B2ISI Team Bacterial Biodiversity And In Situ Interactions

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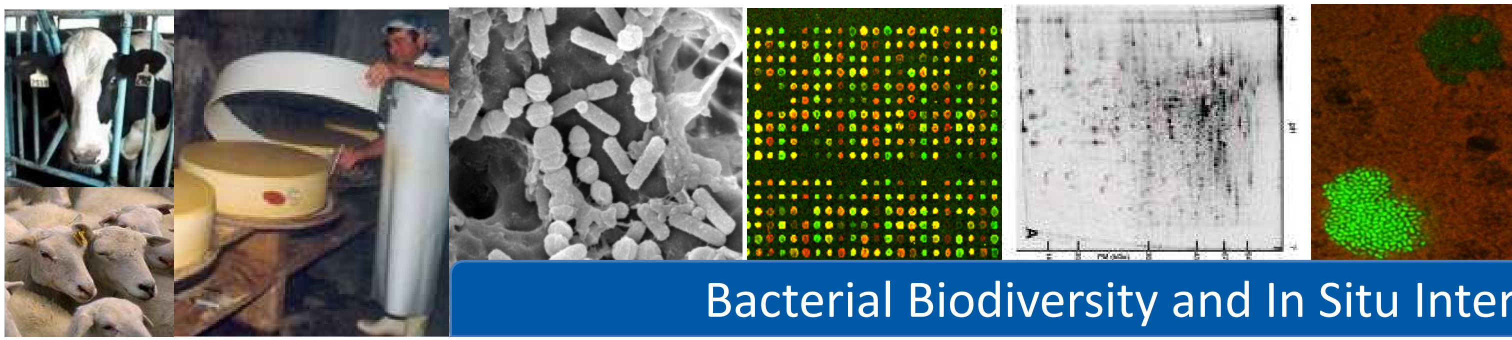
UMR STLO
Evaluation 2006-2010

B2ISI Team

Bacterial Biodiversity
And *In Situ* Interactions

Viçosa, April 2011



Bacterial Biodiversity and In Situ Interactions

P. *freudenreichii* ecological niches

Soil & grass

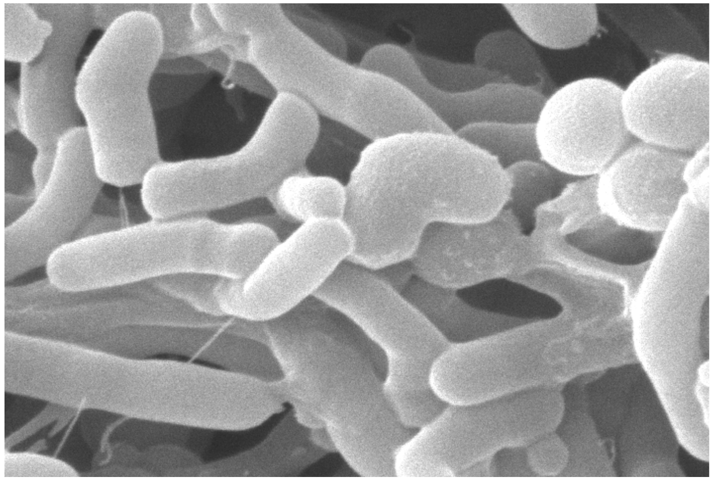
Cow, sheep, deer, goat rumen

Cow dung

milk

Emmental

PROPIONIC ACID BACTERIA



Group of Gram positive bacteria

Variable morphology (chinese characters)

Irregular rods (diam., 0.5 to 0.8 µm; length, 1 to 5 µm)

Catalase$^-$, non spore-forming, non-motile,

anaerobe-aerotolerante, mesophilic

Fermentation of sugars into propionic acid

In the cheese:

3 lactate $\longrightarrow$ 2 propionate + 1 acetate + 1 CO_2 + 1 H_2O

Pathogenic propionic acid bacteria

Propionibacterium acnes

Dairy propionic acid bacteria

Propionibacterium freudenreichii ssp *freudenreichii*

Propionibacterium freudenreichii ssp *shermanii*

Propionibacterium jensenii

Propionibacterium acidi propionici

Propionibacterium thoneii

Role of the PAB

Involved in the ripenning of the swiss type cheeses

Growth in warm curing room

Cell density : 10^3 to 10^6 at 24H

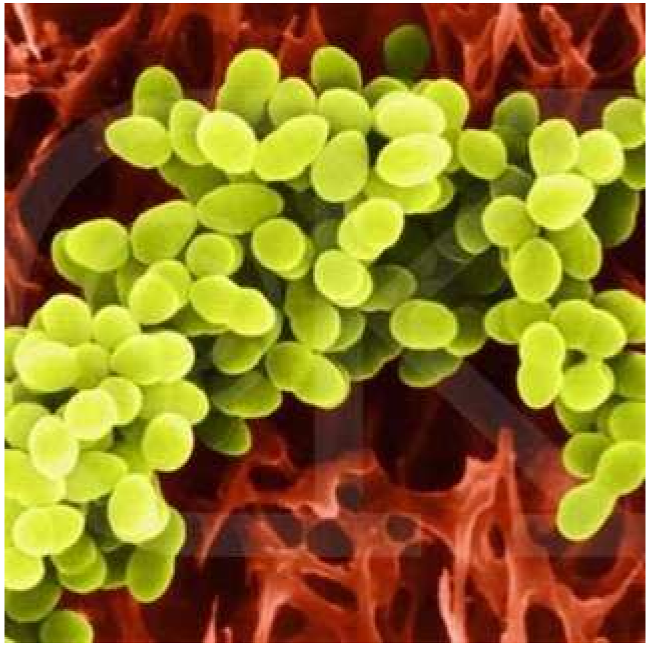
10^9 in warm curing room

Eye formation in some swiss type cheese: release of $C0_2$

Development of the flavor :

Short chain fatty acid, propionic acid and acetic acid, acetaldhehyde, propionaldehyde, ethanol, propanol, diacetyl…

STAPHYLOCOCCUS AUREUS

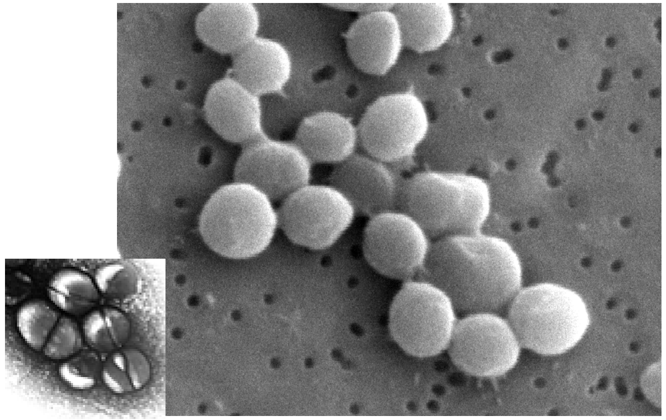


In 1878, first observation by Koch

In 1880, *Staphylococcus* is recognized as a genus by Pasteur

In 1884, Rosenberg cultures the first Staphylococci on solid medium

STAPHYLOCOCCUS AUREUS



Spherical bacterium (coccus). Gram-positive.
Grape-like clusters. Catalase+
Facultative anaerobe.
Growth in a wide range of temperatures (18-40°C) and salt concentrations (10% NaCl).
Resist to lysozyme
Some strains produce highly heat-stable toxins.

Humans and animals are the primary reservoirs (nasal passages and throats and on the hair and skin of 50 percent or more of healthy individuals).
Causes numerous infections in humans and animals.

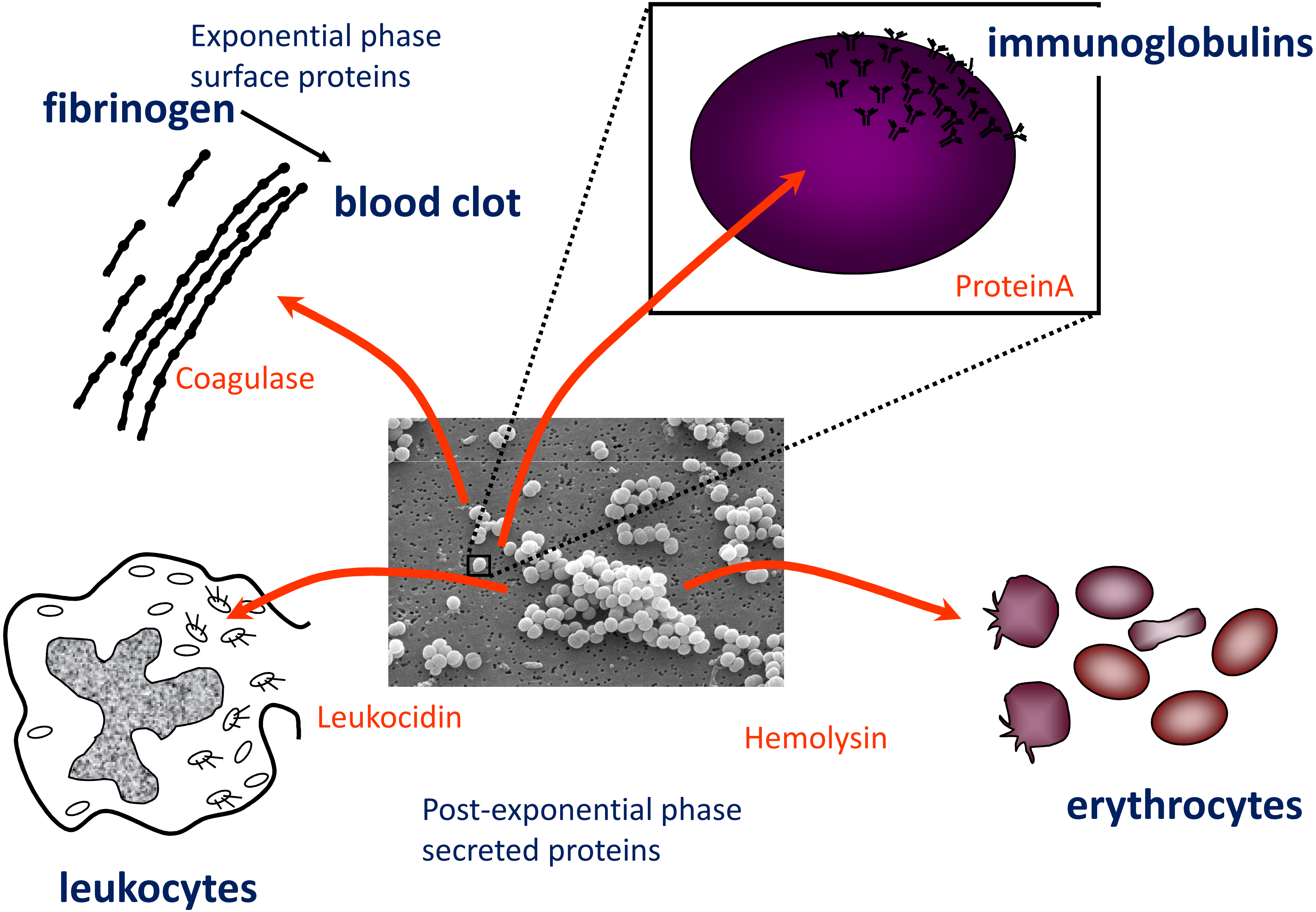
- Major cause of nosocomial infections
  - Extensively studied (virulence factors, bacteria-host interactions, antibiotic resistance…)
- More than 20 complete genome sequences available for *S. aureus* (only one bovine strain and one ovine strain)

