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

Yves Le Loir

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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

Milk & Egg Science and Technology



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

130-140 persons

83 tenure staff

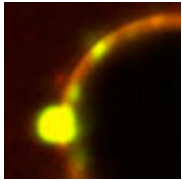
(39 researchers and professors;
44 technicians and engineers)

~25 Docs and Post-Docs / year

~15 Students (Master)

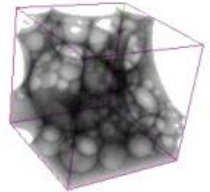
16 members of R&D dept (private sector)

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**

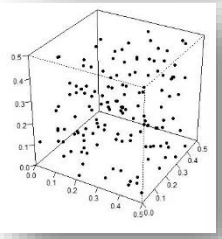
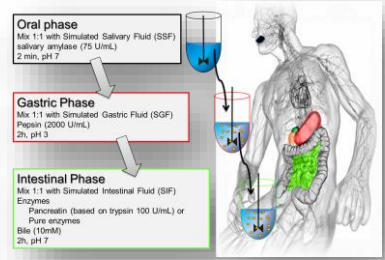


Analyze **transfers** during **technological processes**

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



Organization



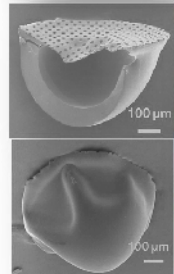
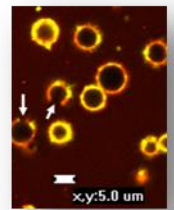
Vice directors **Director**

MICROBIO **ISF** **BN**

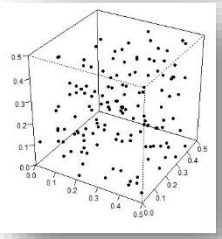
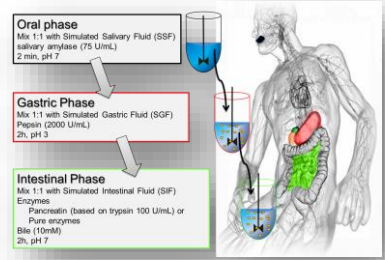
PSM **SMCF**

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

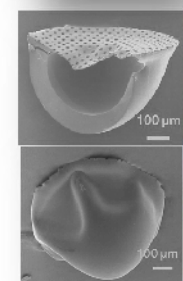
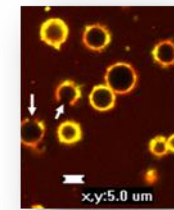

SMCF


Dairy Platform
PFL


PFL



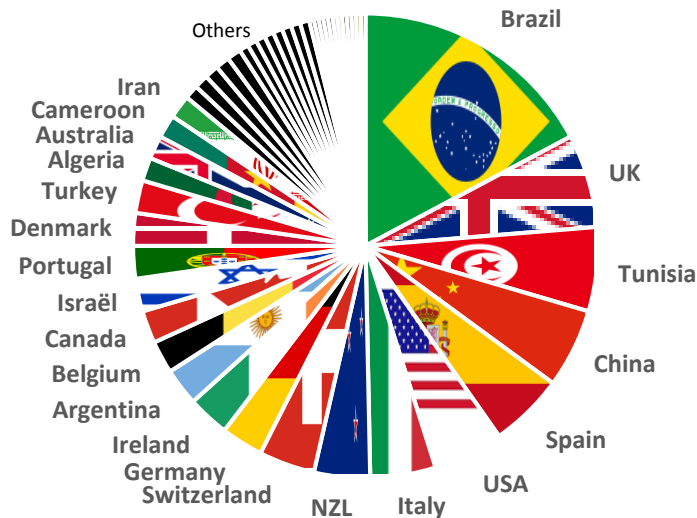
SAPHIRE
Administration,
Informatics & Infrastructure, Doc & Com



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
- SD²P[®] (31 licences), 8 countries
- Milk Salt[®] (7 licences)

International Collaborations (2014-2018)

Intensified

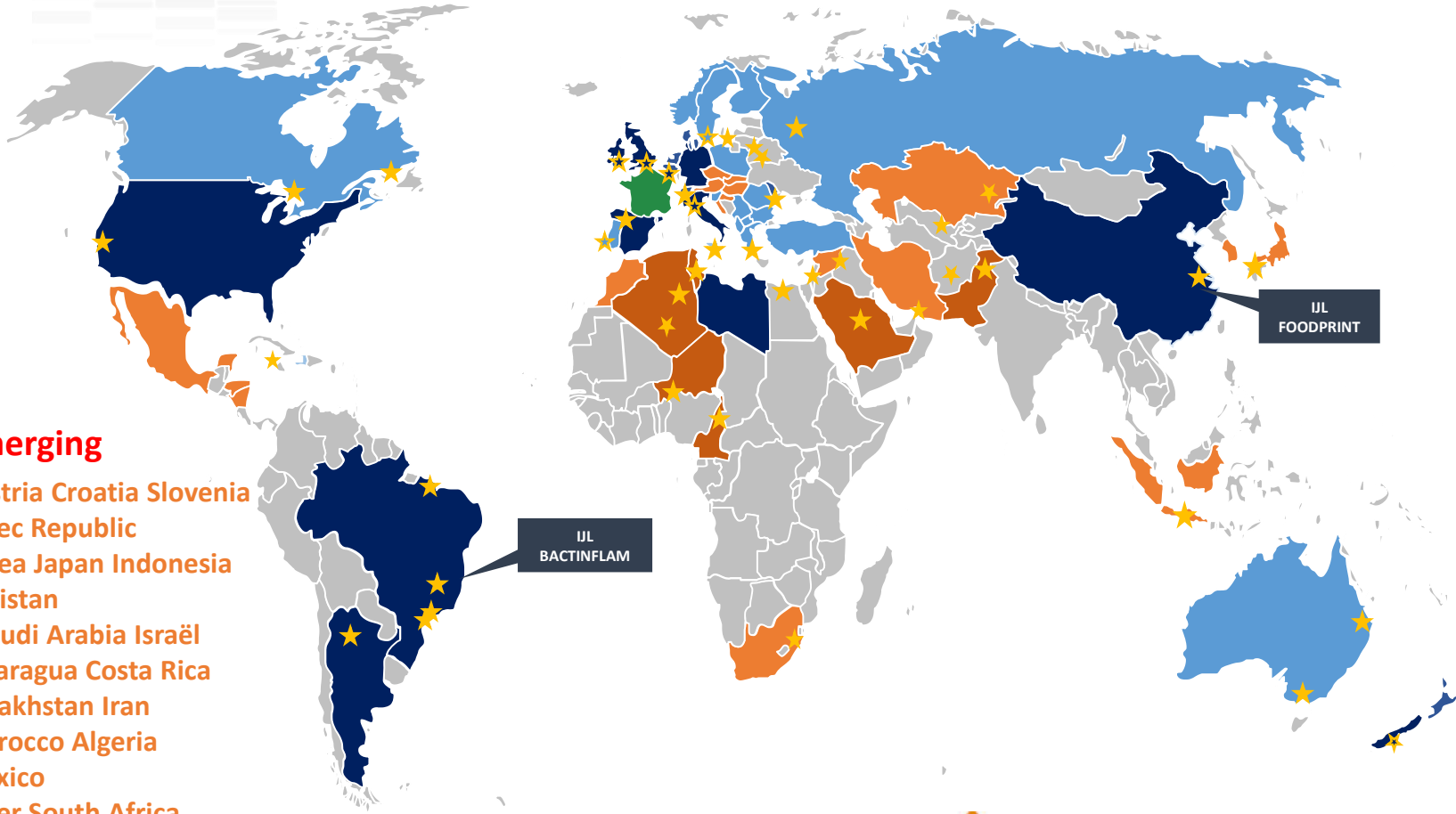
- Brazil
- Great Britain
- Tunisia
- China
- Spain
- USA
- New-Zeland
- Switzerland
- Italy
- Germany
- Argentina
- Ireland

