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## Vésicules extracellulaires de *Staphylococcus aureus*. Composition et rôle en contexte mammite

Yves Le Loir

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Yves Le Loir. Vésicules extracellulaires de *Staphylococcus aureus*. Composition et rôle en contexte mammite. 1ère Rencontre Op+Lait-INRA, Nov 2019, Saint Hyacinthe, Canada. hal-02390195

**HAL Id: hal-02390195**

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Submitted on 5 Jun 2020

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# Science and Technology of Milk and Eggs (STLO)

INRA-AGROCAMPUS Ouest

Yves Le Loir



[www.rennes.inra.fr/stlo](http://www.rennes.inra.fr/stlo)

# Milk & Egg Science and Technology



**Departments: MICA, CEPIA, P3AN  
Carnot Institute « Qualiment » (since 2016)**

**130-140 persons**

**83 tenure staff**

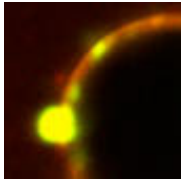
(39 researchers and professors;  
44 technicians and engineers)

**~25 Docs and Post-Docs / year**

**~15 Students (Master)**

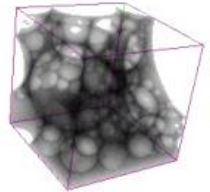
**16 members of R&D dept (private sector)**

# STLO: main research areas



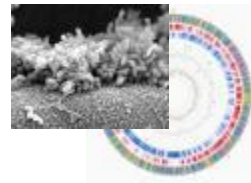
Increase the knowledge on **molecular and supramolecular structure** of milk and egg components

Understand protein-protein, protein-lipid, protein-mineral **interactions** leading to expected technological and bioactivity **functionalities** and understand their **digestion**

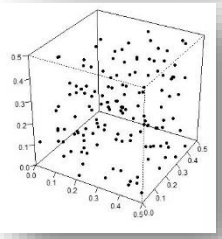
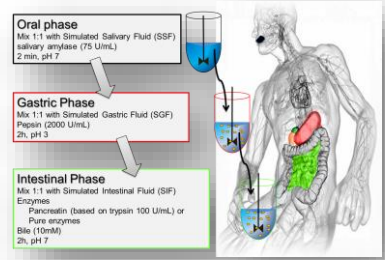


Analyze **transfers** during **technological processes**

Characterize **interactions between bacterial communities/environment** in relationship with product quality, food safety and probiotic activity



# Organization



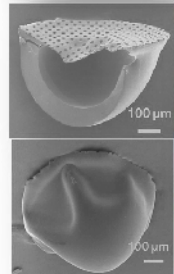
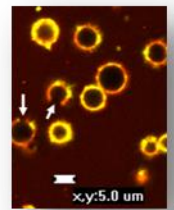
**Vice directors** **Director**

**MICROBIO** **ISF** **BN**

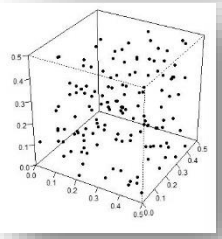
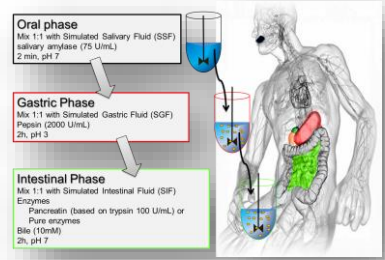
**PSM** **SMCF**

**Biological Resource Center CIRM-BIA** **Dairy Platform PFL**


**SAPHIRE**  
**Administration, Informatics & Infrastructure, Doc & Com**





# Organization




**Vice directors**      **Director**


  
**MICROBIO**


  
**ISF**


  
**BM**


**2 transverse axes:**  
 - optimisation of infant formulas  
 - animal-plant mix

  
**Biological Resources Center**  
**CIRM-BIA**

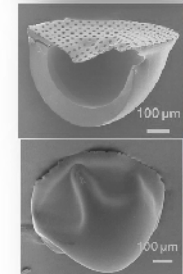
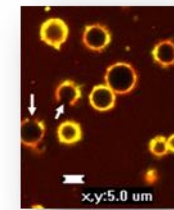
  
**SMCF**

  
**Dairy Platform**  
**PFL**

  
**PFL**



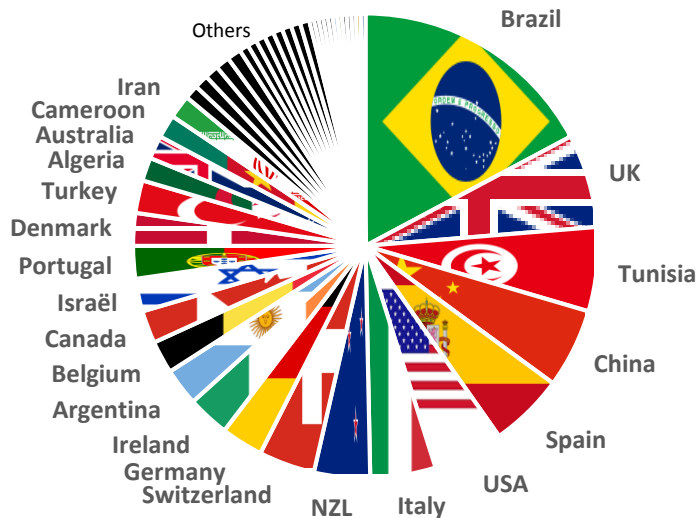
**SAPHIRE**  
**Administration,**  
**Informatics & Infrastructure, Doc & Com**



# PRODUCTION & VALORISATION (2014-2018)

376 papers in peer-reviewed journals  
(74% in Q1)

- 88 % with partners outside STLO
- 48 % with foreign partners



104 papers dedicated to dissemination towards professionals and large public.

- 86 Book chapters
- 9 Book editions

- 654 Conferences (514 with proceedings)
- 203 Posters

- 5 patents incl. 2 softwares
- SD<sup>2</sup>P<sup>®</sup> (31 licences), 8 countries
- Milk Salt<sup>®</sup> (7 licences)

# International Collaborations (2014-2018)

## Intensified

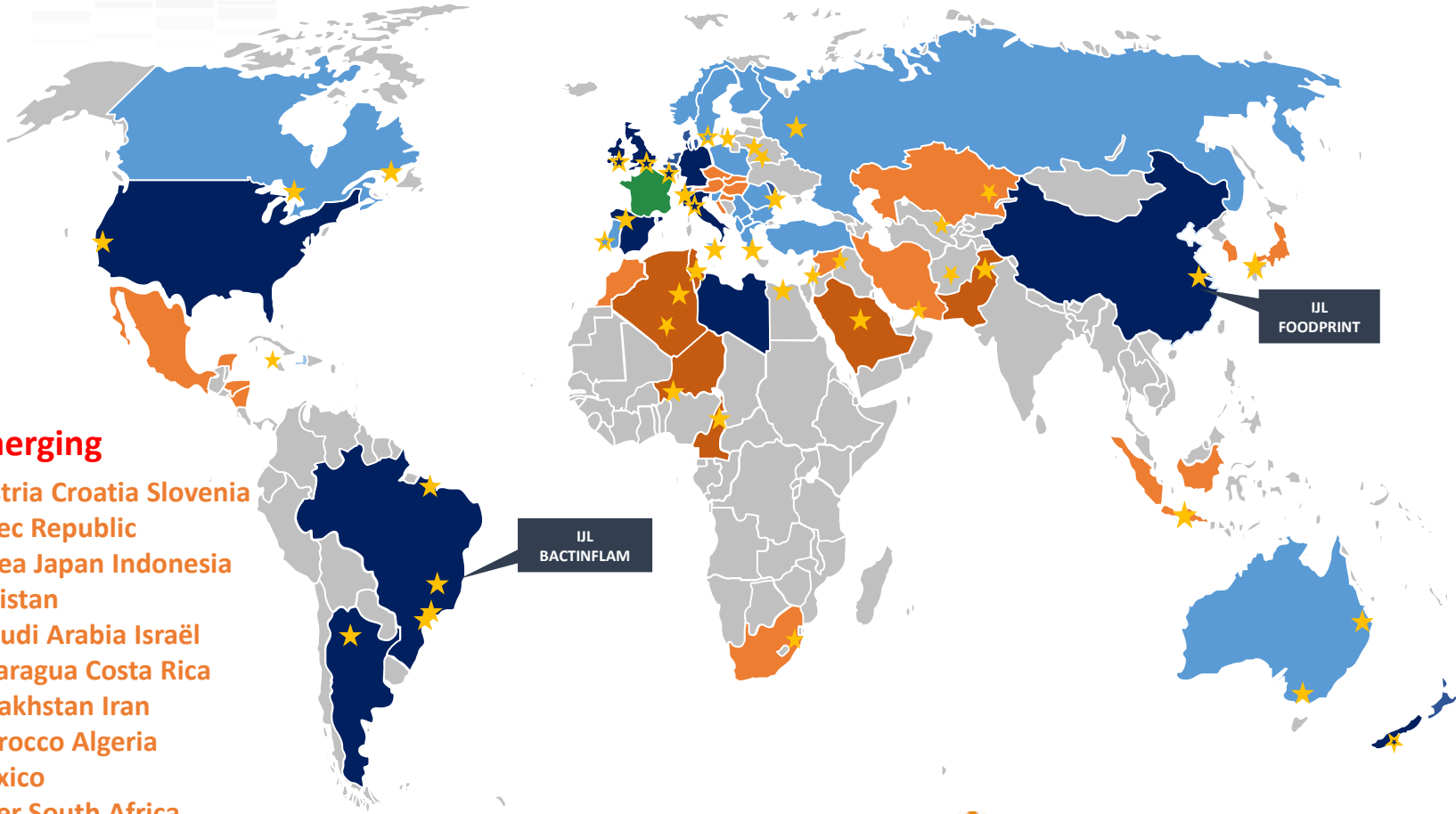
- Brazil
- Great Britain
- Tunisia
- China
- Spain
- USA
- New-Zeland
- Switzerland
- Italy
- Germany
- Argentina
- Ireland