DIVERSE SPECTRUM OF DISEASES

Skin abscess
Eye infection
Wound infection
Mastitis
involved
Septic arthritis
Osteomyelitis
Kidney abscess, nephritis
Septicemia

numerous virulence factors

Staphylococcal scalded skin syndrome	Exfoliatins
Toxic shock syndrome	TSST1 (and enterotoxins)
Staphylococcal food poisoning	Enterotoxins


Exponential phase
surface proteins
fibrinogen
blood clot
immunoglobulins
ProteinA
Coagulase
Leukocidin
Hemolysin
Post-exponential phase
secreted proteins
erythrocytes
leukocytes

Context

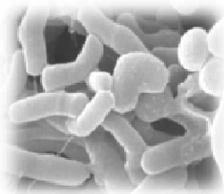
S. aureus

Animal Health
Mastitis

Food Safety
SFP Outbreaks

Human Health
Probiotics

Cheese Technology
Ripenning



P. freudenreichii

Our goals

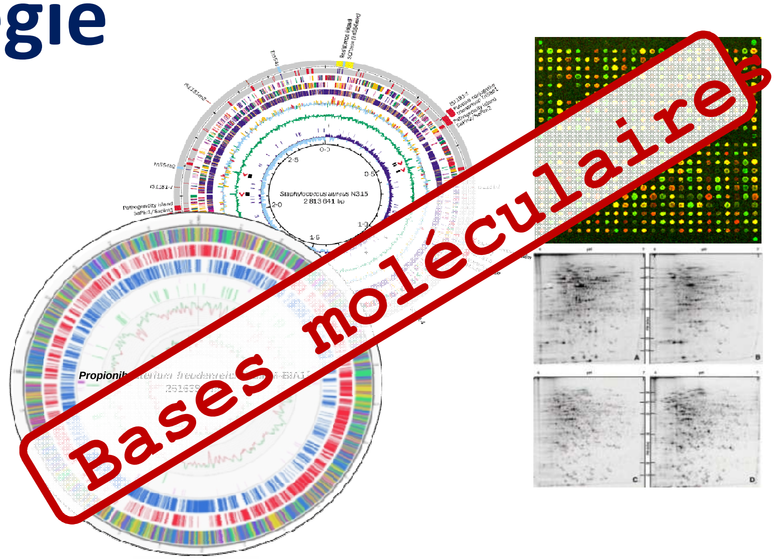
To understand the mechanisms leading to **biodiversity** in two species:

- Host **adaptation** and **virulence** (*S. aureus*)
- **Ripenning** and **probiotic** capabilities (*P. freudenreichii*).

To understand ***in situ*** **interactions, cheese** matrix or with the **host** (Digestive tract or mammary gland).