Ongoing

- Canada
- Australia
- Turkey
- Denmark

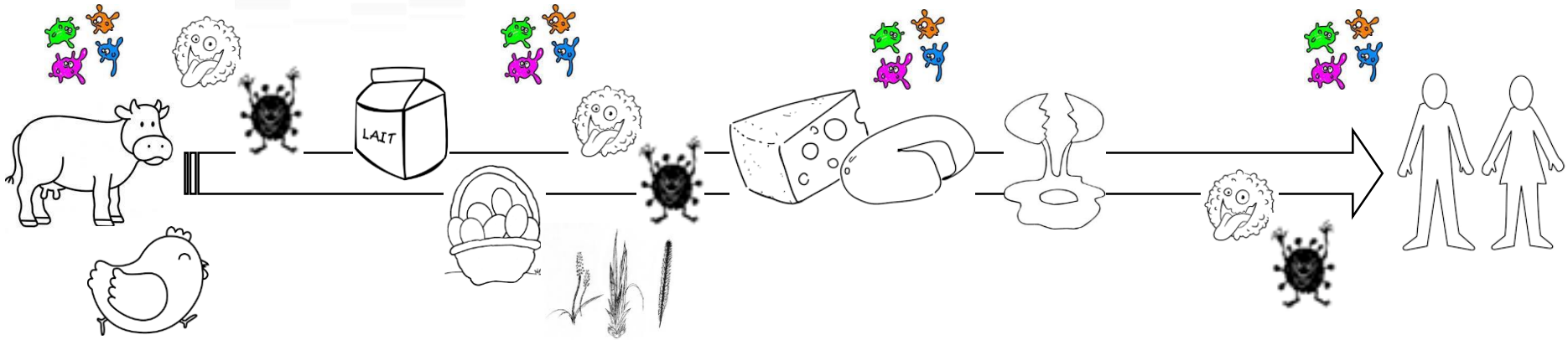
Emerging

- 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

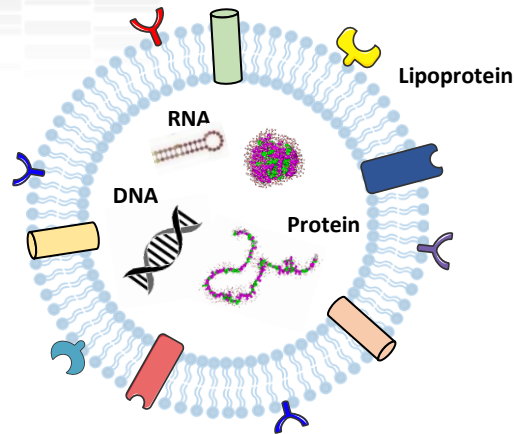


UMR1253 STLO

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

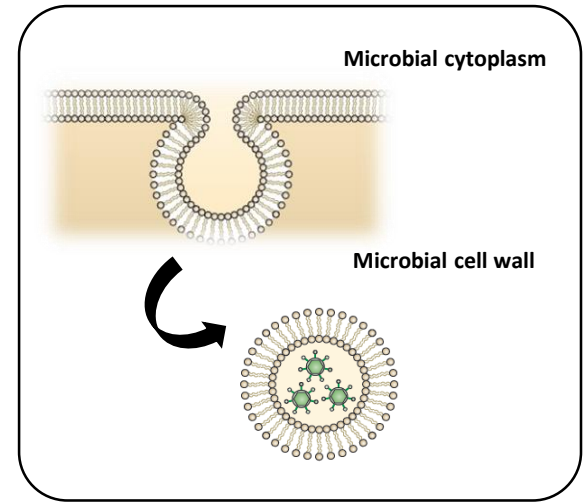
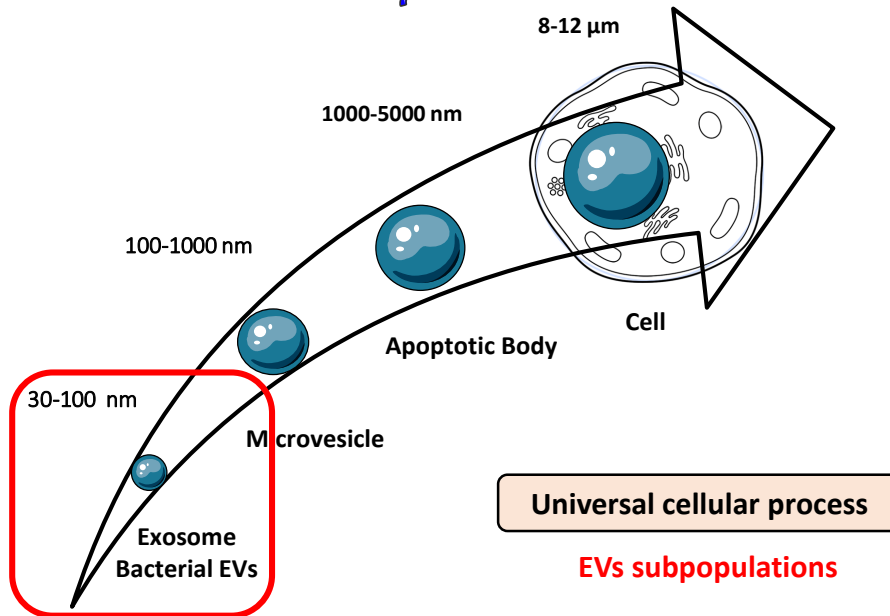


Extracellular Vesicles



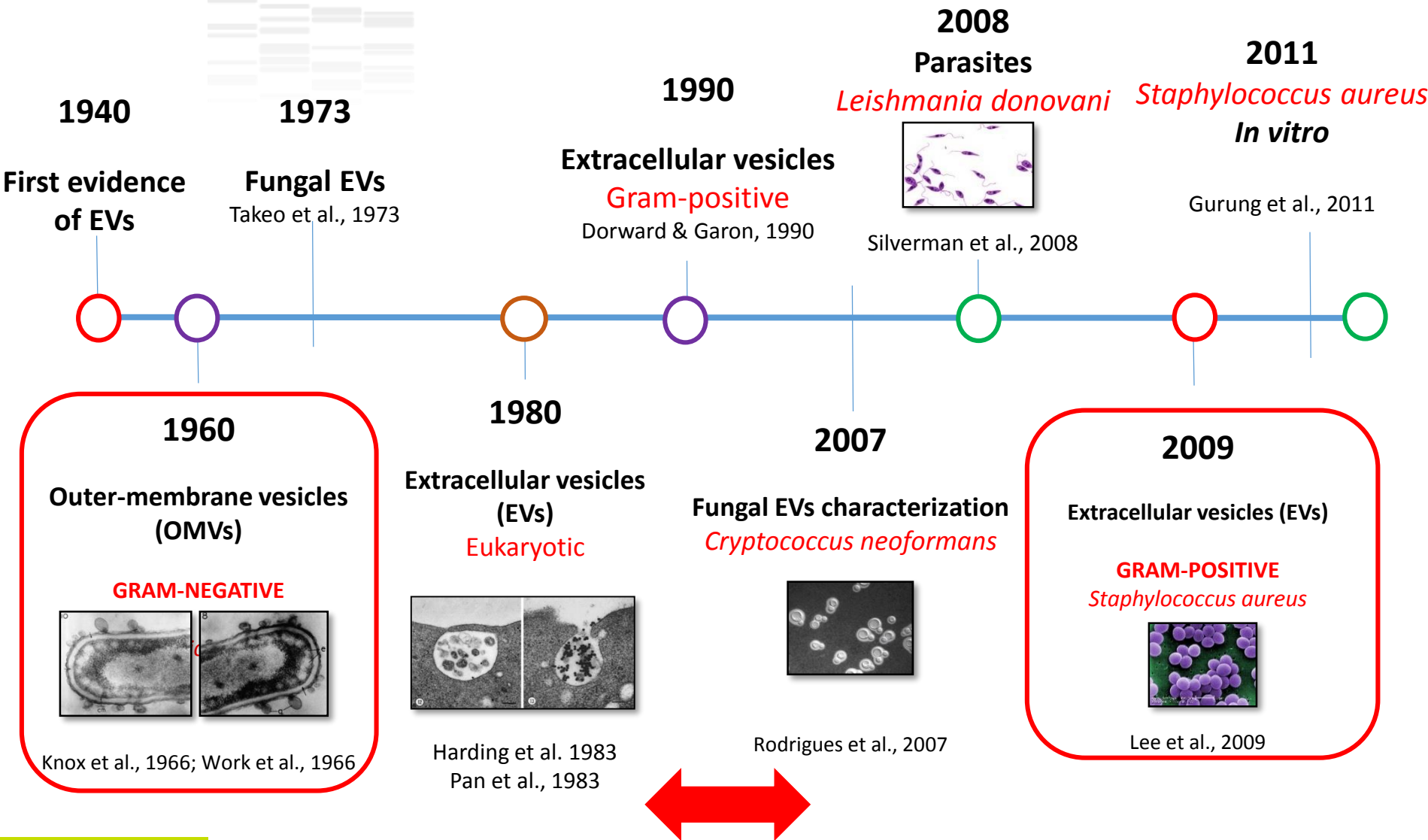
- ✓ Nano-sized particles
- ✓ Phospholipid bilayer