## Ongoing

- Canada
- Australia
- Turkey
- Denmark

## Emerging

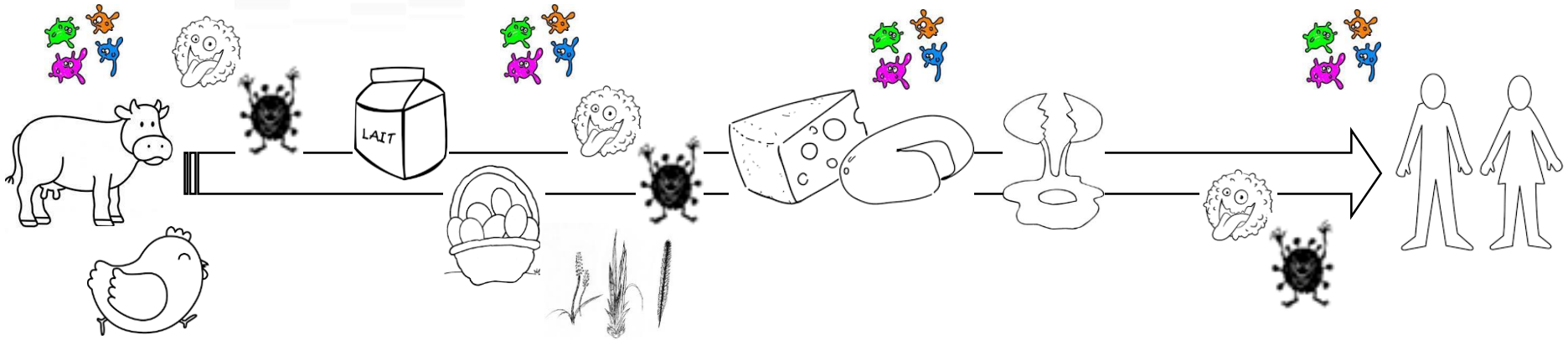
- Austria Croatia Slovenia
- Tchec Republic
- Corea Japan Indonesia
- Pakistan
- Saoudi Arabia Israël
- Nicaragua Costa Rica
- Kasakhstan Iran
- Morocco Algeria
- Mexico
- Niger South Africa
- Cameroon



International Research Network **INFOGEST**



# Microbio team



**Understand the way bacterial communities work to control and use their potential towards target functionalities**

## Animal Health



Offer alternatives to antibiotic treatments

## Sustainability, food safety and quality



Develop safe, healthy, sustainable and hedonic fermented foods  
Reduce the safety and spoiling risks

## Health and wellbeing



Develop functional food against modern lifestyle diseases



# Vésicules extracellulaires de *Staphylococcus aureus*. Composition et rôle en contexte mammite



Natayme R. Tartaglia

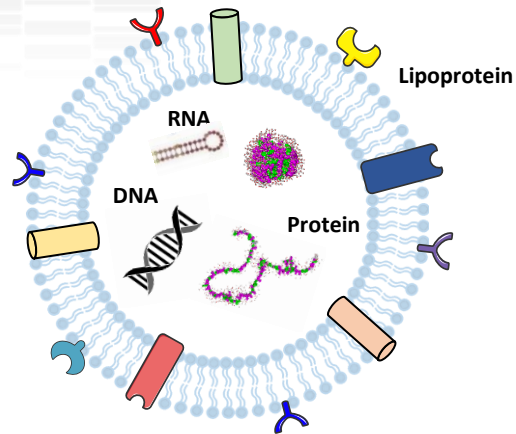


UMR1253 STLO

Science et Technologie du Lait et de l'Oeuf  
INRA Bretagne-Normandie

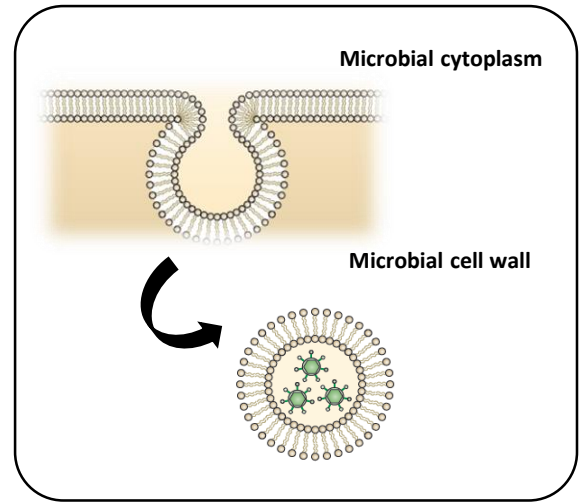
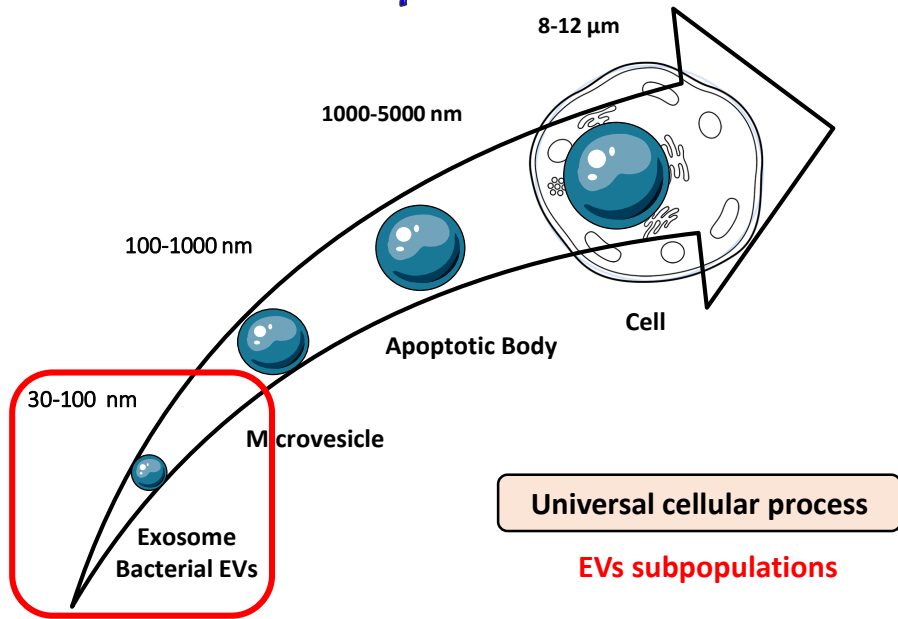


# Extracellular Vesicles



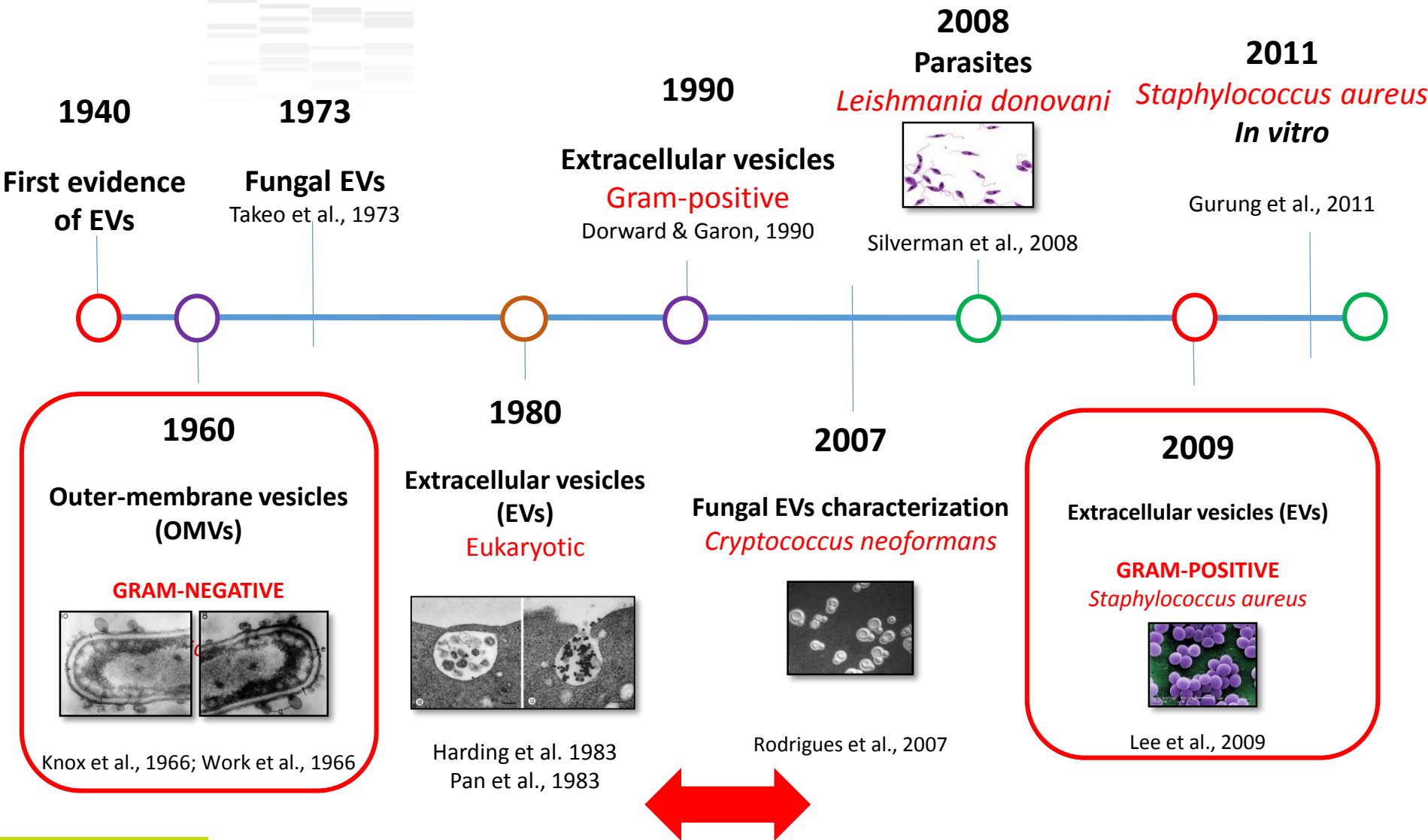
- ✓ Nano-sized particles
- ✓ Phospholipid bilayer