To control the expression of these bacterial potentials (technological, probiotic or pathogenic) *in situ*

Stratégie



Animation scientifique

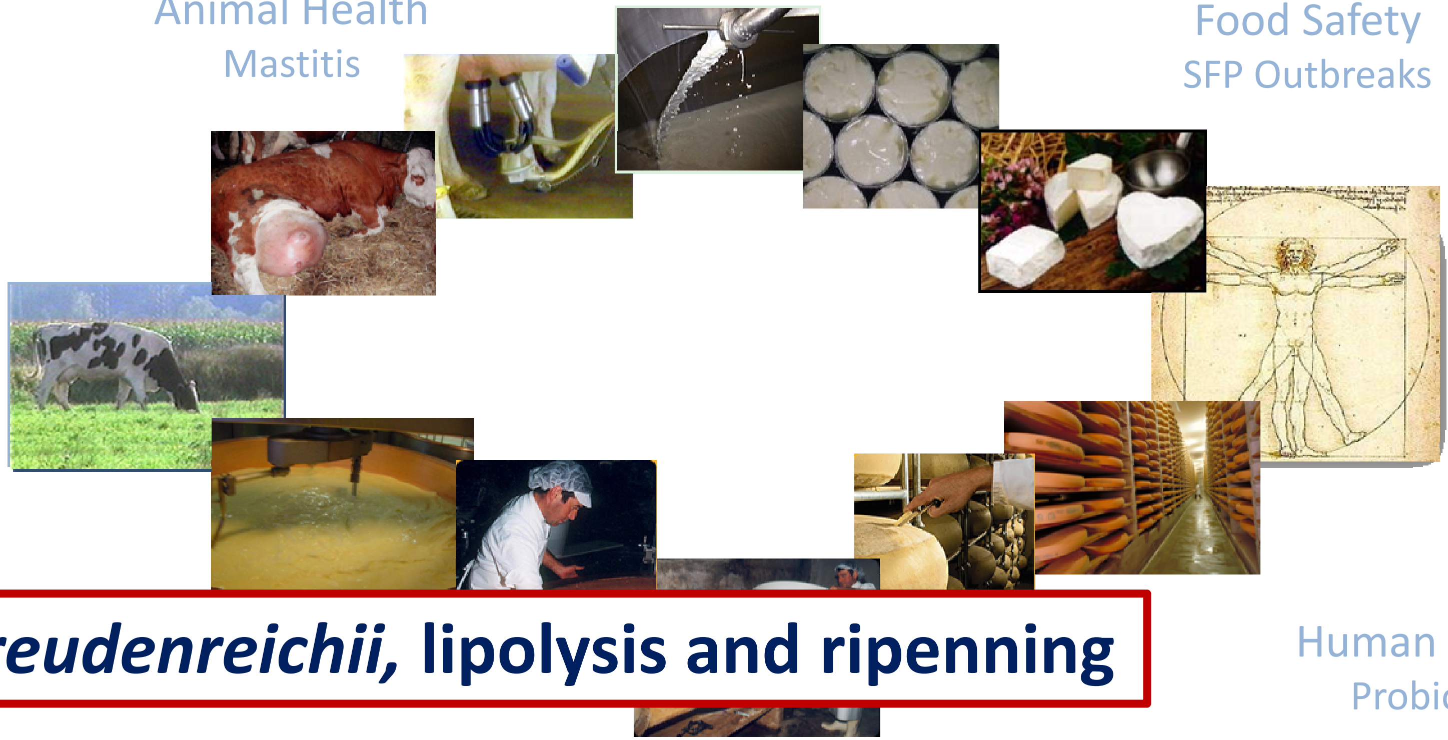
Valorisation, montage de projets

Highlights
Perspectives

S. aureus

Animal Health
Mastitis

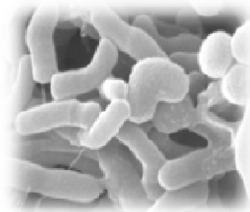
Food Safety
SFP Outbreaks



***P. freudenreichii*, lipolysis and ripenning**

Human Health
Probiotics

Cheese technology
Ripenning



P. freudenreichii

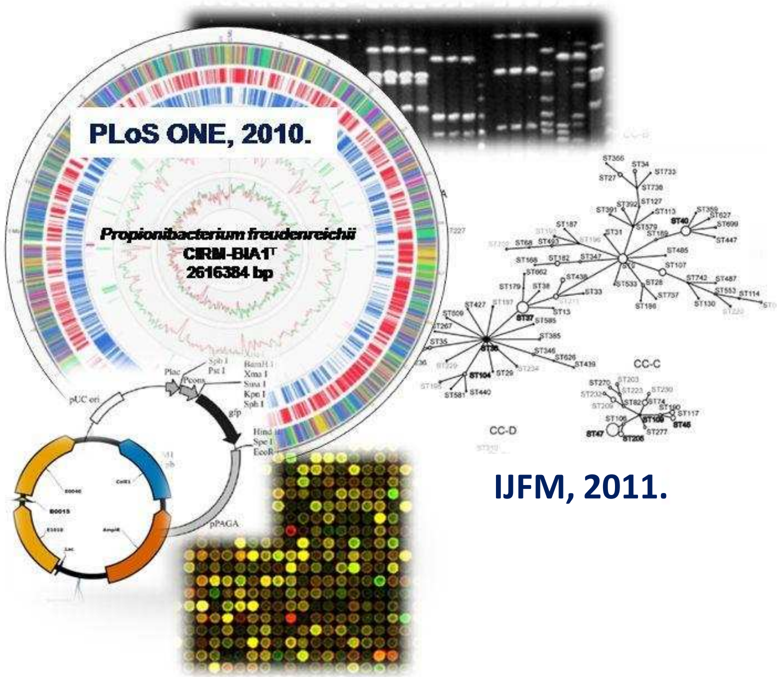
Lipolysis: essential role in the development of cheese flavor
In emmental (swiss-type cheese), *P. freudenreichii* responsible for > 95% lipolysis