Proteins
Lipids
DNA
RNA

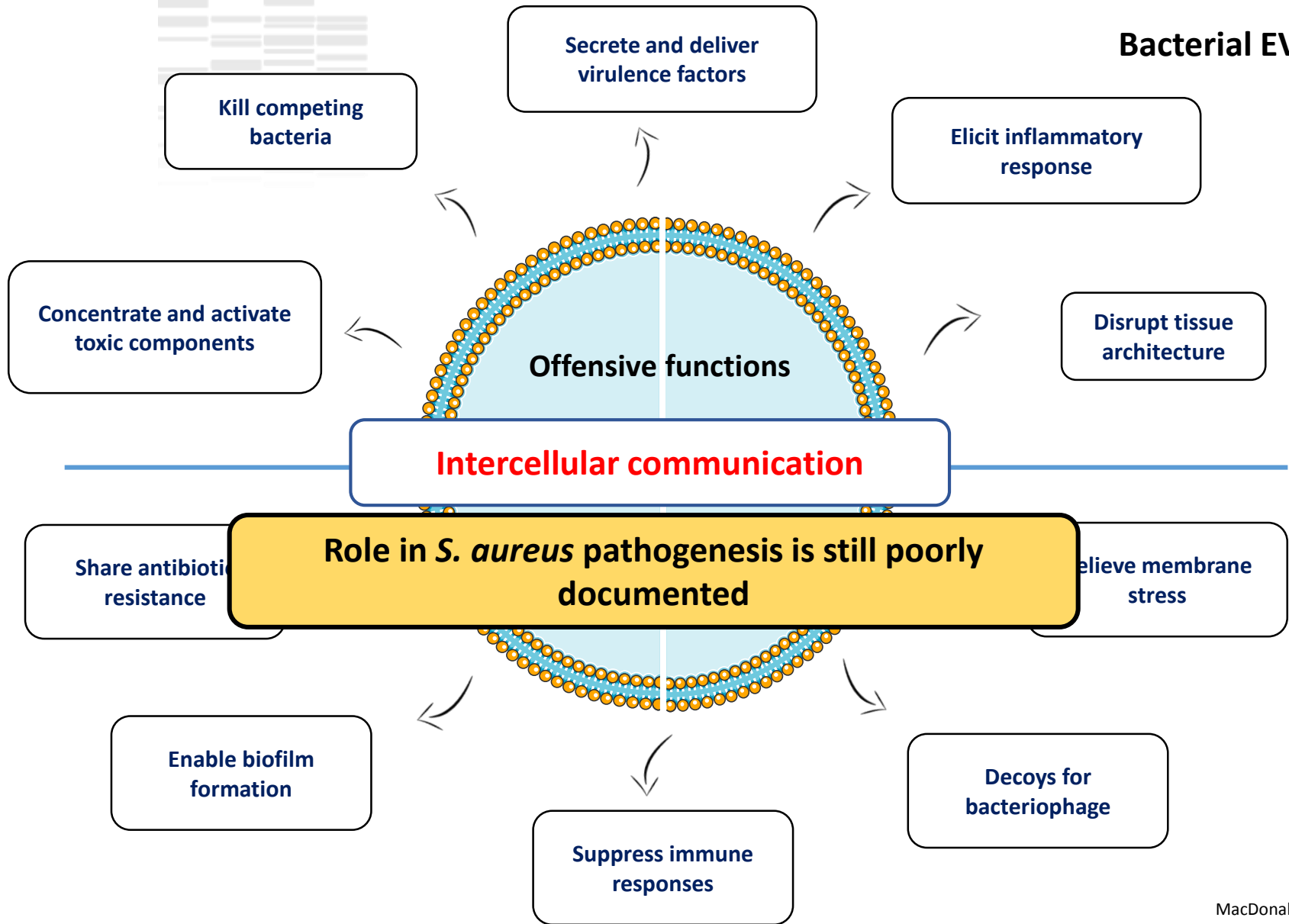


Brown et al., 2015; Lee et al., 2009

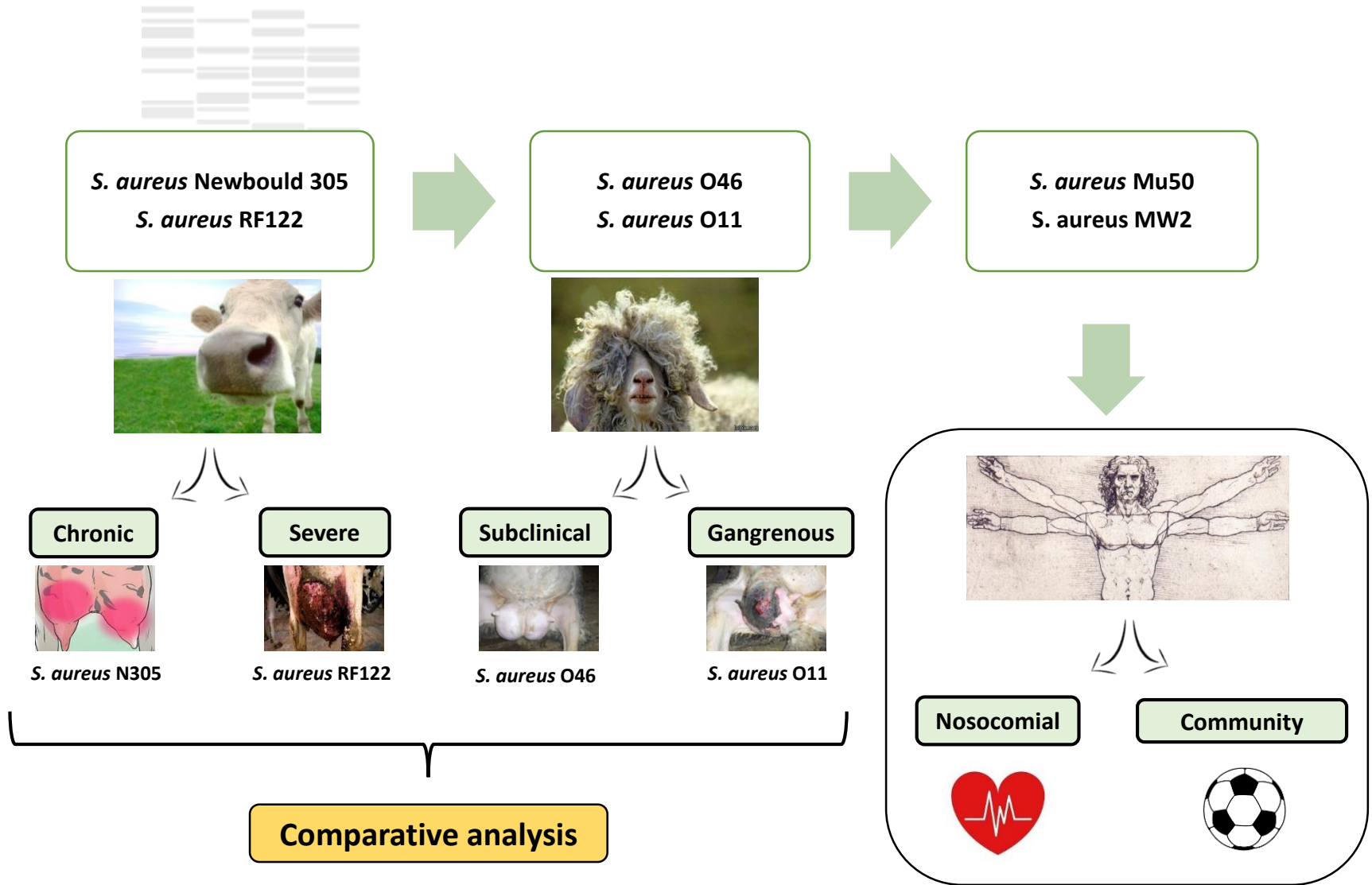
Extracellular vesicle discovery timeline



