**

- Infection capability influenced by SEC & TSST
- Protein profiles in BME-cells altered



**Detection of SEA production:**

- Monitoring in real-time
- Population heterogeneity



GFP

***S. aureus* SEA:**

- Production is strain dependent
- Regulation linked to bacteriophage life cycle



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# Acknowledgement

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## **SEA-project:**

Food safety group  
Master students

<http://www.tmb.lth.se/>

## **GFP-project:**

Magnus Carlquist  
Claes von Wachenfeldt  
Jakob Engman  
LP3 - Lund Protein Production Platform

# Thank you!

## **Mastitis project:**

Karin Artursson  
Lihong Liu  
Jonas Bergquist  
SciLifeLab, Uppsala University



The Swedish Research Council Formas

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