Huge amount of cheese
- France: 1st emmental producer worldwide, 250 000 t/year and 13.5% of the total cow milk production

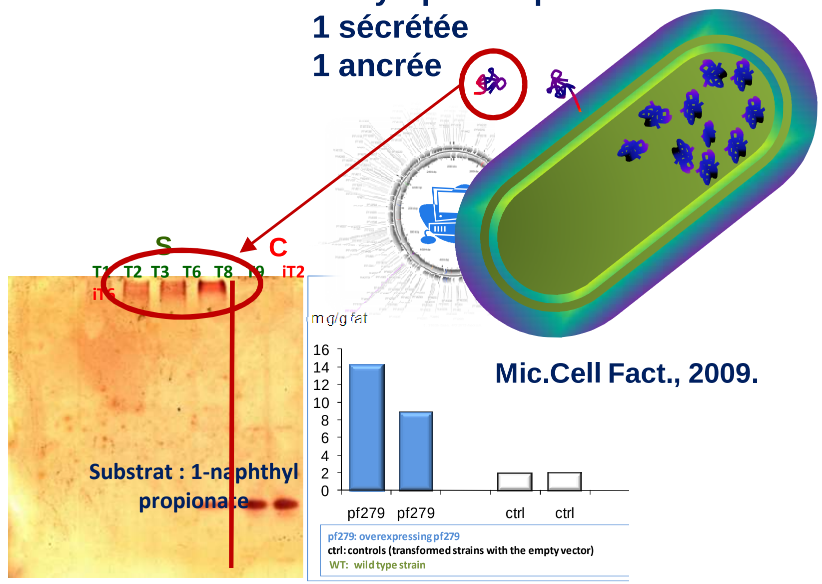
Lipolysis level is strain-dependant

Our input:
- Knowledge and access to high biodiversity / CIRM
- Integrated appraoch from gene to cheese

To identify the molecular bases of the biodiversity to enhance criteria for starter selection



Analyse *in silico* : 12 candidats estérases
10 cytoplasmiques
1 sécrétée
1 ancrée



AEM, 2010.

Identification d'une estérase sécrétée

Origine de la souche-dépendance du caractère lipolytique.
Suivi de l'expression (ciblée et globale) pendant l'affinage.

CERELA CONICET MIG Valio plateforme LAIT CIRM-BIA D2R

S. aureus

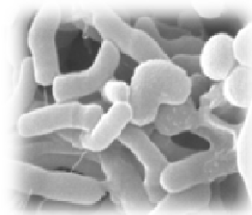
Animal health
Mastitis

Food Safety
SFP Outbreaks

Potentiel probiotique de *P. freudenreichii*

Human Health
Probiotics

Cheese technology
Ripenning



P. freudenreichii

Probiotics to limit some pathologies: digestives tract diseases or immunological disorders (allergy, MICI)

Dairy PAB have unic capabilities:

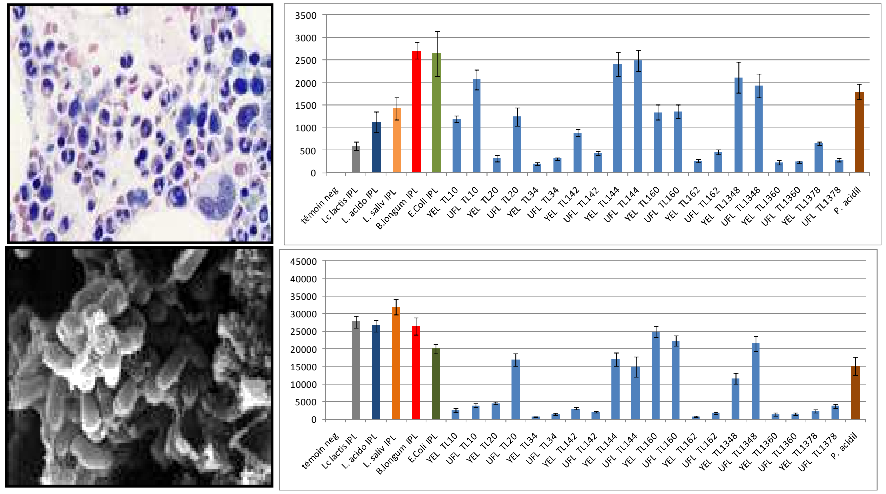
- **modulation of the host microbiota** (bifidogenic)
- **hardiness**
- **apoptosis induction** (pre-cancer cells)
- **modulation of immune response**