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

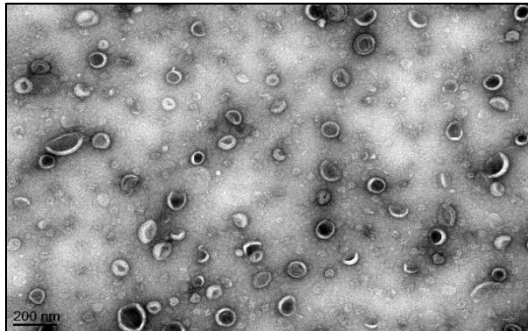


S. aureus O46
S. aureus O11

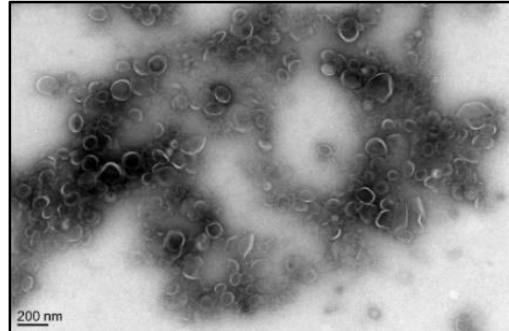


S. aureus Mu50
S. aureus MW2

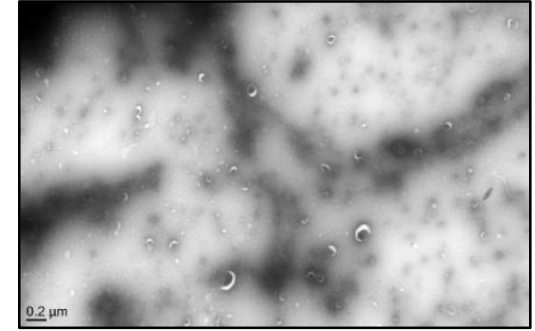
S. aureus N305



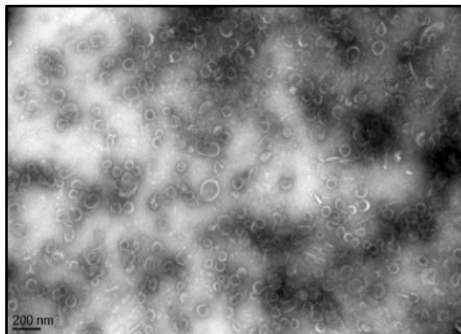
S. aureus O46



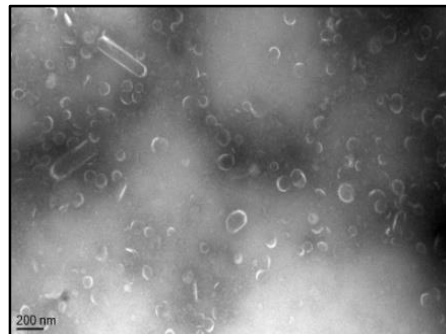
S. aureus MW2



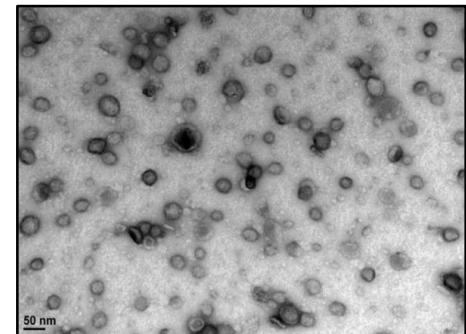
S. aureus RF122



S. aureus O11



S. aureus Mu50



Bovine

Ovine

Human

EVs characterization



S. aureus N305
S. aureus RF122

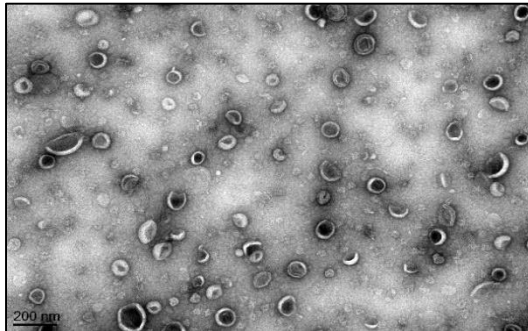


S. aureus O46
S. aureus O11

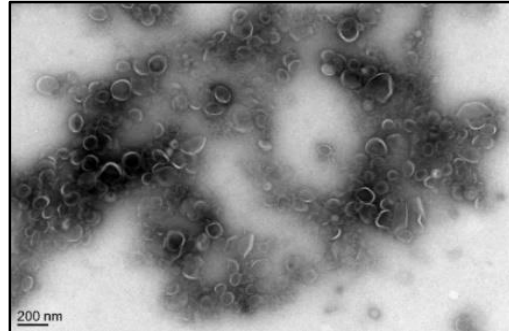


S. aureus Mu50
S. aureus MW2

S. aureus N305



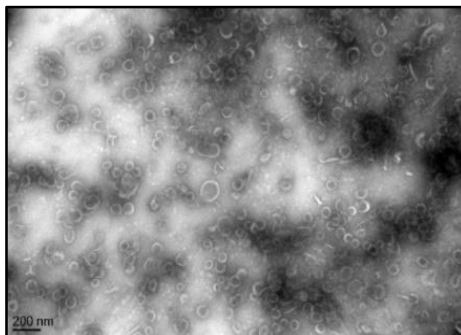
S. aureus O46



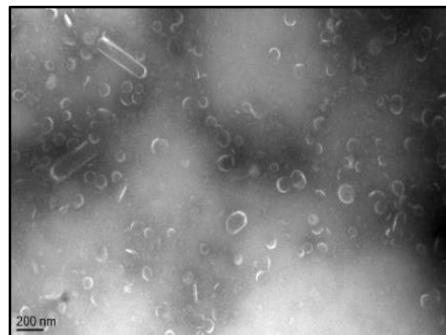
S. aureus MW2



S. aureus RF122



S. aureus O11



Bovine

Ovine

Human

261 proteins identified

Distribution of COGs



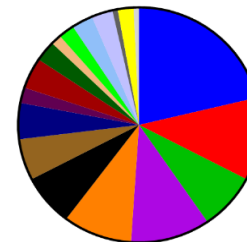
S. aureus N305
S. aureus RF122



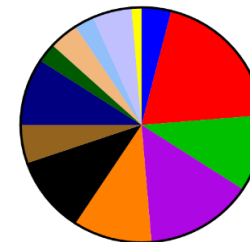
S. aureus O46
S. aureus O11



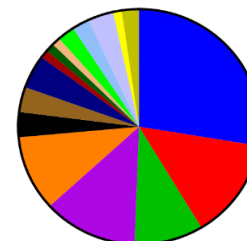
S. aureus Mu50
S. aureus MW2



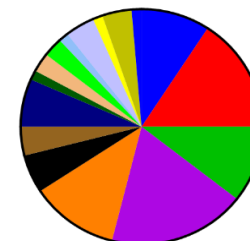
S. aureus N305



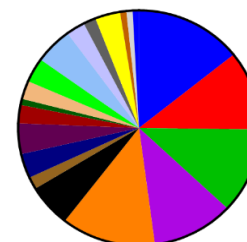
S. aureus RF122



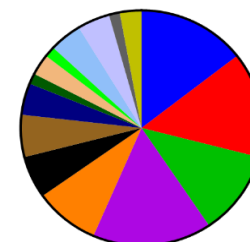
S. aureus O46



S. aureus O11



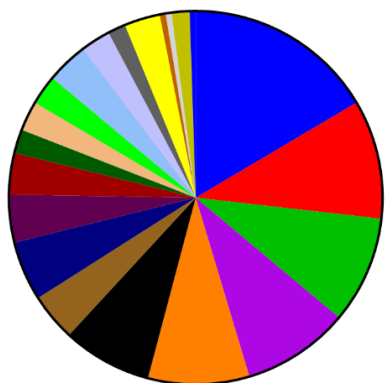
S. aureus Mu50



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



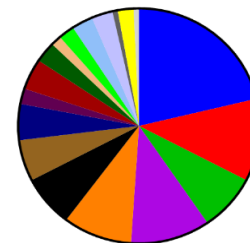
S. aureus N305
S. aureus RF122



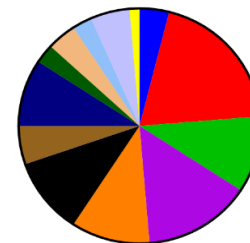
S. aureus O46
S. aureus O11



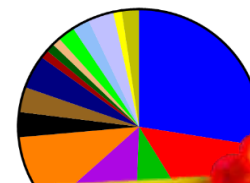
S. aureus Mu50
S. aureus MW2



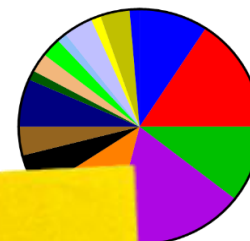
S. aureus N305



S. aureus RF122



S. aureus O11

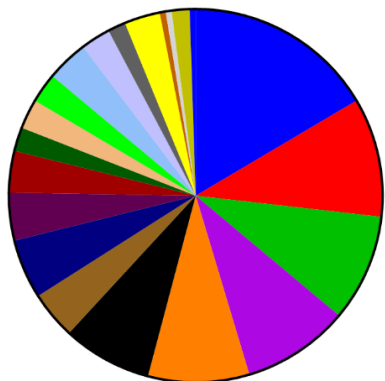


COGs distribution varies from a strain to another

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
- 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