Proteins  
Lipids  
DNA  
RNA

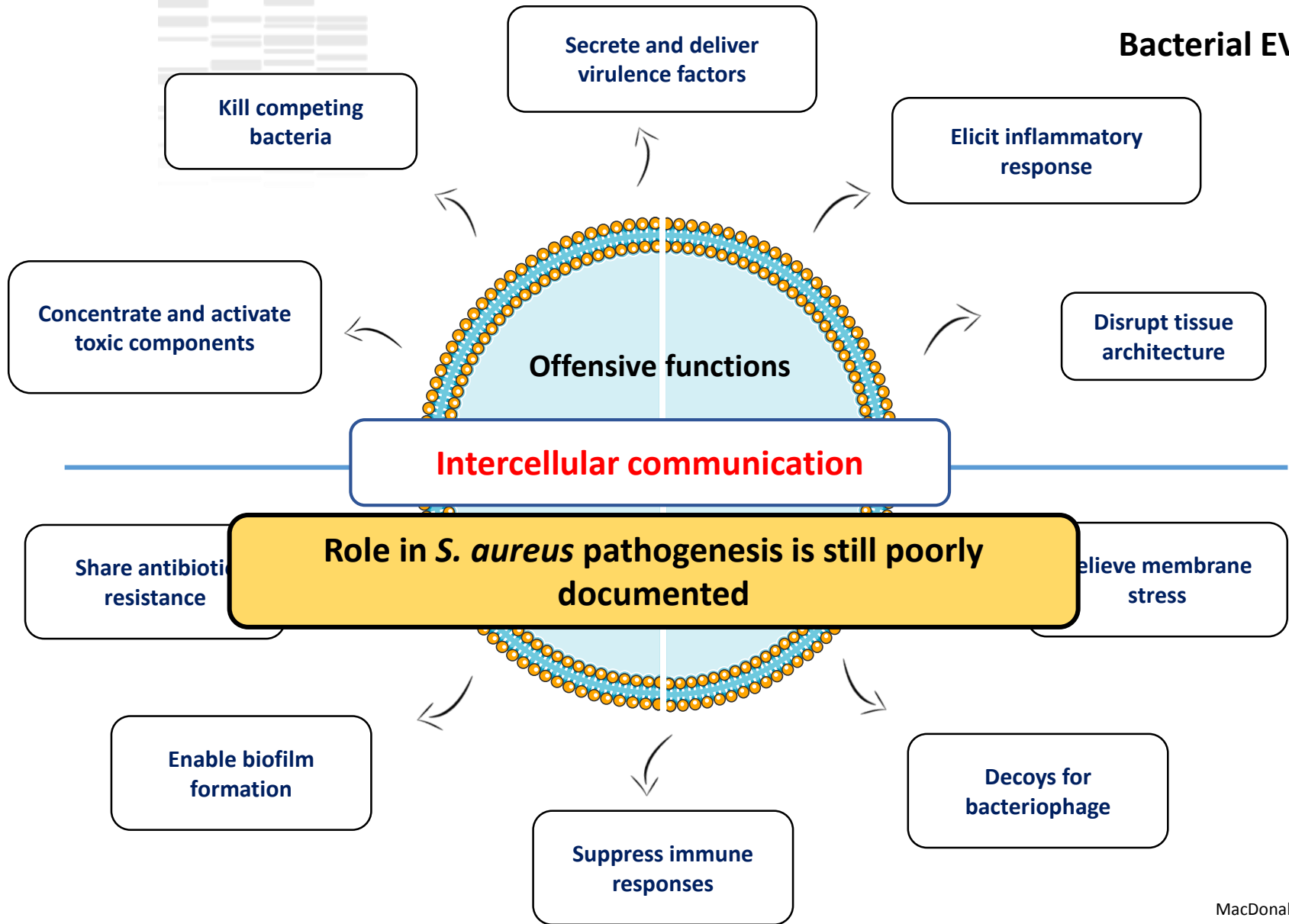


Brown et al., 2015; Lee et al., 2009

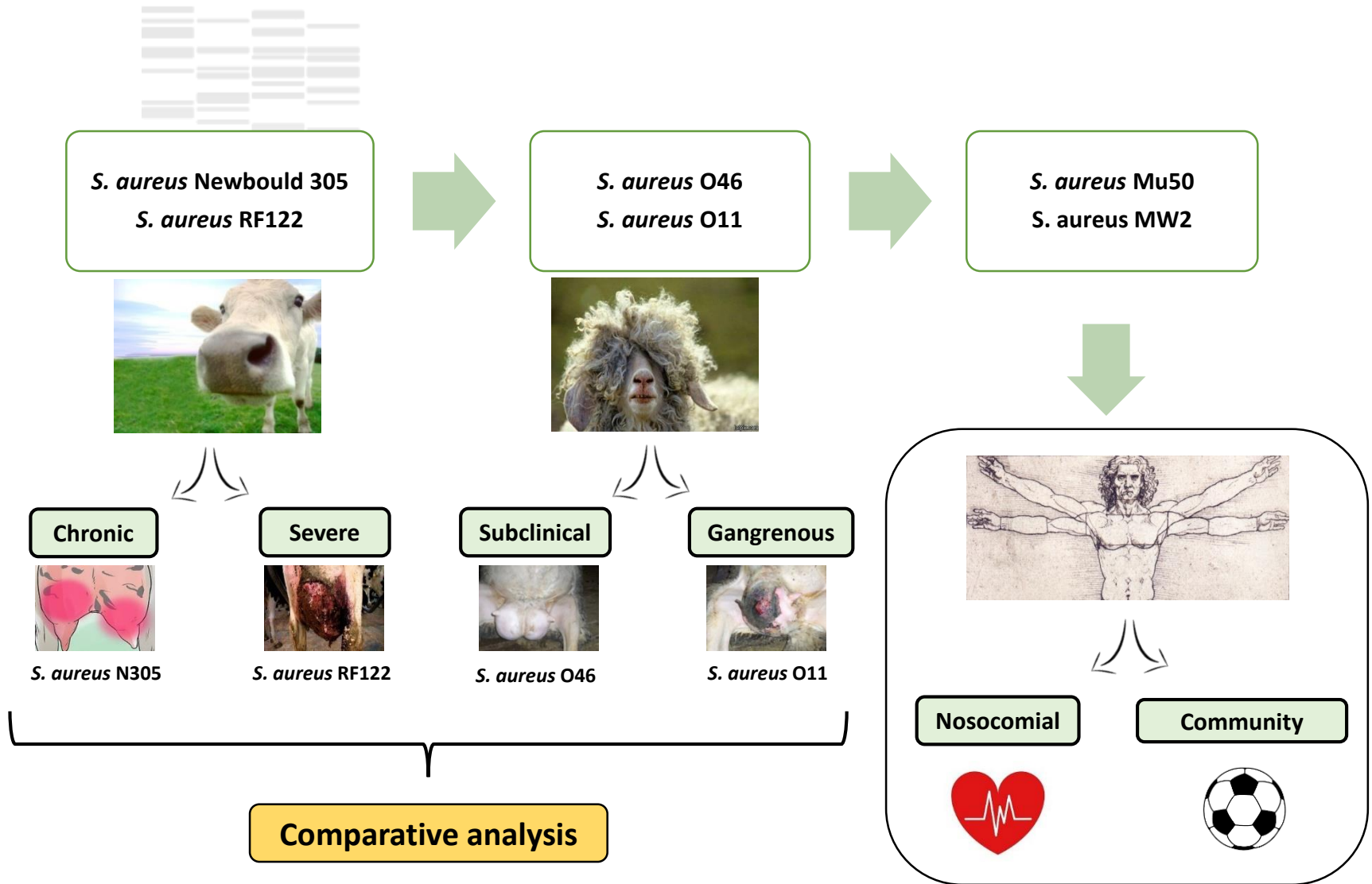
# Extracellular vesicle discovery timeline



# Bacterial EVs



MacDonald et al., 2012



Bouchard *et al.*, 2012; Prasad and Newbould, 1968; Herron *et al.*, 2002; Herron-Olson *et al.*, 2007  
 Le Maréchal *et al.*, 2011; Vautor *et al.*, 2009; Kuroda *et al.*, 2001; Baba *et al.*, 2002

# EVs characterization



*S. aureus* N305  
*S. aureus* RF122

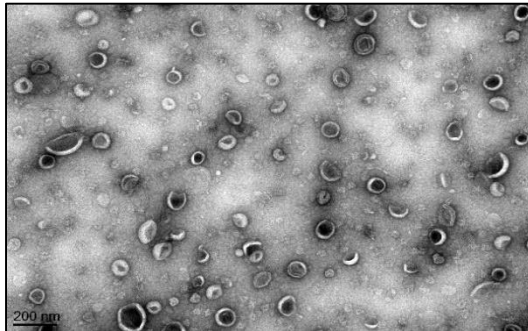


*S. aureus* O46  
*S. aureus* O11

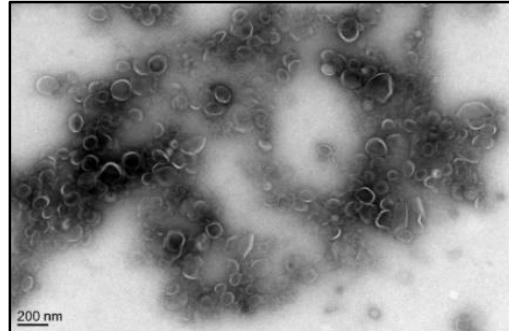


*S. aureus* Mu50  
*S. aureus* MW2

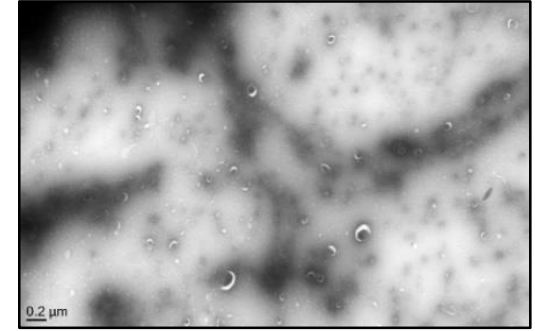
*S. aureus* N305



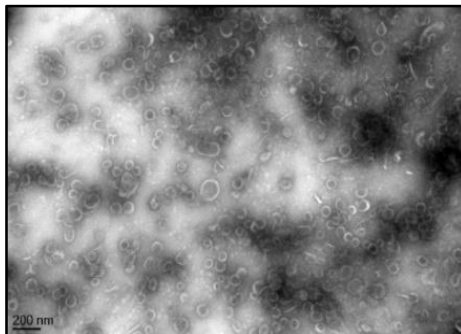
*S. aureus* O46



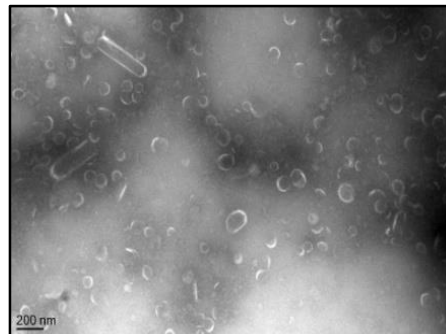
*S. aureus* MW2



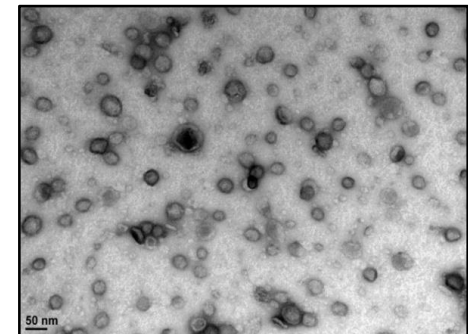
*S. aureus* RF122



*S. aureus* O11



*S. aureus* Mu50



**Bovine**

**Ovine**

**Human**

# EVs characterization



*S. aureus* N305  
*S. aureus* RF122

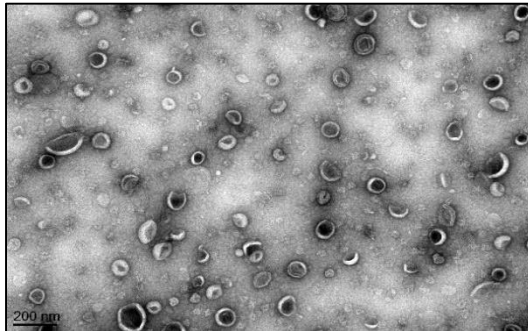


*S. aureus* O46  
*S. aureus* O11

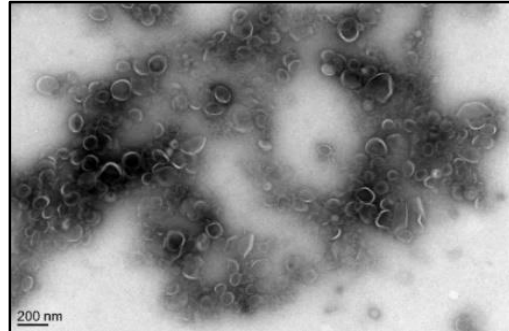


*S. aureus* Mu50  
*S. aureus* MW2

*S. aureus* N305



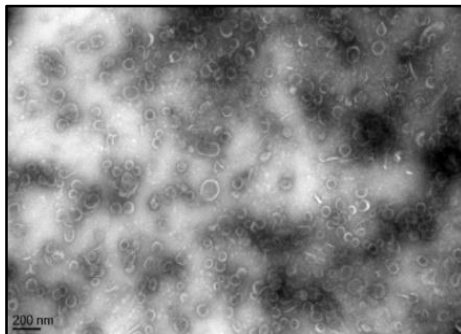
*S. aureus* O46



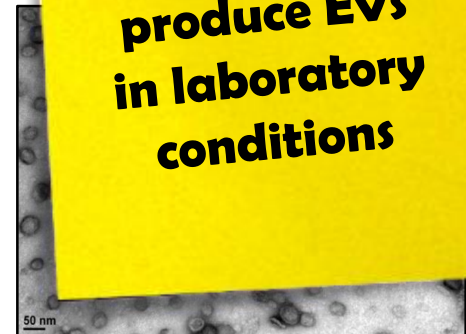
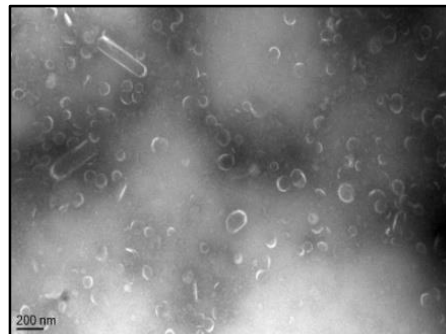
*S. aureus* MW2



*S. aureus* RF122



*S. aureus* O11



**Bovine**

**Ovine**

**Human**



261 proteins identified

## Distribution of COGs



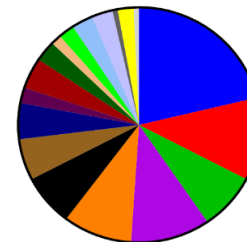
*S. aureus* N305  
*S. aureus* RF122



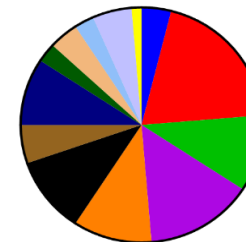
*S. aureus* O46  
*S. aureus* O11



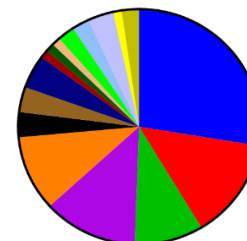
*S. aureus* Mu50  
*S. aureus* MW2



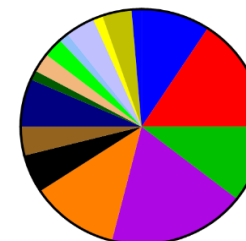
*S. aureus* N305



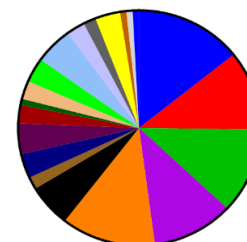
*S. aureus* RF122



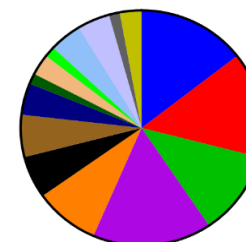
*S. aureus* O46



*S. aureus* O11



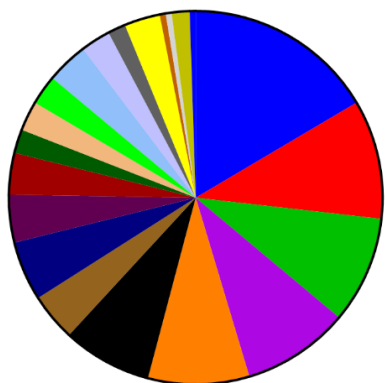
*S. aureus* Mu50



*S. aureus* MW2



- Translation, ribosomal structure and biogenesis
- Energy production and conversion
- Cell wall/membrane/envelope biogenesis
- Inorganic ion transport and metabolism
- Carbohydrate transport and metabolism
- Function unknown
- Amino acid transport and metabolism
- Posttranslational modification, protein turnover, chaperones
- Lipid transport and metabolism
- Coenzyme transport and metabolism
- General function prediction only
- Defense mechanisms
- Transcription
- Signal transduction mechanisms
- Intracellular trafficking, secretion, and vesicular transport
- Replication, recombination and repair
- Cell cycle control, cell division, chromosome partitioning
- Nucleotide transport and metabolism
- Secondary metabolites biosynthesis, transport and catabolism
- Mobilome: prophages, transposons
- Cell motility



261 proteins identified

## Distribution of COGs



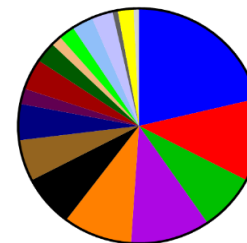
*S. aureus* N305  
*S. aureus* RF122



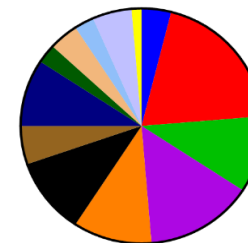
*S. aureus* O46  
*S. aureus* O11



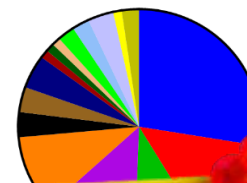
*S. aureus* Mu50  
*S. aureus* MW2



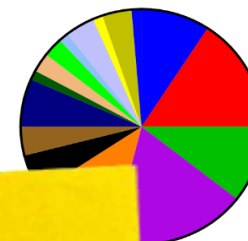
*S. aureus* N305



*S. aureus* RF122



*S. aureus* O11

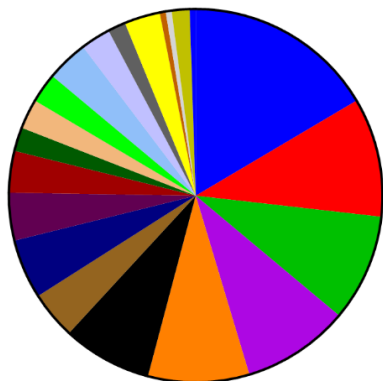


**COGs distribution varies from a strain to another**

*S. aureus* Mu50

*S. aureus* MW2

- Translation, ribosomal structure and biogenesis
- Energy production and conversion
- Cell wall/membrane/envelope biogenesis
- Inorganic ion transport and metabolism
- Carbohydrate transport and metabolism
- Function unknown
- Amino acid transport and metabolism
- Posttranslational modification, protein turnover, chaperones
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- Defense mechanisms
- Transcription
- Signal transduction mechanisms
- Intracellular trafficking, secretion, and vesicular transport
- Replication, recombination and repair
- Cell cycle control, cell division, chromosome partitioning
- Nucleotide transport and metabolism
- Secondary metabolites biosynthesis, transport and catabolism
- Mobilome: prophages, transposons
- Cell motility



261 proteins identified

## Distribution of COGs



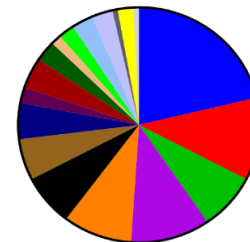
*S. aureus* N305  
*S. aureus* RF122



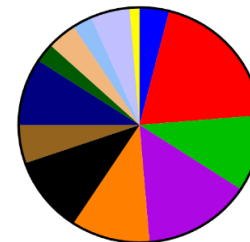
*S. aureus* O46  
*S. aureus* O11



*S. aureus* Mu50  
*S. aureus* MW2

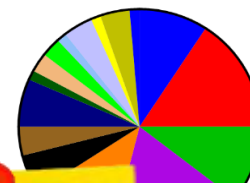
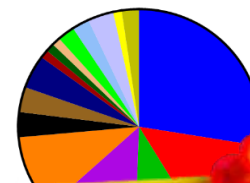
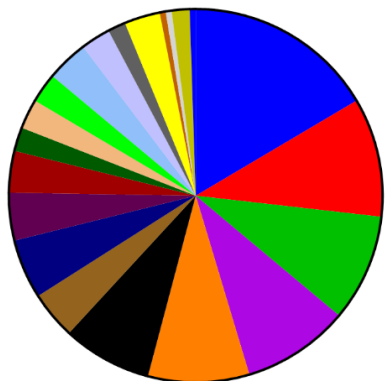


*S. aureus* N305



*S. aureus* RF122

- Translation, ribosomal structure and biogenesis
- Energy production and conversion
- Cell wall/membrane/envelope biogenesis
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- Carbohydrate transport and metabolism
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- Lipid transport and metabolism
- Coenzyme transport and metabolism
- General function prediction only
- Defense mechanisms
- Transcription
- Signal transduction mechanisms
- Intracellular trafficking, secretion, and vesicular transport
- Replication, recombination and repair
- Cell cycle control, cell division, chromosome partitioning
- Nucleotide transport and metabolism
- Secondary metabolites biosynthesis, transport and catabolism
- Mobilome: prophages, transposons
- Cell motility



O11



**No correlation with host origin**

*S. aureus* Mu50

*S. aureus* MW2

# Do EVs induce an effect *in vivo*?

**Mating**

2 weeks

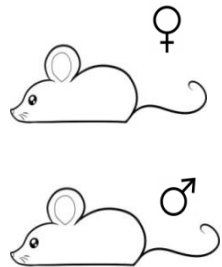
1 week

12 days

24 hours

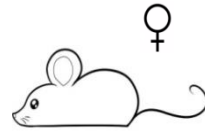
**Sacrifice**

**Dissection**

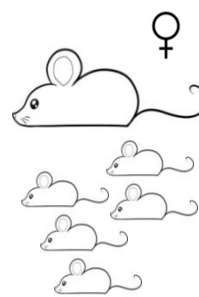


CD-1

Female and male mice



Isolated



Intraductal inoculation

40 CD-1

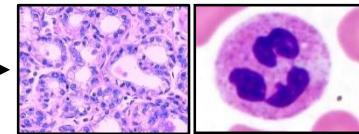
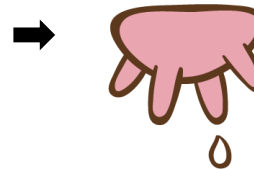
**Control groups**

- ✓ PBS
- ✓ *S. aureus* N305 (117 CFU)
- ✓ *S. aureus* N305 HK (100 CFU)
- ✓ LTA (10µg)

**Experimental groups**

- ✓ EVs 1µg
- ✓ EVs 10µg

Mammary tissue



Hematoxylin and eosin stain



Cytokines assay

# Do EVs induce an effect *in vivo*?



Negative control

Positive controls

Tests

PBS

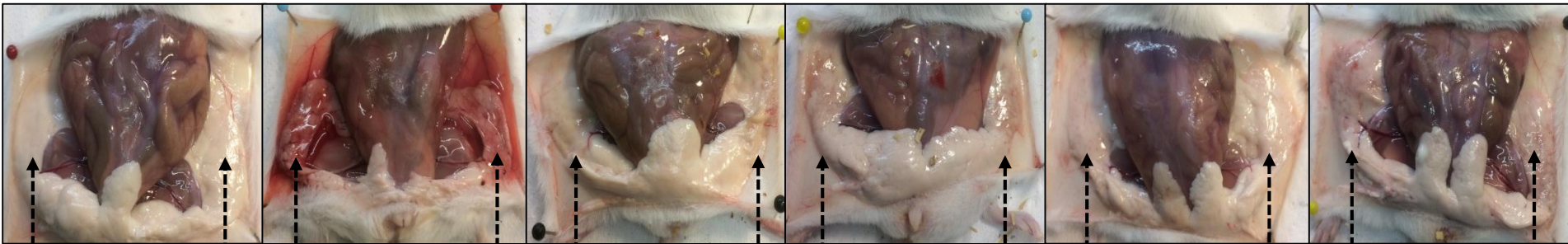
Living N305

HK N305

LTA

EVs 1  $\mu$ g

EVs 10  $\mu$ g



Healthy mammary glands

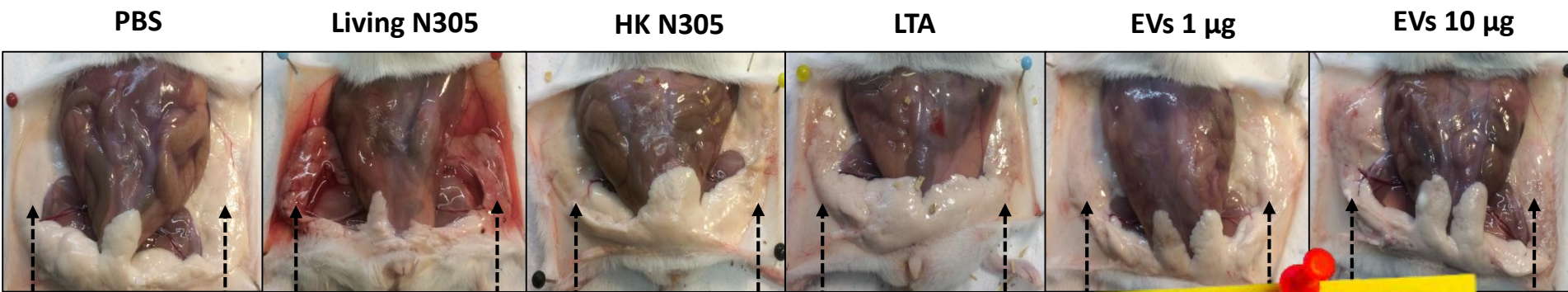
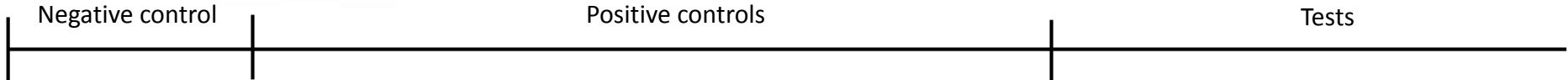
Severe lesion (hemorrhage)

Moderate lesions

Moderate lesions



# Do EVs induce an effect *in vivo*?



Healthy mammary glands

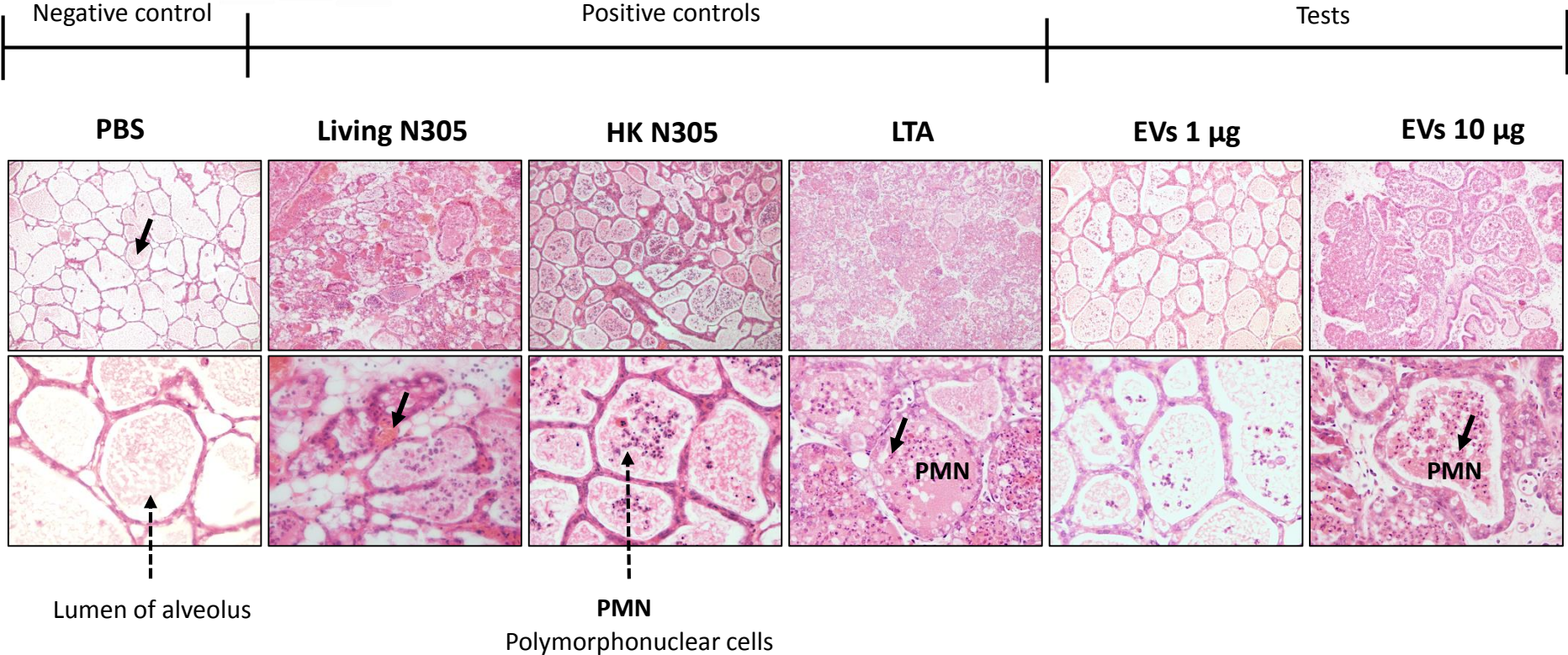
Severe lesion (hemorrhage)

Moderate lesions

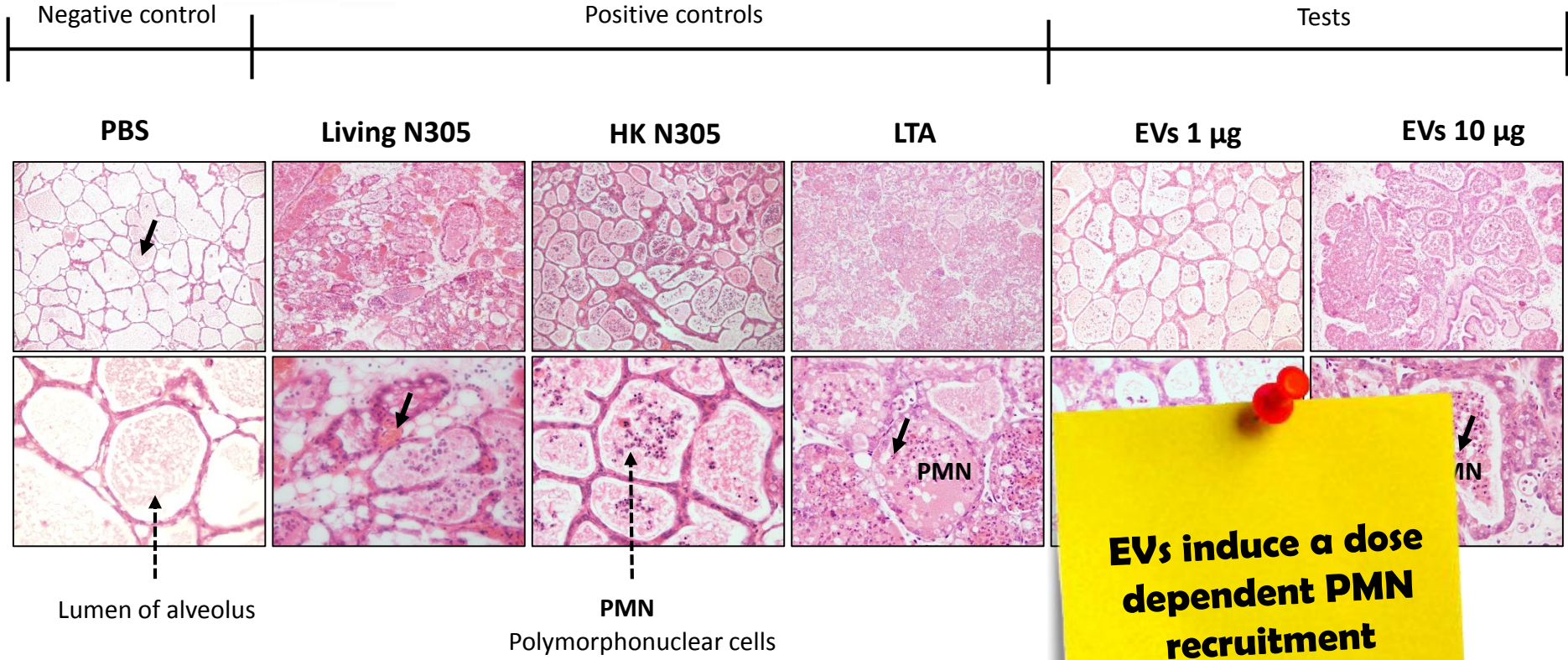
**A dose-dependent inflammation of mammary glands induced by EVs**



Do EVs induce an effect *in vivo*?



# Do EVs induce an effect *in vivo*?

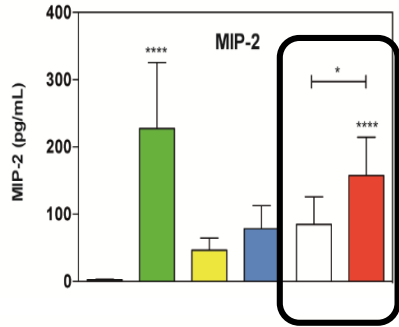


**EVs induce a dose dependent PMN recruitment**

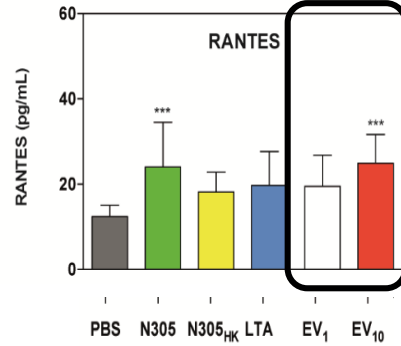
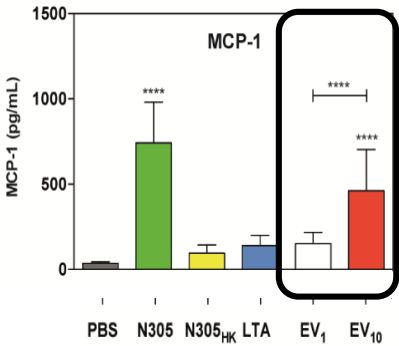
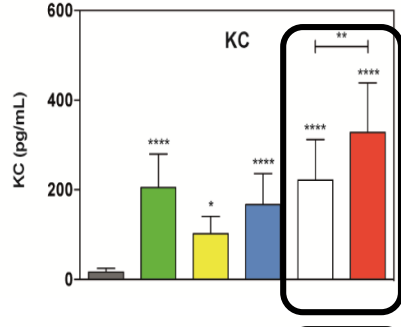


PBS
  N305
  N305<sub>HK</sub>
 LTA
  EV<sub>1</sub>
 EV<sub>10</sub>

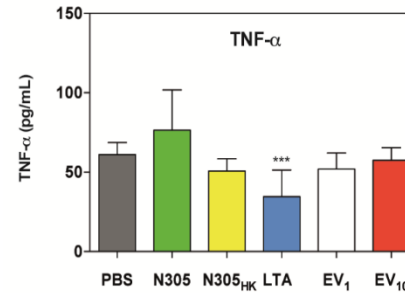
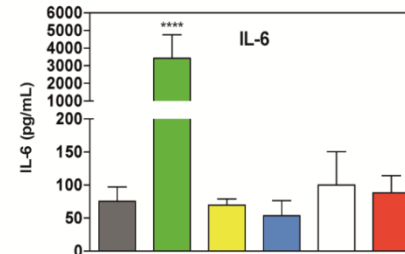
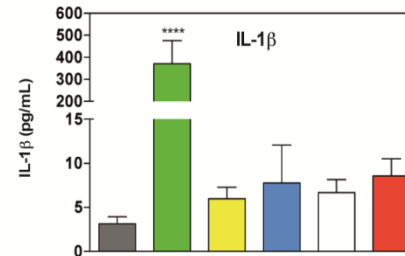
### Macrophage inflammatory protein 2



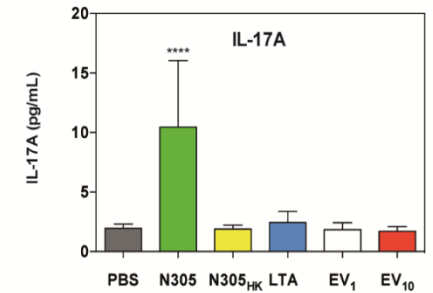
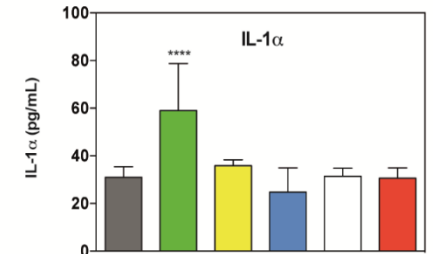
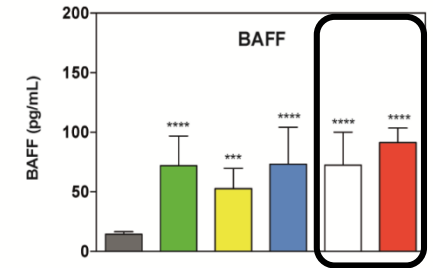
### Keratinocyte chemoattractant



### Monocyte chemoattractant protein 1

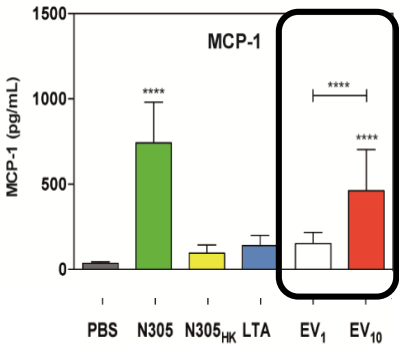
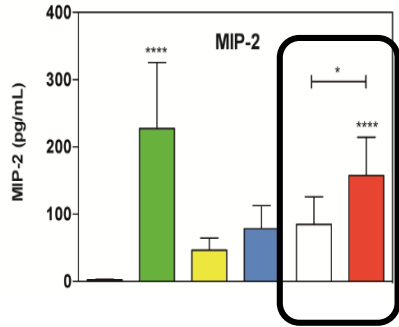


### B-cell activating factor



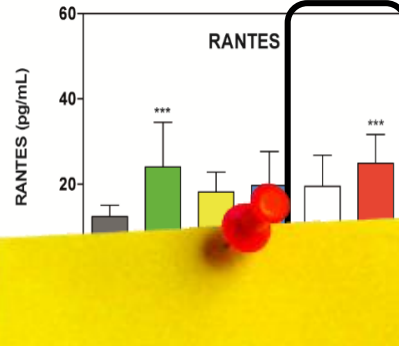
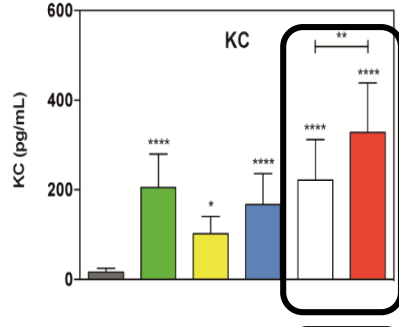
PBS
  N305
  N305<sub>HK</sub>
 LTA
  EV<sub>1</sub>
 EV<sub>10</sub>

### Macrophage inflammatory protein 2

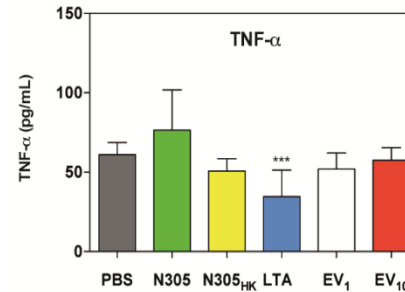
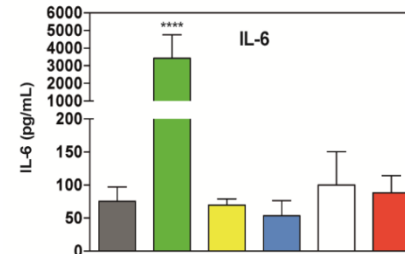
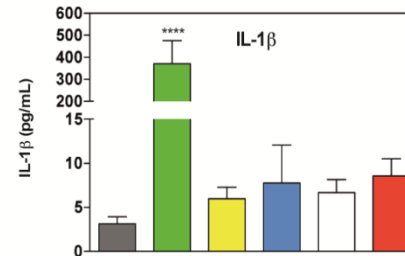


### Monocyte chemoattractant protein 1

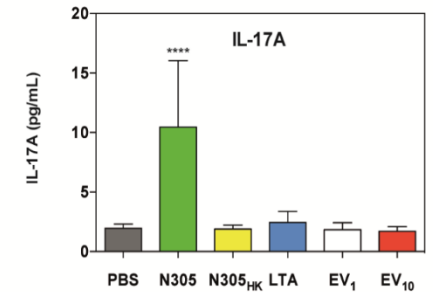
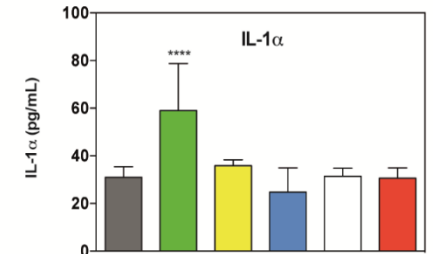
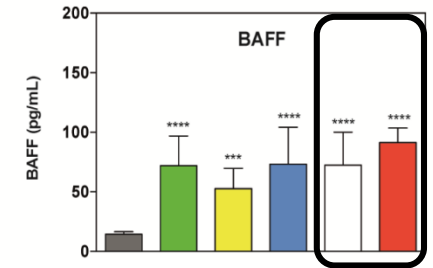
### Keratinocyte chemoattractant



**Dose-dependant induction of chemokines**

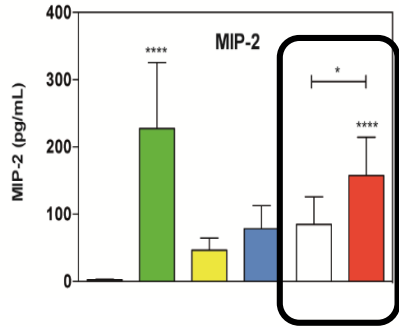


### B-cell activating factor

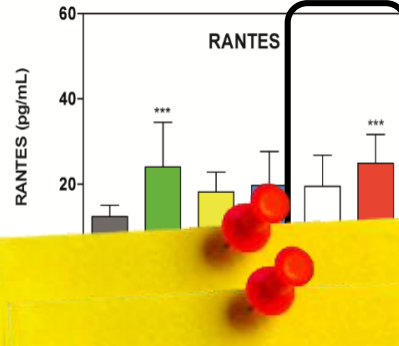
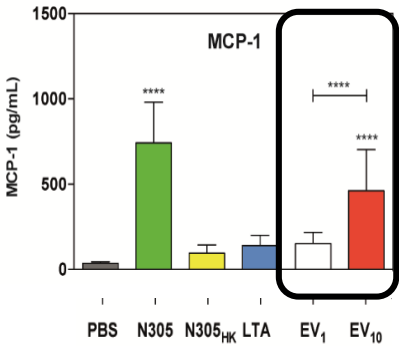
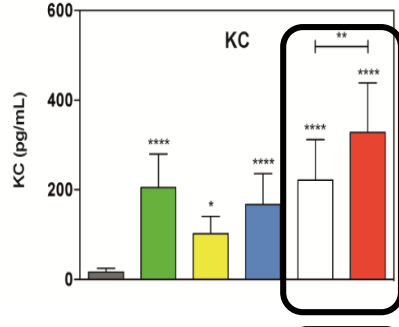


PBS
  N305
  N305<sub>HK</sub>
 LTA
  EV<sub>1</sub>
 EV<sub>10</sub>

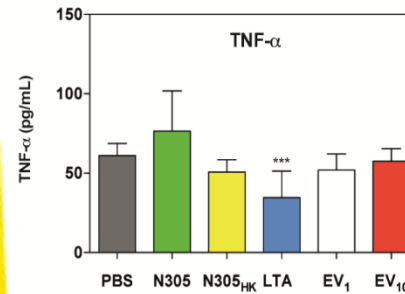
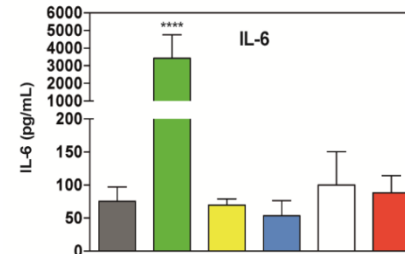
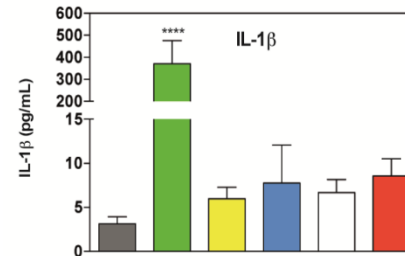
### Macrophage inflammatory protein 2



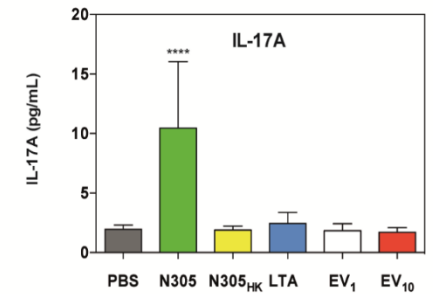
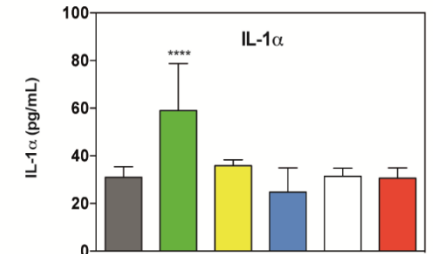
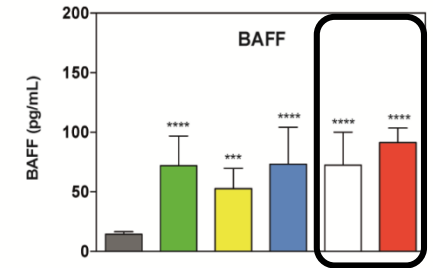
### Keratinocyte chemoattractant



### Monocyte chemoattractant protein 1



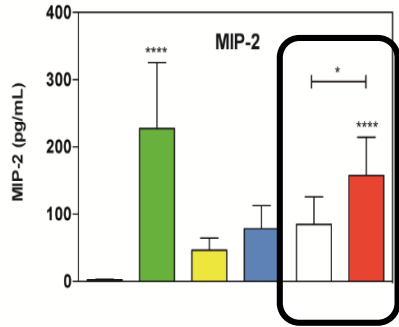
### B-cell activating factor



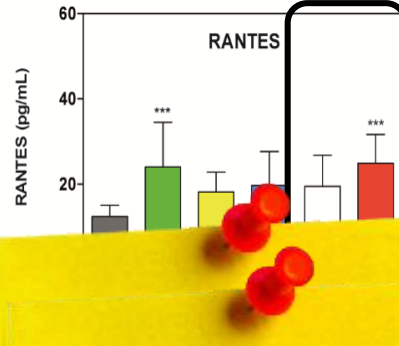
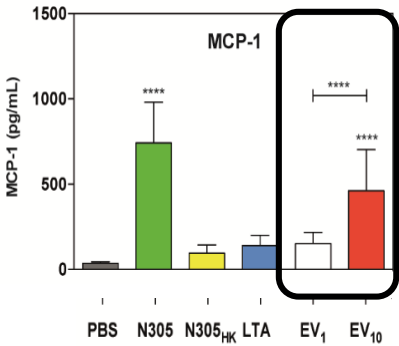
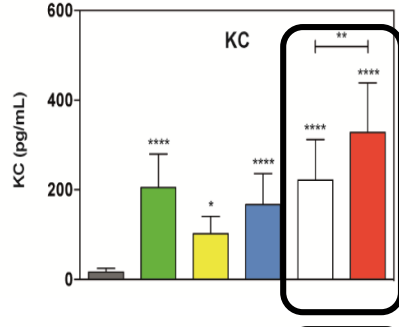
Chemoattractant activity

PBS
  N305
  N305<sub>HK</sub>
 LTA
  EV<sub>1</sub>
 EV<sub>10</sub>

### Macrophage inflammatory protein 2



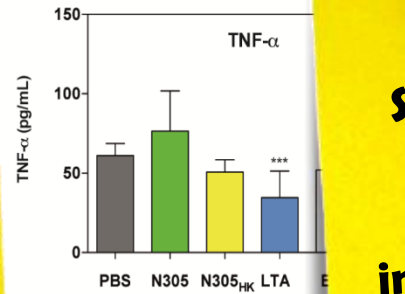
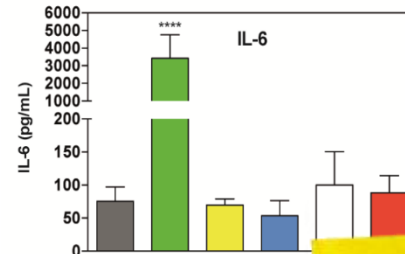
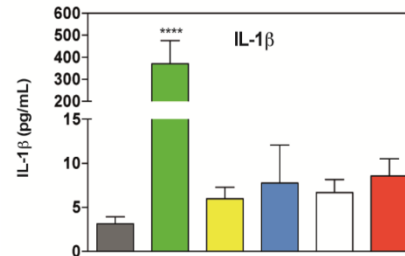
### Keratinocyte chemoattractant



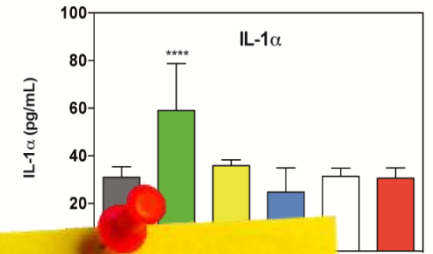
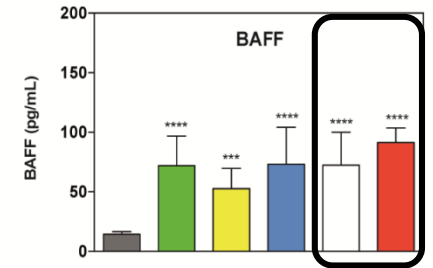
### Monocyte chemoattractant protein 1



**Chemoattractant activity**



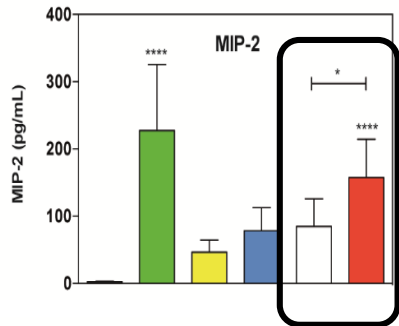
### B-cell activating factor



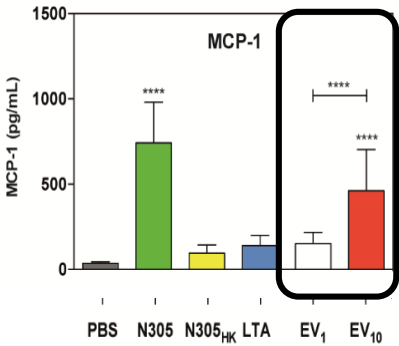
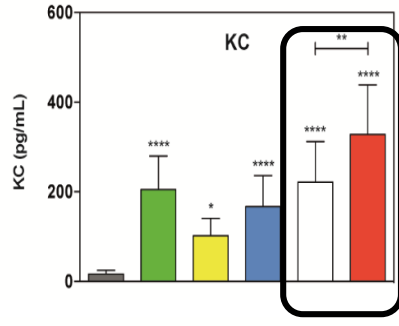
**Slight and dose-dependent induction of BAFF**