Our input:

- **Knowledge and access to high biodiversity / CIRM**
- **From gene to cell culture… via cheese.**

Understand the biodiversity (probiotic effects)
Demonstrate that traditional fermented products can be probiotic

Impact sur le système immunitaire

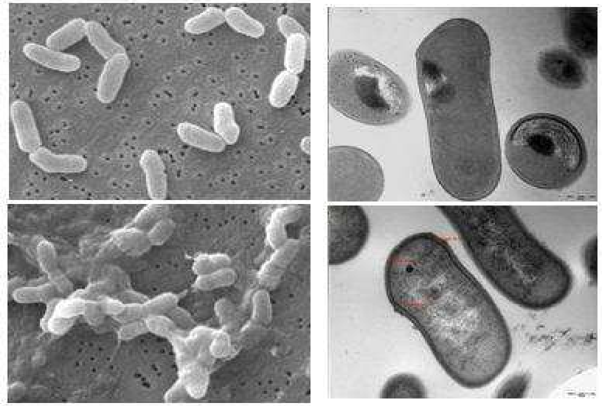


IL-10

TNF-α

AEM, 2010a.

Composés et propriétés de surface



AEM, 2010b. AEM, 2009.

Démonstration *in vitro* et *in vivo* de la capacité anti-inflammatoire.
Origine de la souche-dépendance, lien avec les propriétés de surface.

ANR

SURFING
Starter Surface against Inflammation of the Gut

Rôle des protéines de surface de *L. delbrueckii et P. freudenreichii* lans la modulation de l'inflammation intestinale

S. aureus

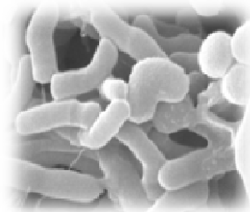
Animal Health
Mastitis

Food Safety
SFP Outbreaks

Staphylococcal diversity
Infection accuteness / bacteria-host interaction

Human Health
Probiotics

Cheese technology
Ripenning



P. freudenreichii

Mastitis induce huge economic losses in milk production

1st cause of antibiotic used in dairy herds

~5 k€ / year / 100 cows i.e. ~200 M€ in France

***S. aureus* = major agent**

Prevention and Treatment can still be optimized

Our input:

Good knowledge of *S. aureus* isolated from ruminants.

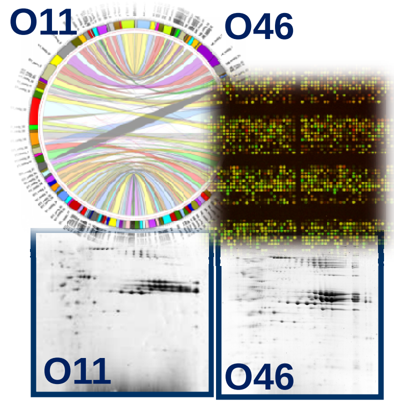
JB, 2008

Vet. Mic., 2009

Better understanding of bacteria-host interactions

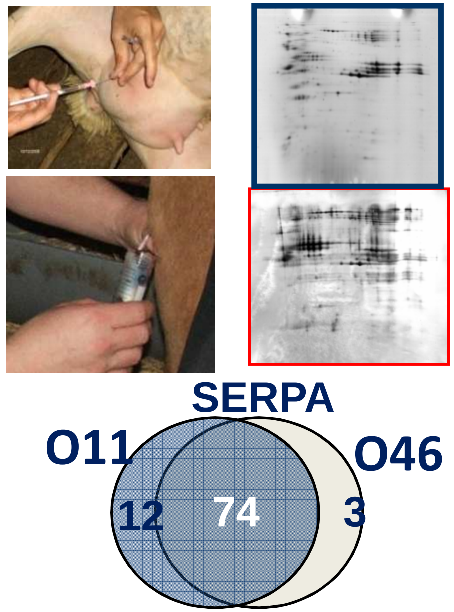
Targets for prevention strategies

Vet. Res., 2009



JB, accepted

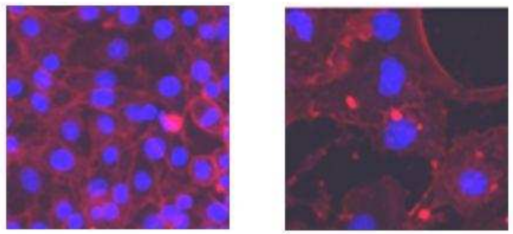
PLoS ONE, submitted



Identification de protéines produites en contexte mammite Vet. Res., 2011

Différenciation de *S. aureus* induisant des mammites modérées ou sévères.