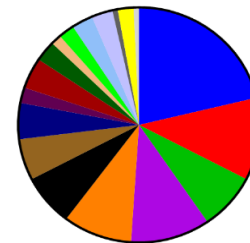
S. aureus N305
S. aureus RF122



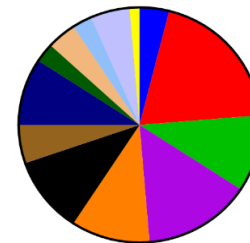
S. aureus O46
S. aureus O11



S. aureus Mu50
S. aureus MW2

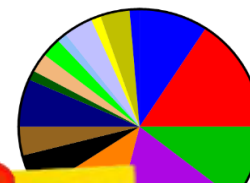
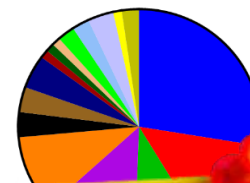
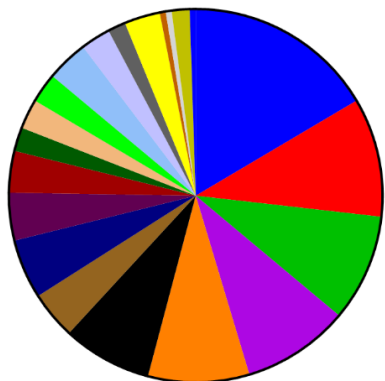


S. aureus N305



S. aureus RF122

- 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
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- Lipid transport and metabolism
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- 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

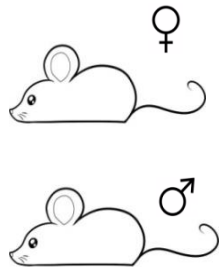
1 week

12 days

24 hours

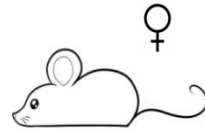
Sacrifice

Dissection

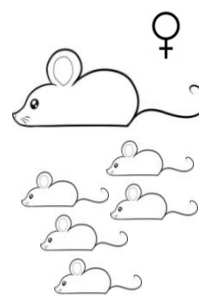


CD-1

Female and male mice



Isolated



40 CD-1

Intraductal inoculation

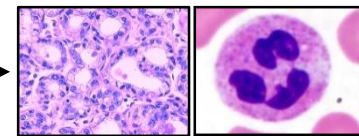
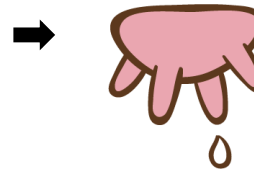
Control groups

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

Experimental groups

- ✓ EVs 1µg
- ✓ EVs 10µg

Mammary tissue

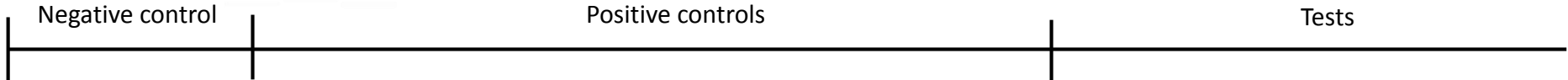


Hematoxylin and eosin stain



Cytokines assay

Do EVs induce an effect *in vivo*?



Negative control	Positive controls			Tests	
PBS	Living N305	HK N305	LTA	EVs 1 µg	EVs 10 µg
Healthy mammary glands	Severe lesion (hemorrhage)	Moderate lesions	Moderate lesions	Moderate lesions	Moderate lesions

A yellow arrow points from the 'EVs 1 µg' column to the 'EVs 10 µg' column, indicating a comparison or progression.



Do EVs induce an effect *in vivo*?

Negative control

Positive controls

Tests

PBS

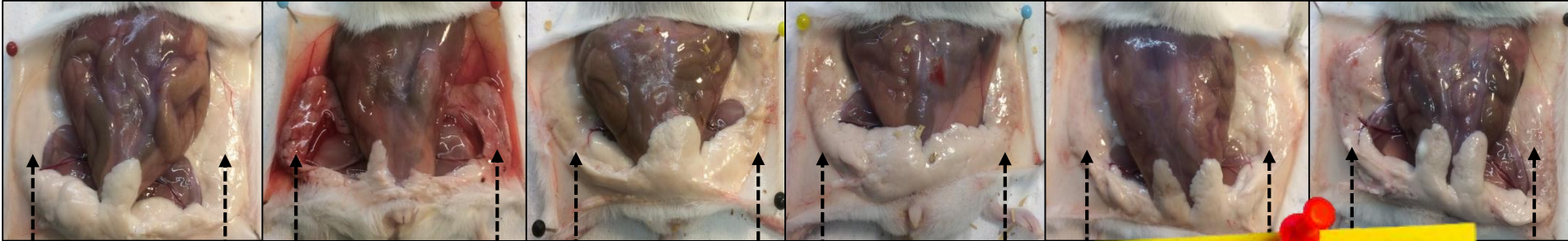
Living N305

HK N305

LTA

EVs 1 µg

EVs 10 µg



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*?

Negative control

Positive controls

Tests

PBS

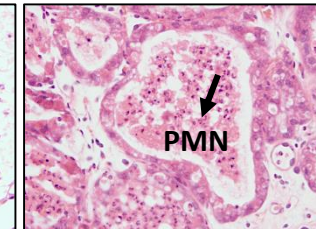
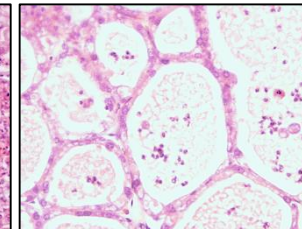
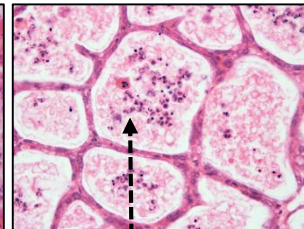
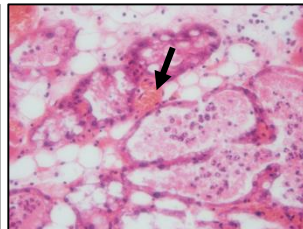
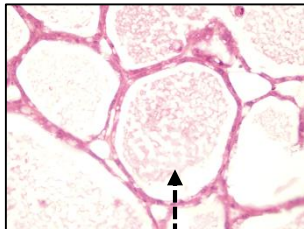
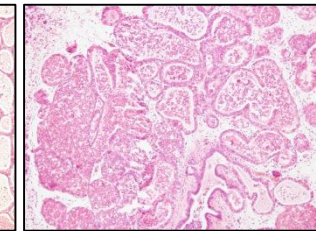
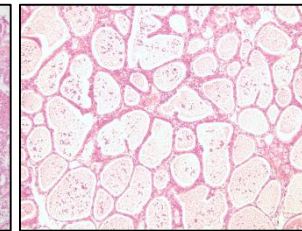
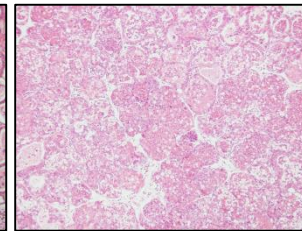
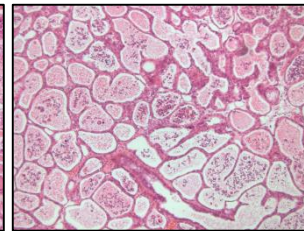
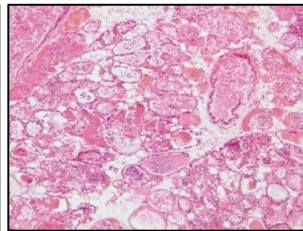
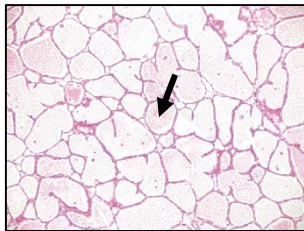
Living N305

HK N305

LTA

EVs 1 μ g

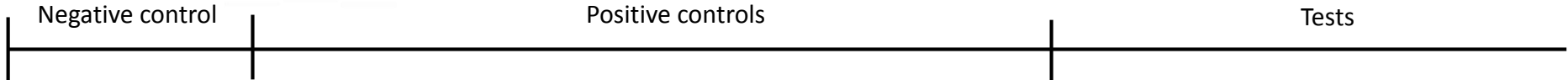
EVs 10 μ g



Lumen of alveolus

PMN
Polymorphonuclear cells

Do EVs induce an effect *in vivo*?