PBS
  N305
  N305<sub>HK</sub>
 LTA
  EV<sub>1</sub>
 EV<sub>10</sub>

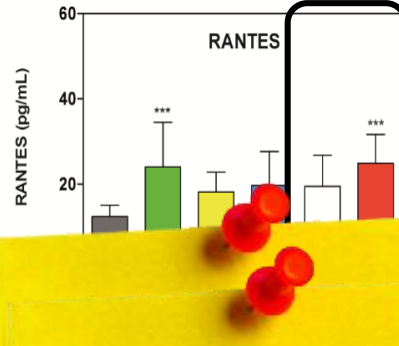
### Macrophage inflammatory protein 2



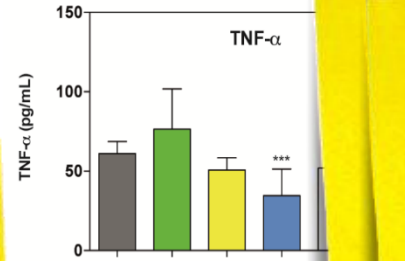
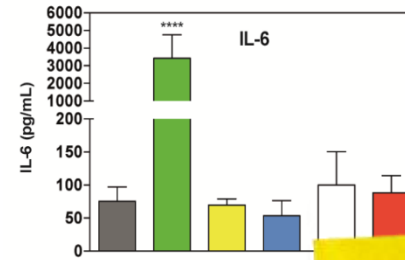
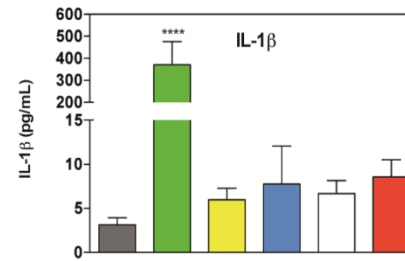
### Keratinocyte chemoattractant



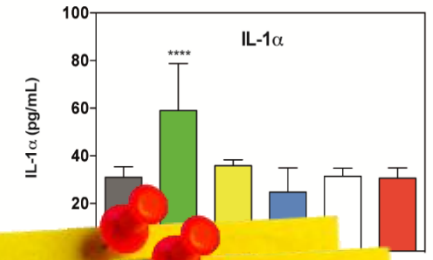
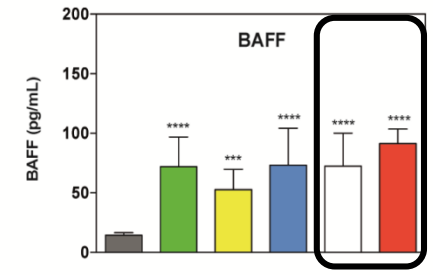
### Monocyte chemoattractant protein 1



**Chemoattractant activity**



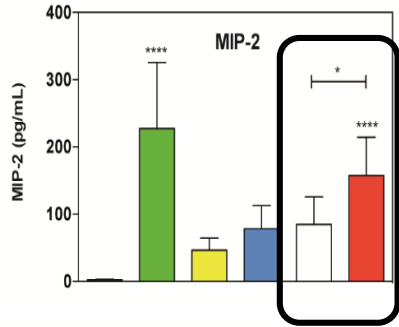
### B-cell activating factor



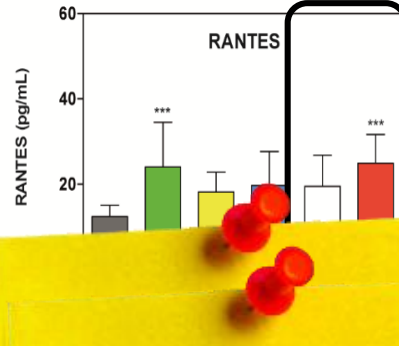
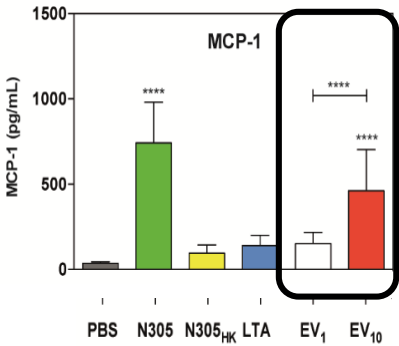
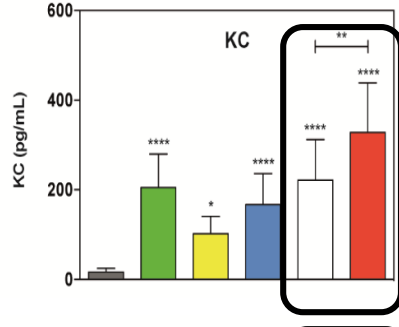
**No or weak response for other cytokines**

PBS
  N305
  N305<sub>HK</sub>
 LTA
  EV<sub>1</sub>
 EV<sub>10</sub>

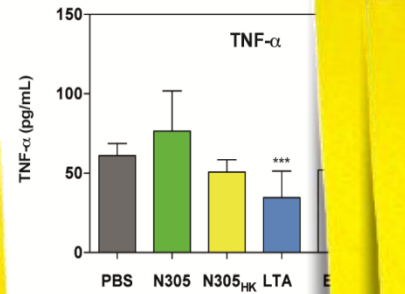
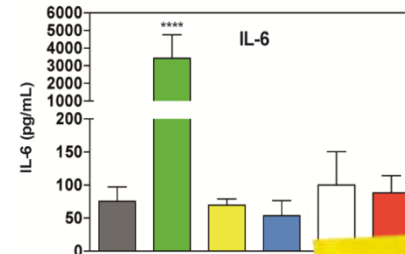
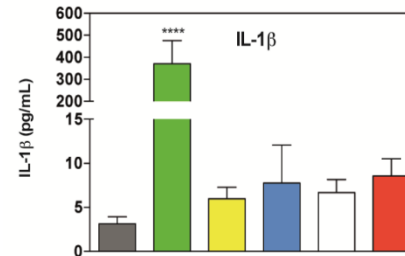
### Macrophage inflammatory protein 2



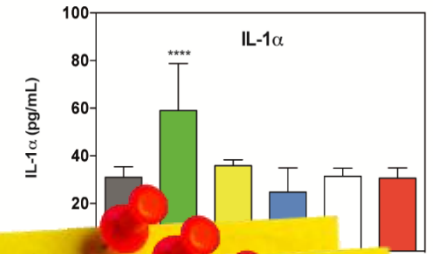
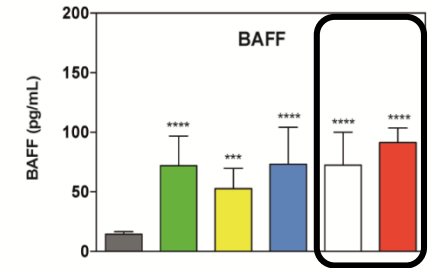
### Keratinocyte chemoattractant



### Monocyte chemoattractant protein 1



### B-cell activating factor




**Chemoattractant activity**

**EVs << live N305**

**Cellular response**

# Conclusions

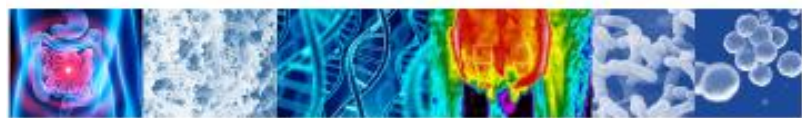
## EVs act as a “proxy” in Gram positive pathogens?

- ✓ *S. aureus* N305 EVs are non cytotoxic *in vitro*
  - ✓ *S. aureus* N305 EVs modulate the epithelial immune response *in vitro*
  - ✓ *S. aureus* N305 EVs induce a dose-dependent **PMN recruitment** after intramammary infection
  - ✓ *S. aureus* N305 EVs induce a dose-dependent production of **chemokines** after intramammary infection
- 

What about other Gram positive bacteria (food-grade and probiotic bacteria)?



# BactInflam Summer School



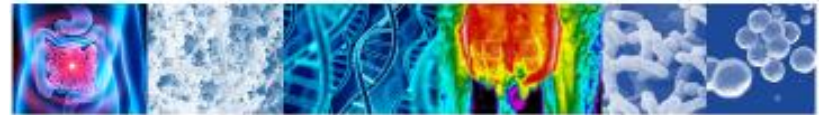
June 29<sup>th</sup>-July 3<sup>rd</sup> 2020  
Rennes, France



Interplay between bacteria and the immune system, seen through the prism of inflammation, immune maturation or immune tolerance starts to be better understood, and breakthrough applications based on this new knowledge are now available on the market.

The BactInflam summer school builds on longstanding collaborations between INRA teams and the Federal University of Minas Gerais, in Brazil. The BactInflam International Associated Laboratory gathers French and Brazilian scientists in immunology, microbiology and bioinformatics.  
<https://www6.inra.fr/bactinfram>

# BactInflam Summer School



A 5-day summer school. One topic per day.

Talks on...

- D1. Microbiomes: tools to investigate their composition
- D2. Inflammation process: Mechanisms and role of bacteria
- D3. Inflammatory diseases in human and animal health
- D4. Bacteria and inflammation: those that trigger, those that alleviate
- D5. Applications: microbial levers to mitigate inflammatory diseases

+ **Tutor-guided sessions** to develop a research project on a topic related to BactInflam themes.

+ **Round-table** with representatives of the academic and private sectors

More information and registration on:  
<http://workshop.inra.fr/bactInflam-summarschool2020>

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Jean-Marc Chatel [jean-marc.chatel@inra.fr](mailto:jean-marc.chatel@inra.fr)  
Nathalie Le Marre [nathalie.le-marre@inra.fr](mailto:nathalie.le-marre@inra.fr)

Vasco Azevedo [vasco@icb.ufmg.br](mailto:vasco@icb.ufmg.br)



Under the auspices of:



Société Française de Microbiologie