Effet cytopathique sur cellules épithéliales mammaires

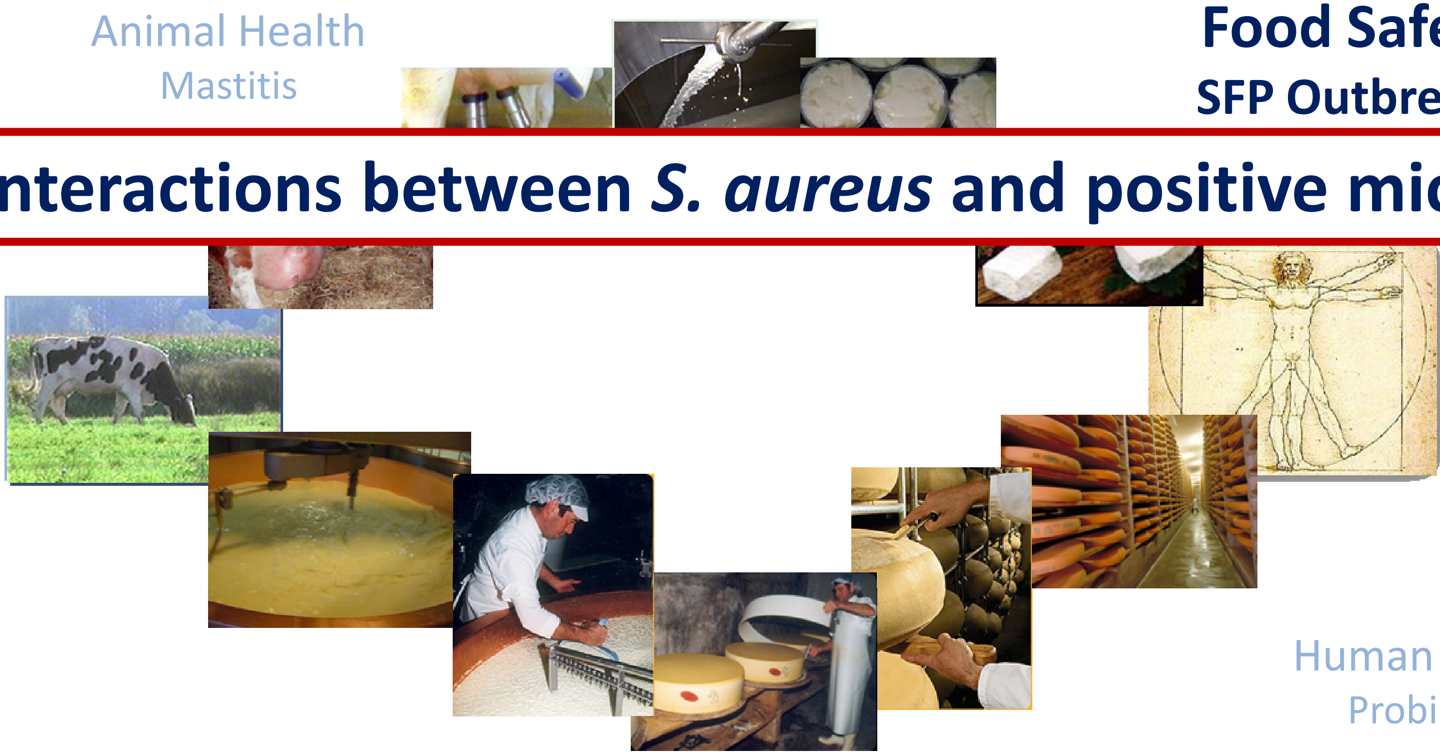


Contrôle **_S. aureus_ 72h**

Réponse des cellules de l'hôte à l'interaction avec *S. aureus*

S. aureus

Animal Health
Mastitis



Food Safety
SFP Outbreaks

Interactions between *S. aureus* and positive microbiota

Human Health
Probiotics

Cheese Technology
Ripenning

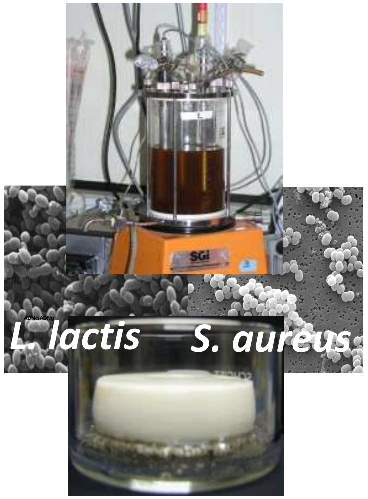
P. freudenreichii

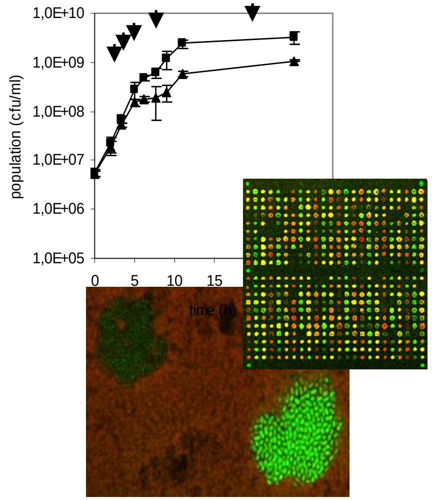
***S. aureus*, 2^{nd} cause of FP outbreaks in France**
Milk and dairy products, 1st foodstuffs involved in SFP outbreaks
Soft and semi-hard cheeses : sensitive technologies