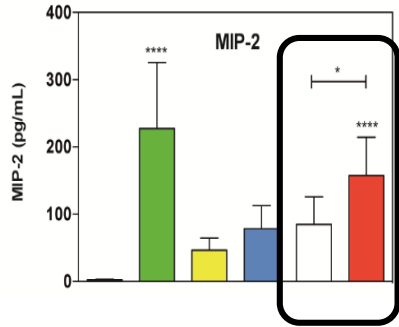


PBS	Living N305	HK N305	LTA	EVs 1 μ g	EVs 10 μ g
Lumen of alveolus		PMN Polymorphonuclear cells			

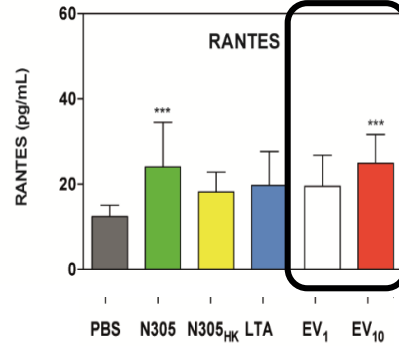
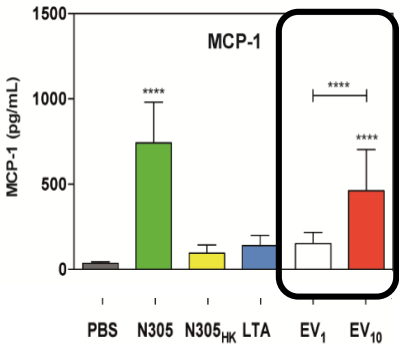
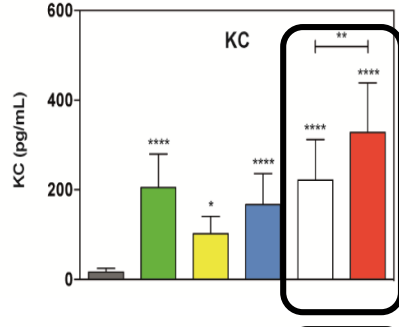
EVs induce a dose dependent PMN recruitment

PBS
 N305
 N305_{HK}
 LTA
 EV₁
 EV₁₀

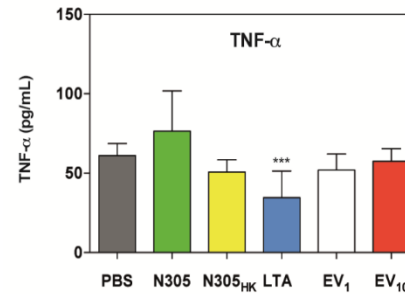
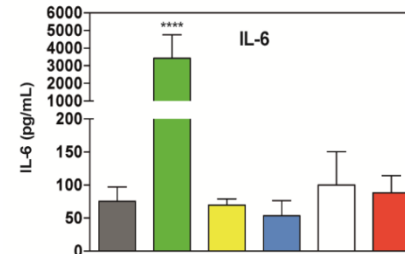
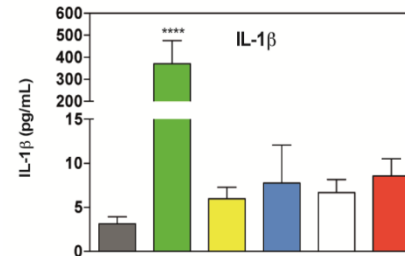
Macrophage inflammatory protein 2



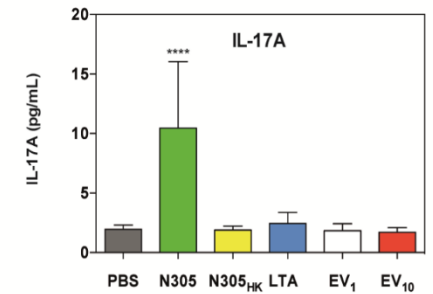
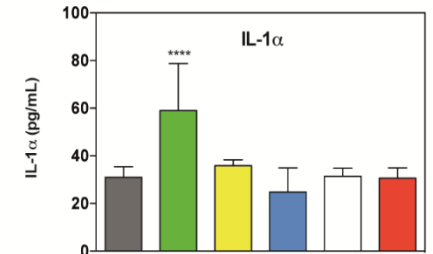
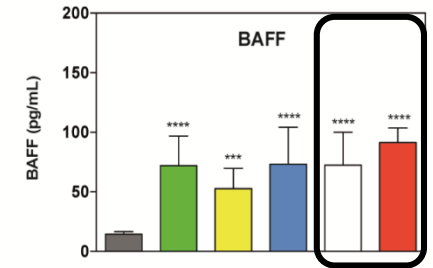
Keratinocyte chemoattractant



Monocyte chemoattractant protein 1

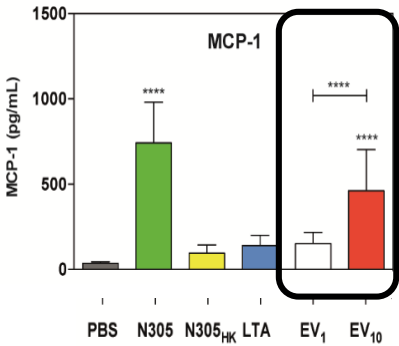
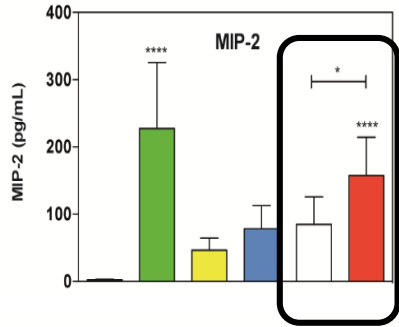


B-cell activating factor



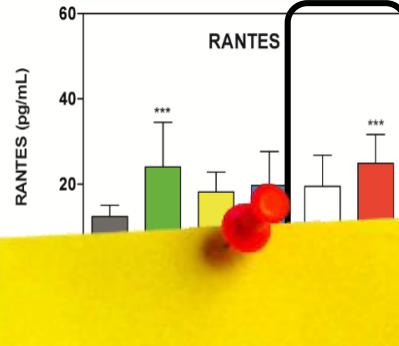
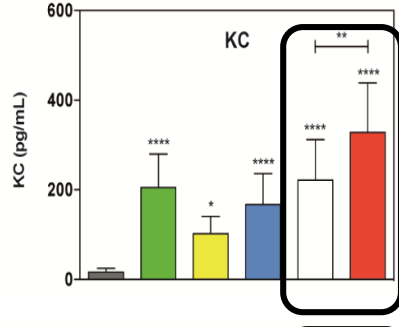
PBS
 N305
 N305_{HK}
 LTA
 EV₁
 EV₁₀

Macrophage inflammatory protein 2

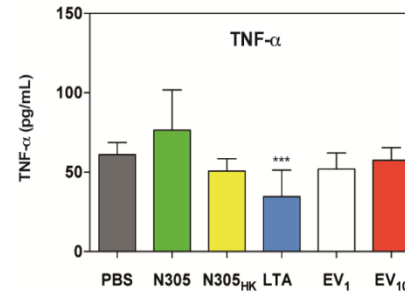
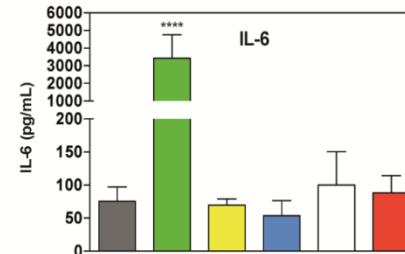
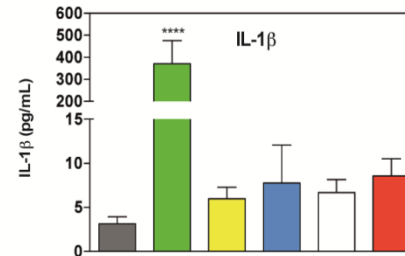


Monocyte chemoattractant protein 1

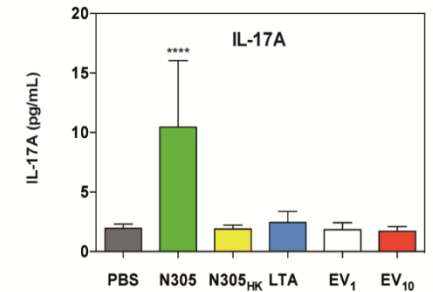
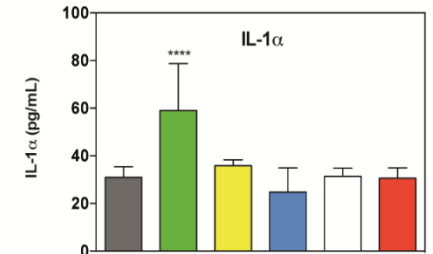
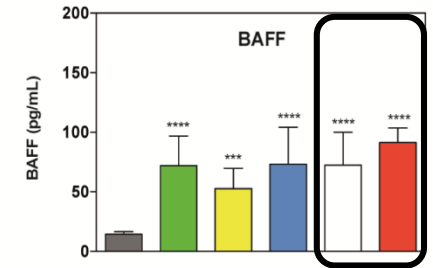
Keratinocyte chemoattractant



Dose-dependant induction of chemokines

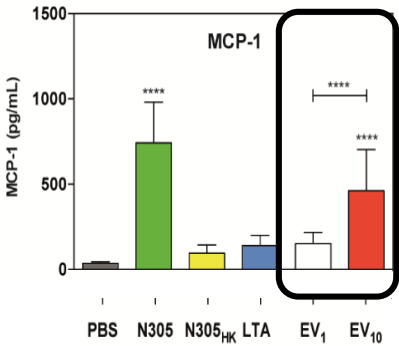
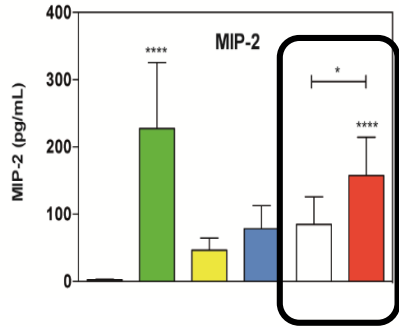