Interactions between LAB and pathogens
Bacteriocin production / growth inhibition
***S. aureus* virulence is tightly controled (QS, environment)**

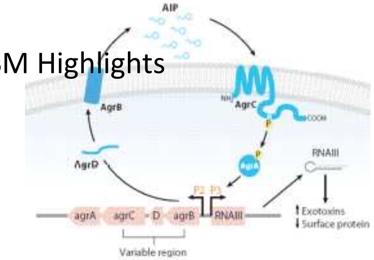
Our input:
inter-species interactions
***In situ* Interactions**
Global transcriptomic approach

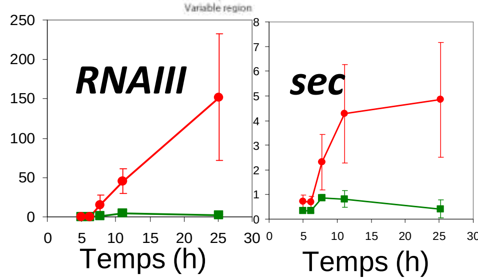
Selection natural-killer or natural-inhibitor starter strains
Application of our results to other contexts
Mastitis prevention





AEM, 2009 / ASM Highlights
AEM, 2009
EMIR, 2011

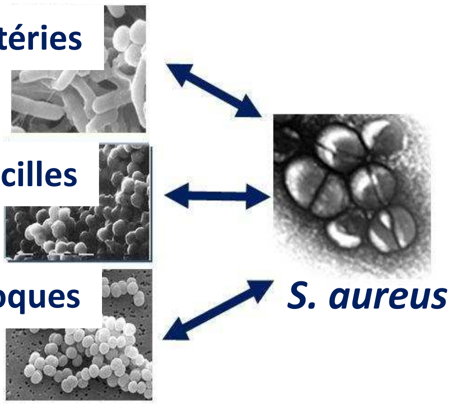


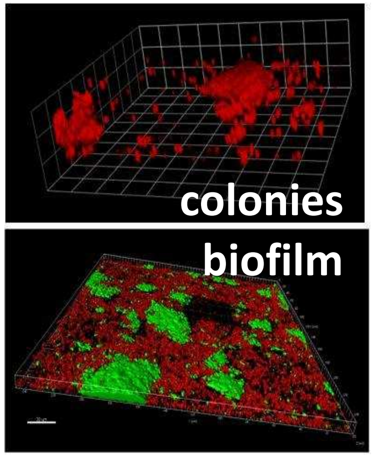


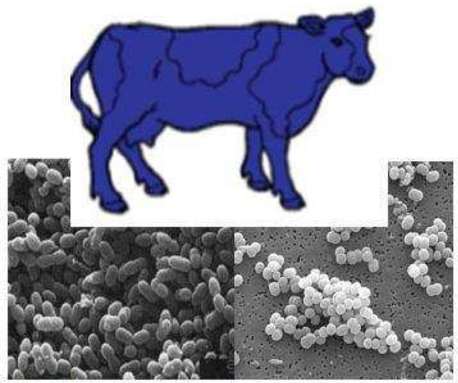
L. lactis inhibe l'expression de la virulence chez *S. aureus* en condition de laboratoire ou en matrice fromagère

AGENCE NATIONALE DE LA RECHERCHE
The NABAB Project
2009 • 2012

Exploration des capacités inhibitrices des écosystèmes naturels contre les contaminations à *S. aureus* en contexte laitier

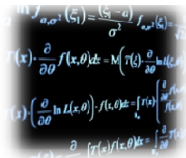






plateforme LAIT
D2R
CIRM-BIA

To sum up…



Modelling
(growth / expression)

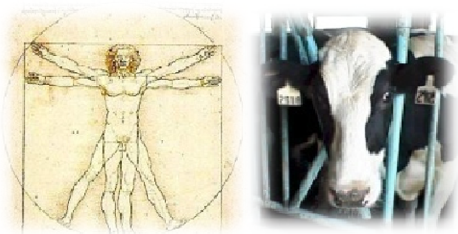
Physiology *Pf* / ripenning

Interactions *Sa* - LAB

Surface properties *Pf* / immunomodulation

Pathogenicity *Sa* / mastitis

Rational Selection
(technological / probiotic / inhibitory)



Host response
(well-being/ prevention)