B-cell activating factor



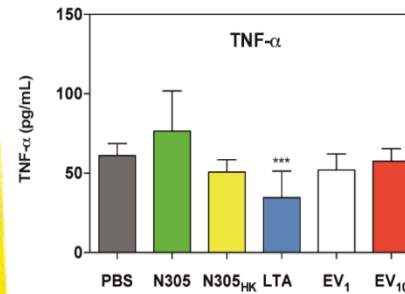
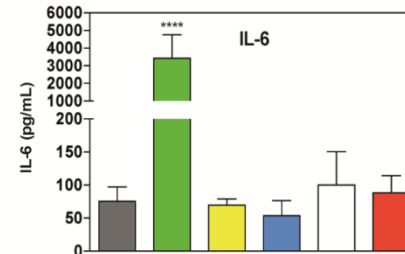
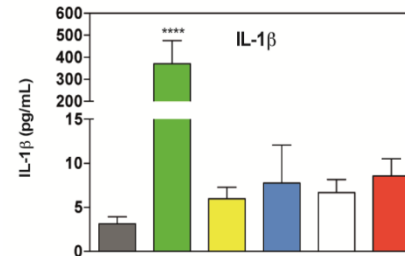
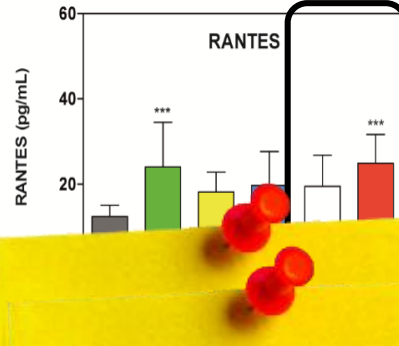
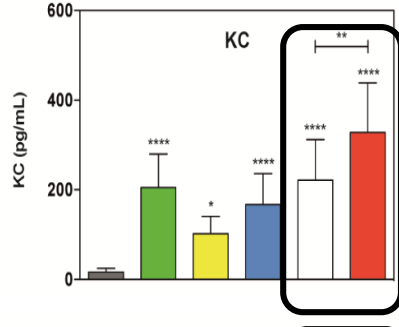
PBS
 N305
 N305_{HK}
 LTA
 EV₁
 EV₁₀

Macrophage inflammatory protein 2

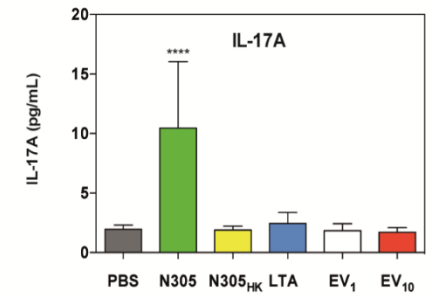
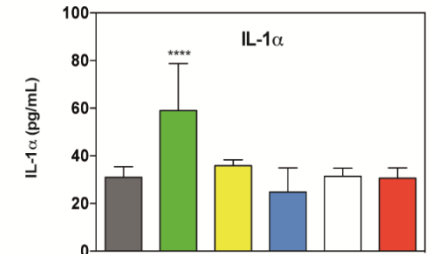
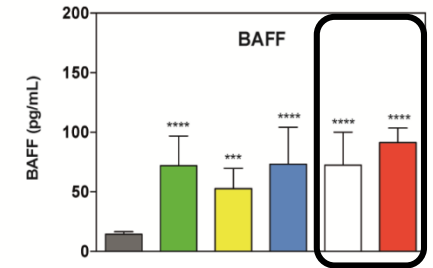


Monocyte chemoattractant protein 1

Keratinocyte chemoattractant



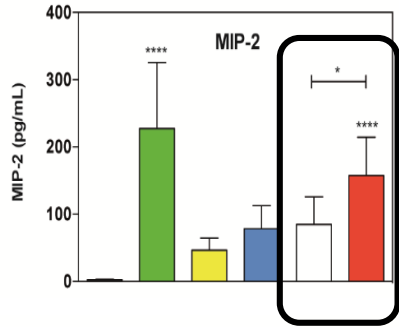
B-cell activating factor



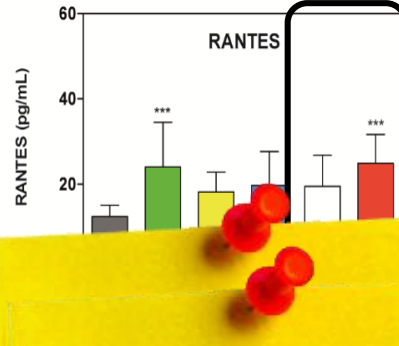
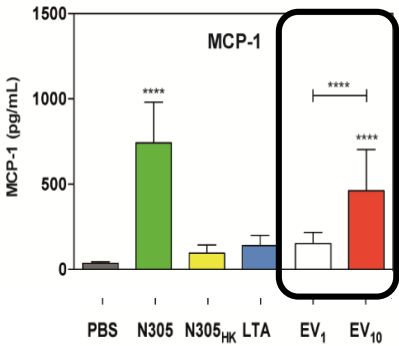
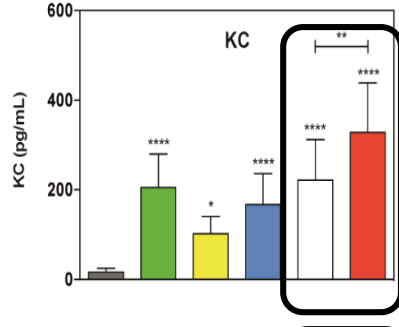
Chemoattractant activity

PBS
 N305
 N305_{HK}
 LTA
 EV₁
 EV₁₀

Macrophage inflammatory protein 2



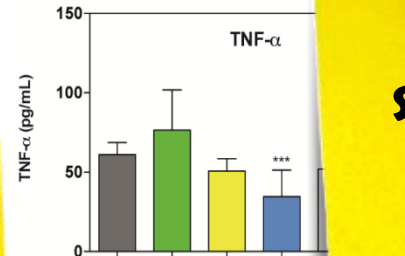
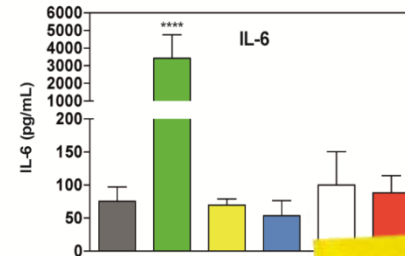
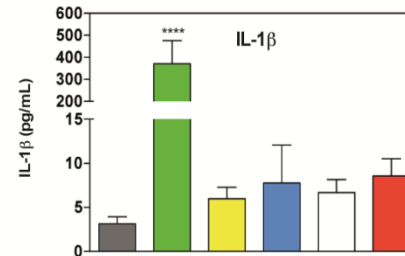
Keratinocyte chemoattractant



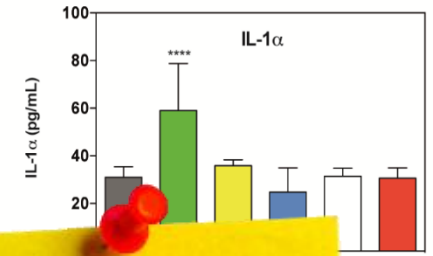
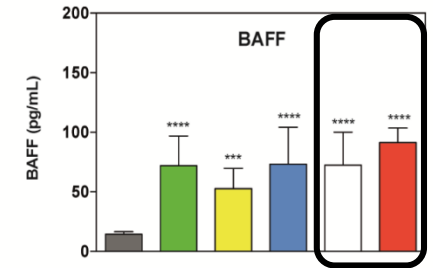
Monocyte chemoattractant protein 1



Chemoattractant activity



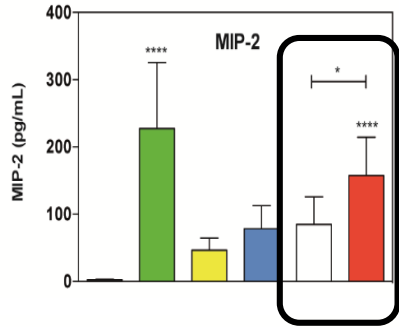
B-cell activating factor



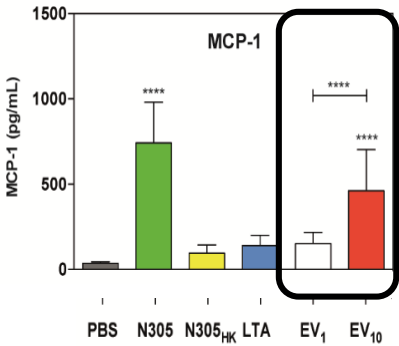
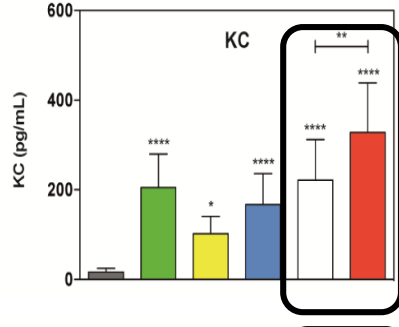
Slight and dose-dependent induction of BAFF

PBS
 N305
 N305_{HK}
 LTA
 EV₁
 EV₁₀

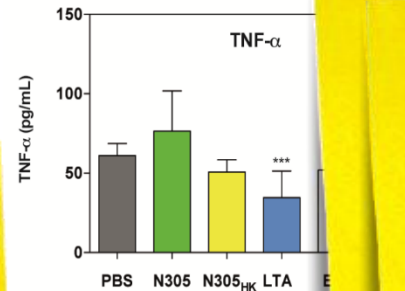
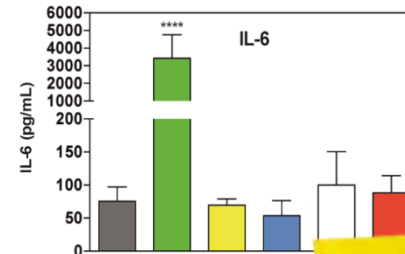
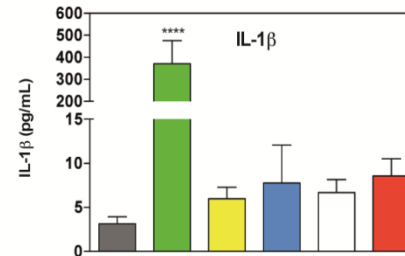
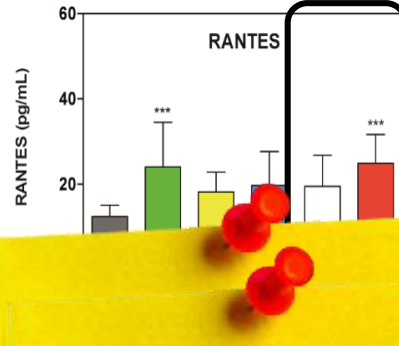
Macrophage inflammatory protein 2



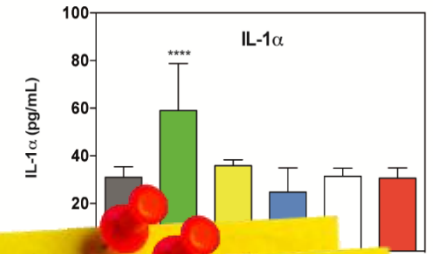
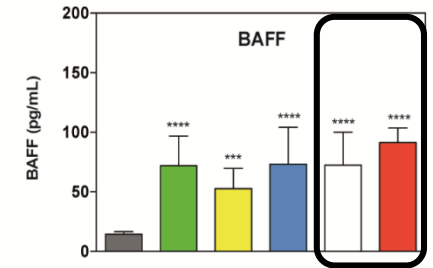
Keratinocyte chemoattractant



Monocyte chemoattractant protein 1



B-cell activating factor

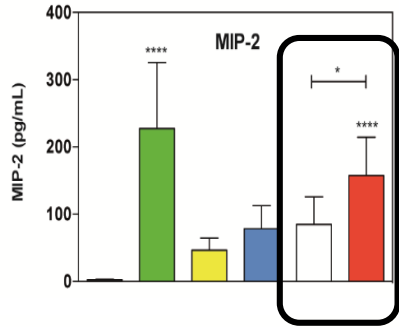


Chemoattractant activity

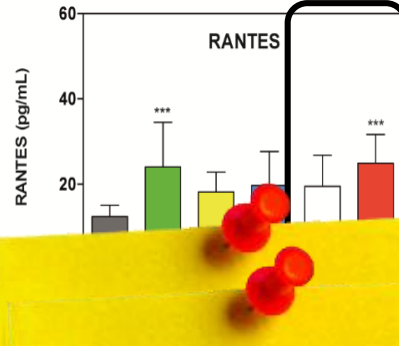
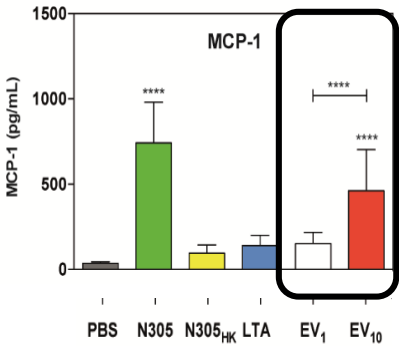
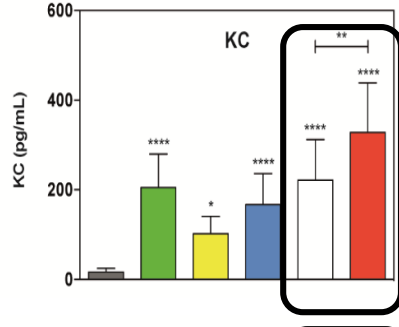
No or weak response for other cytokines

PBS
 N305
 N305_{HK}
 LTA
 EV₁
 EV₁₀

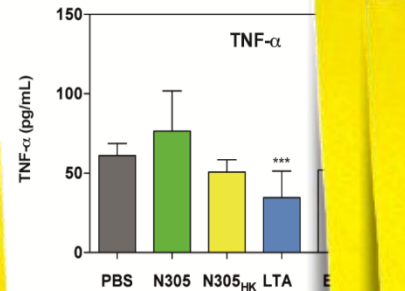
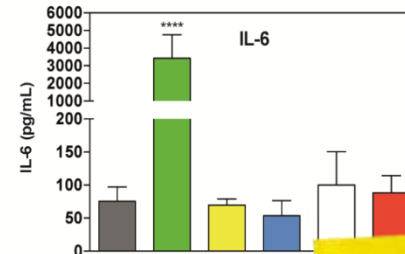
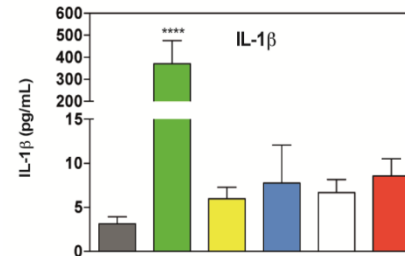
Macrophage inflammatory protein 2



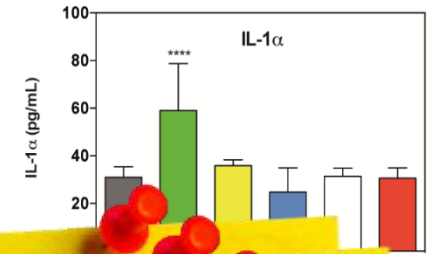
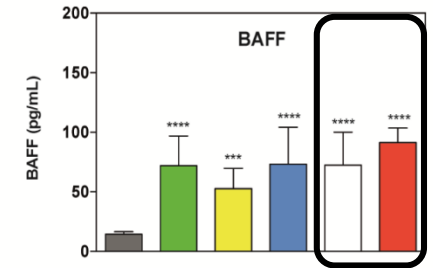
Keratinocyte chemoattractant



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?

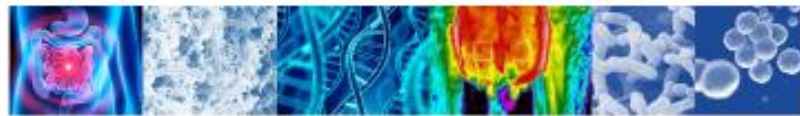
- ✓ *S. aureus* N305 EVs are non cytotoxic *in vitro*
- ✓ *S. aureus* N305 EVs modulate the epithelial immune response *in vitro*
- ✓ *S. aureus* N305 EVs induce a dose-dependent PMN recruitment after intramammary infection
- ✓ *S. aureus* N305 EVs induce a dose-dependent production of chemokines after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



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



BactInflam Summer School



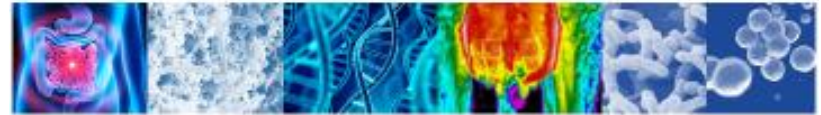
June 29th-July 3rd 2020
Rennes, France



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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Vasco Azevedo vasco@icb.ufmg.br



Under the auspices of:

