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## B2ISI Team Bacterial Biodiversity And In Situ Interactions

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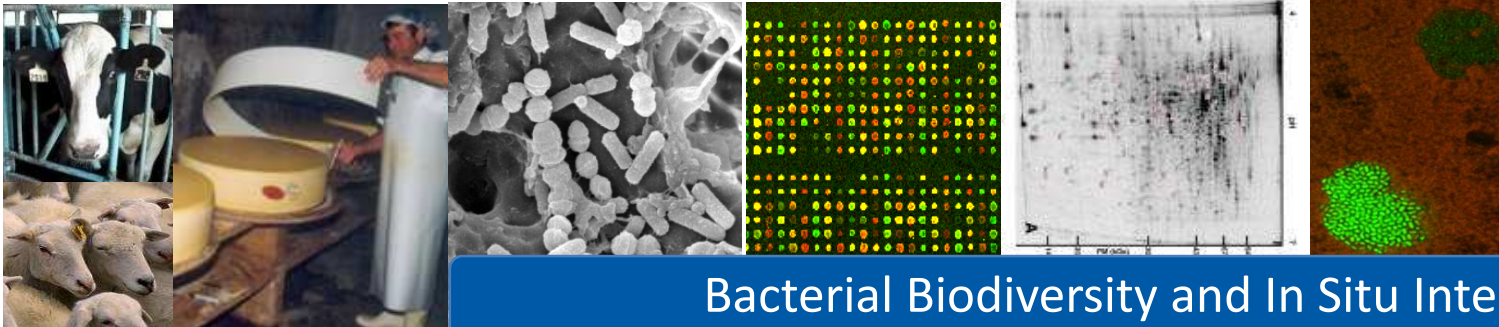
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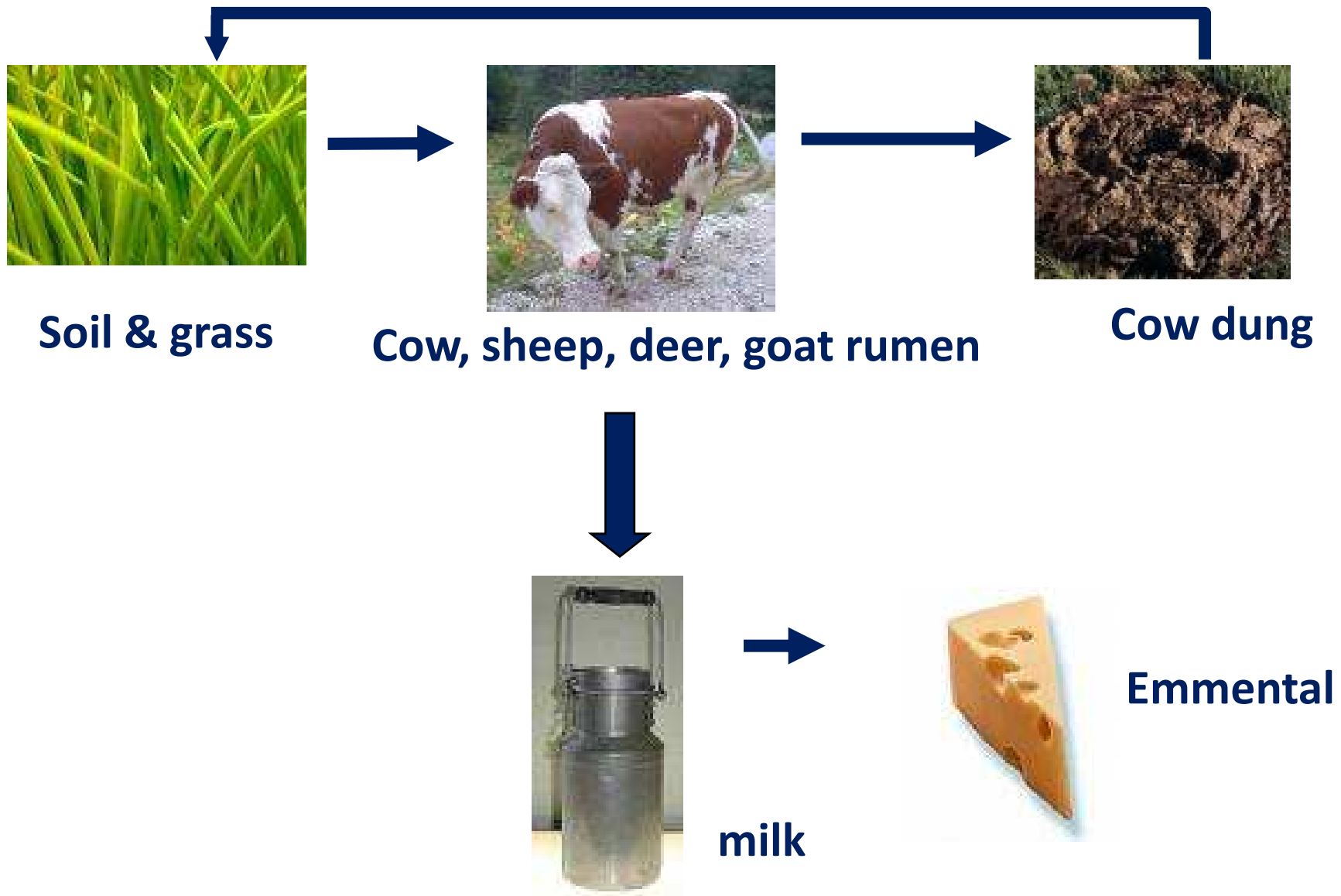
## B2ISI Team

### Bacterial Biodiversity And *In Situ* Interactions

Viçosa, April 2011



# *P. freudenreichii* ecological niches



## PROPIONIC ACID BACTERIA

Group of Gram positive bacteria

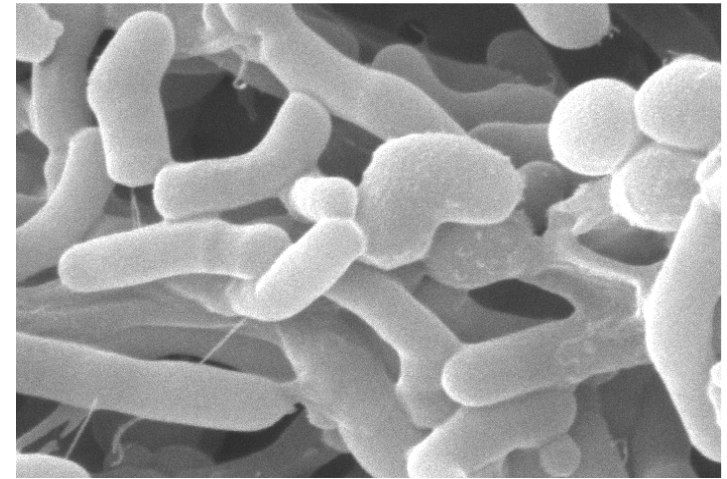
Variable morphology (chinese characters)

Irregular rods (diam., 0.5 to 0.8  $\mu\text{m}$ ; length, 1 to 5  $\mu\text{m}$ )

Catalase<sup>-</sup>, non spore-forming, non-motile,  
anaerobe-aerotolerante, mesophilic

Fermentation of sugars into propionic acid

In the cheese:



## **Pathogenic propionic acid bacteria**

*Propionibacterium acnes*

## **Dairy propionic acid bacteria**

*Propionibacterium freudenreichii* ssp *freudenreichii*

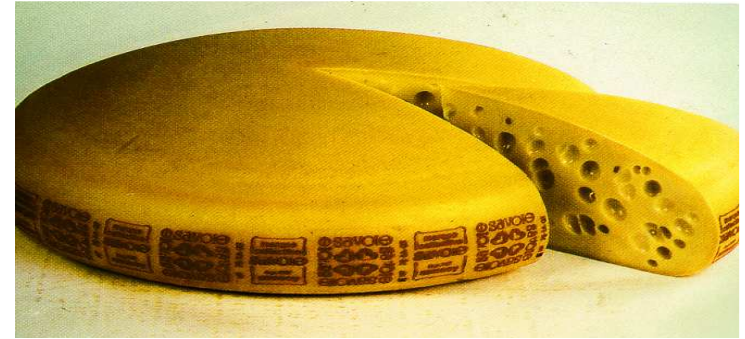
*Propionibacterium freudenreichii* ssp *shermanii*

*Propionibacterium jensenii*

*Propionibacterium acidi propionici*

*Propionibacterium thoneii*

## Role of the PAB



Involved in the ripening 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  $\text{CO}_2$

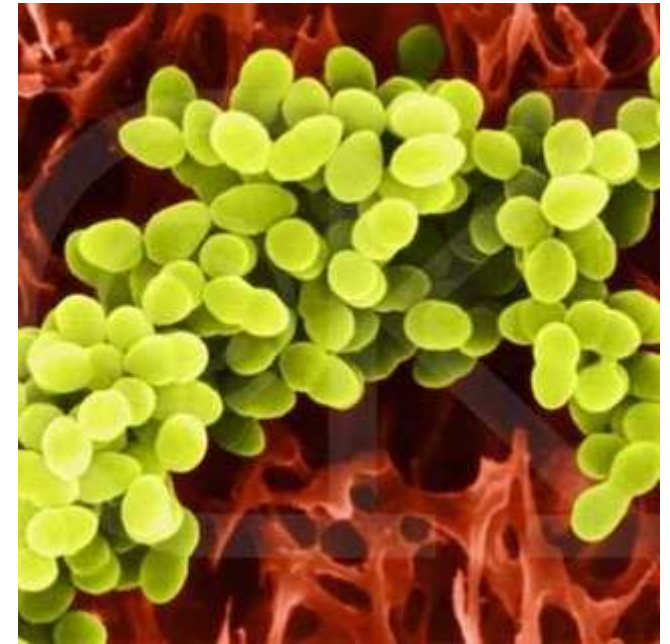
Development of the flavor :

Short chain fatty acid, propionic acid and acetic acid, acetaldehyde, 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.

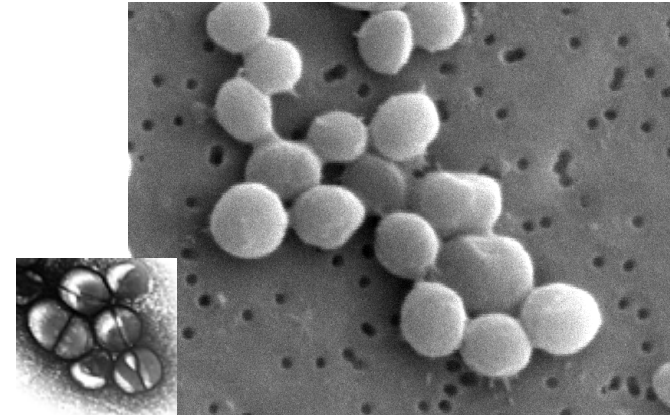
Grape-like clusters. Catalase+

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

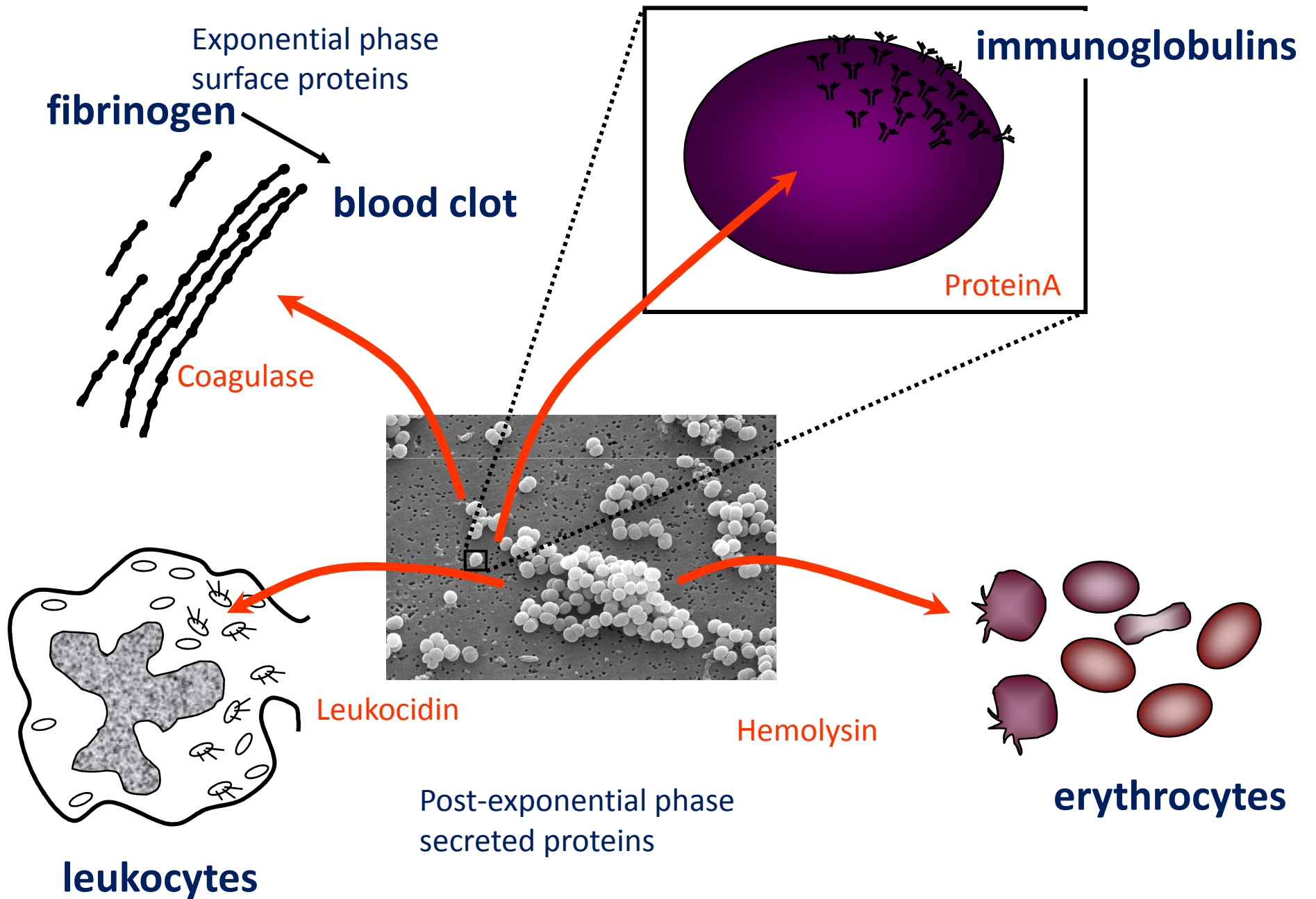
Skin abscess  
Eye infection  
Wound infection  
Mastitis  
involved  
Septic arthritis  
Osteomyelitis  
Kidney abscess, nephritis  
Septicemia



numerous virulence factors

Staphylococcal scalded skin syndrome  
Toxic shock syndrome  
Staphylococcal food poisoning

Exfoliatins  
TSST1 (and enterotoxins)  
Enterotoxins



# Context

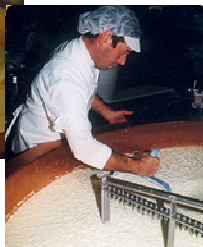
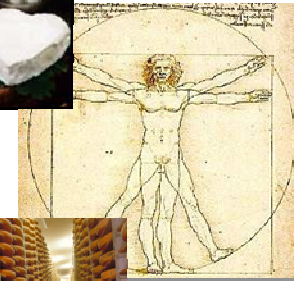
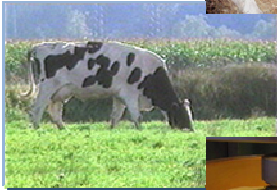


*S. aureus*

Animal Health  
Mastitis

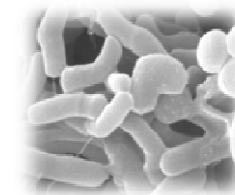


Food Safety  
SFP Outbreaks



Human Health  
Probiotics

Cheese Technology  
Ripening



*P. freudenreichii*

## Our goals

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

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

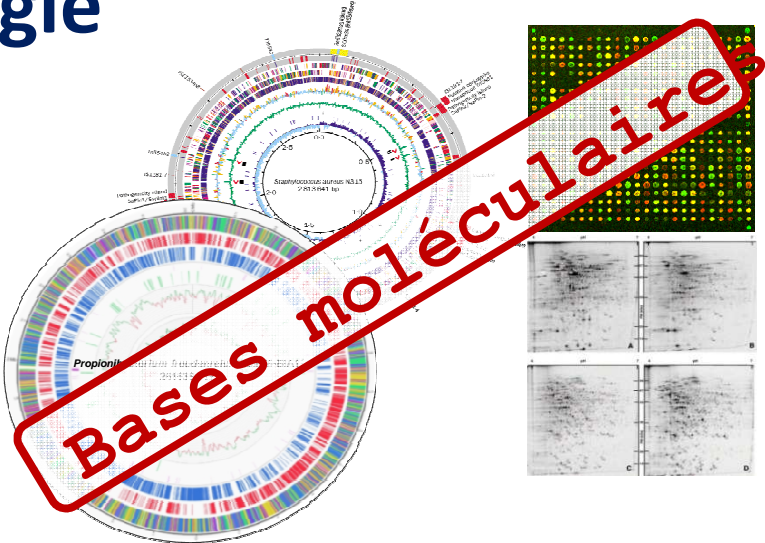
To understand ***in situ* interactions**, **cheese** matrix or with the **host** (Digestive tract or mammary gland).

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

# Stratégie



Diversité



Bases moléculaires



Interactions in situ



Maîtrise

Animation scientifique  
Valorisation, montage de projets

# Highlights Perspectives

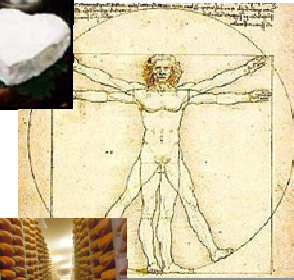


*S. aureus*

Animal Health  
Mastitis

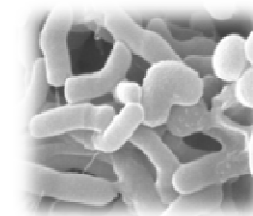


Food Safety  
SFP Outbreaks



***P. freudenreichii*, lipolysis and ripening**

Human Health  
Probiotics



**Cheese technology  
Ripening**

***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: 1<sup>st</sup> emmental producer worldwide, 250 000 t/year and 13.5% of the total cow milk production

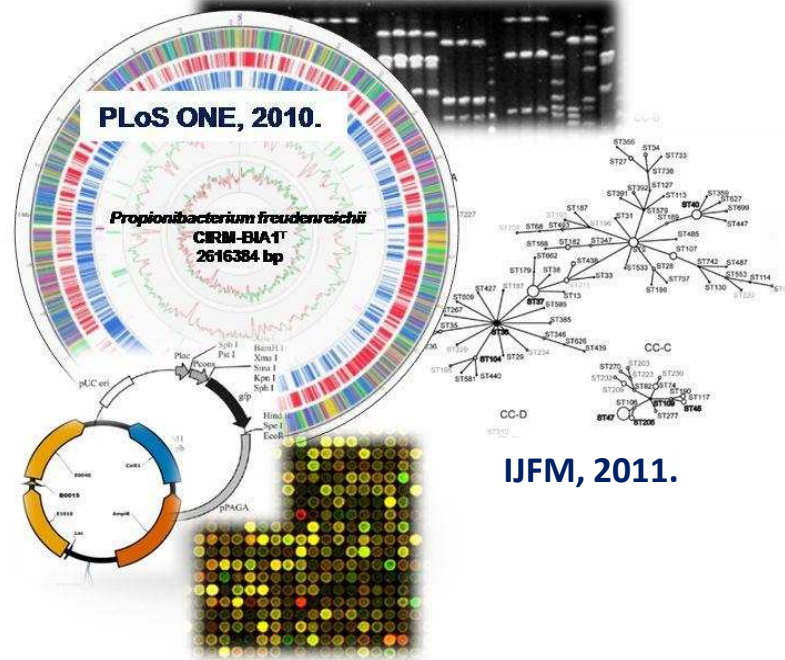
**Lipolysis level is strain-dependant**

**Our input:**

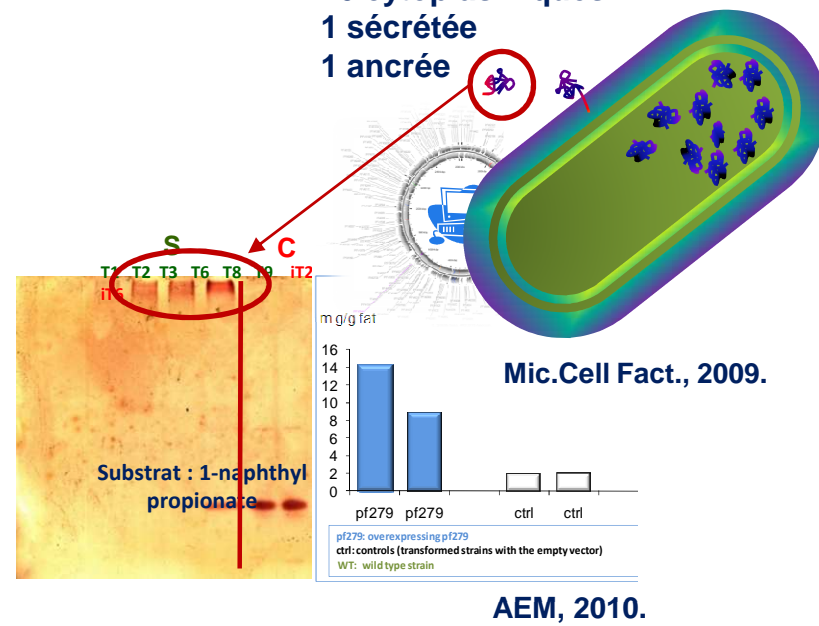
**Knowledge and access to high biodiversity / CIRM**  
**Integrated approach from gene to cheese**

**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



## Identification d'une estérase sécrétée

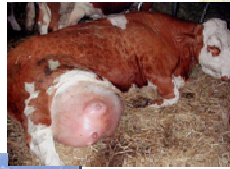
Origine de la souche-dépendance du caractère lipolytique.  
 Suivi de l'expression (ciblée et globale) pendant l'affinage.



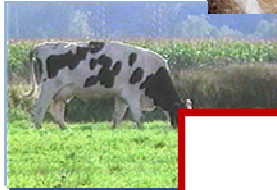
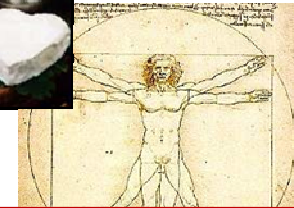


*S. aureus*

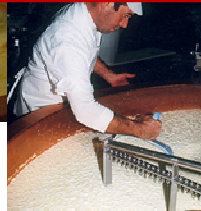
Animal health  
Mastitis



Food Safety  
SFP Outbreaks

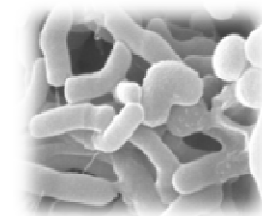


**Potentiel probiotique de *P. freudenreichii***



Human Health  
Probiotics

Cheese technology  
Ripening



*P. freudenreichii*

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

**Dairy PAB have unique capabilities:**

**modulation of the host microbiota (bifidogenic)**

**hardiness**

**apoptosis induction (pre-cancer cells)**

**modulation of immune response**

**Our input:**

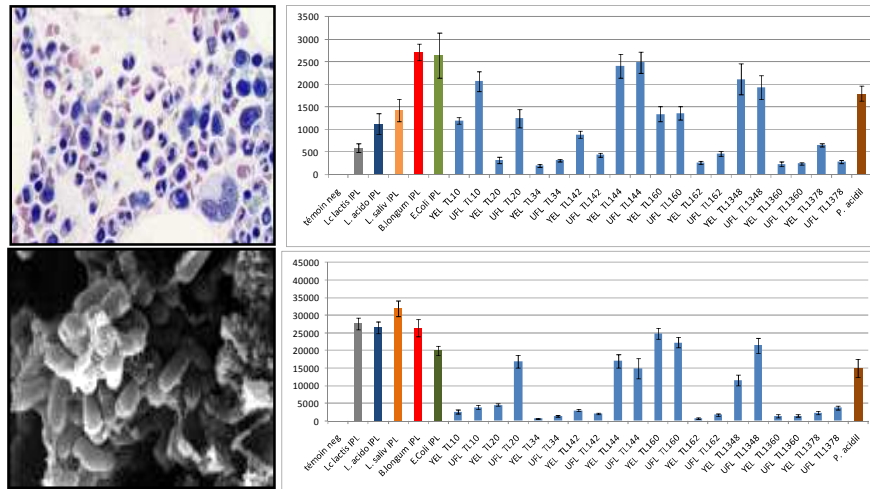
**Knowledge and access to high biodiversity / CIRM**

**From gene to cell culture... via cheese.**

**Understand the biodiversity (probiotic effects)**

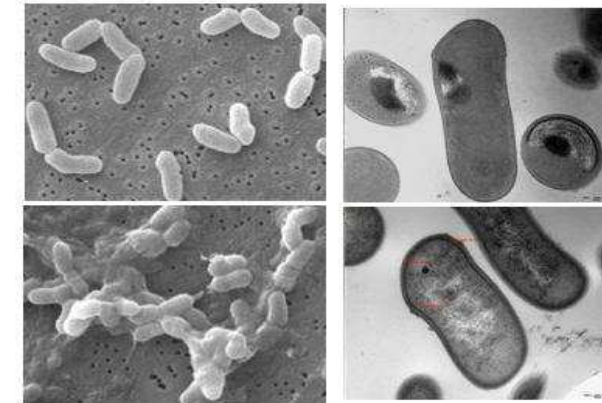
**Demonstrate that traditional fermented products can be probiotic**

## Impact sur le système immunitaire



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.



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





*S. aureus*

Animal Health

Mastitis



Food Safety

SFP Outbreaks

# Staphylococcal diversity

## Infection acuteness / bacteria-host interaction

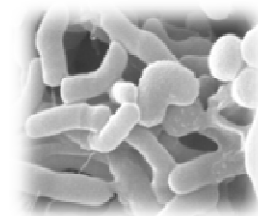


Human Health

Probiotics

Cheese technology

Ripening



*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**

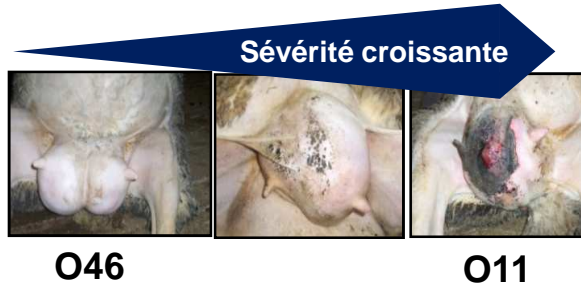
**Our input:**

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

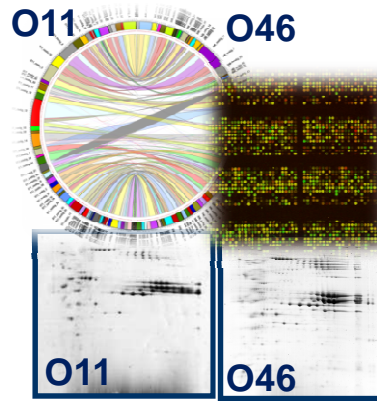
JB, 2008

Vet. Mic., 2009

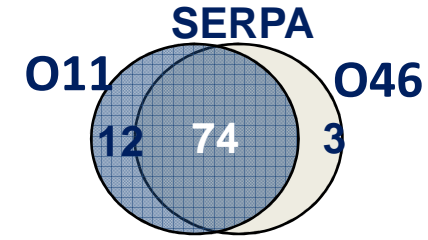
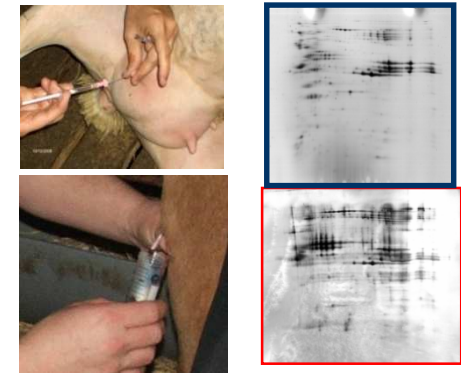
**Better understanding of bacteria-host interactions**  
**Targets for prevention strategies**



Vet. Res., 2009



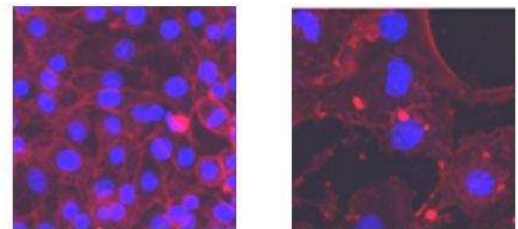
JB, accepted  
PLoS ONE, submitted



**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  
Ripening



*P. freudenreichii*



***S. aureus*, 2<sup>nd</sup> 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 controlled (QS, environment)**

**Our input:**

**inter-species interactions**

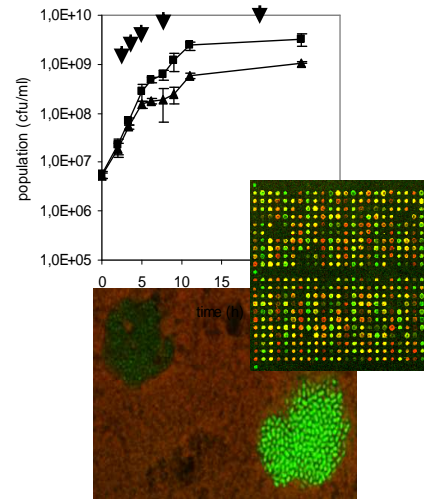
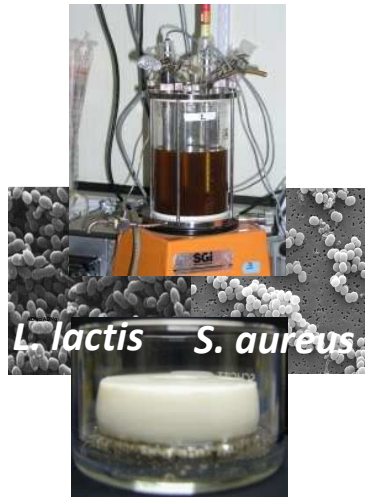
***In situ* Interactions**

**Global transcriptomic approach**

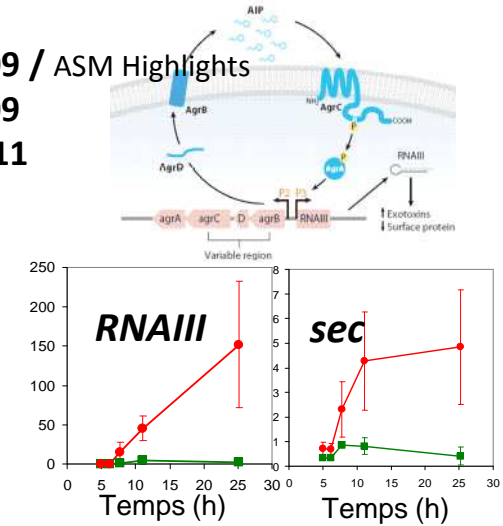
**Selection natural-killer or natural-inhibitor starter strains**

**Application of our results to other contexts**

**Mastitis prevention**



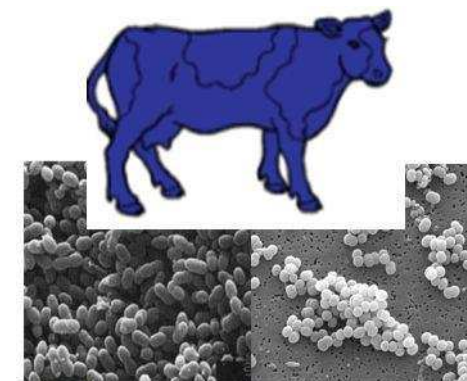
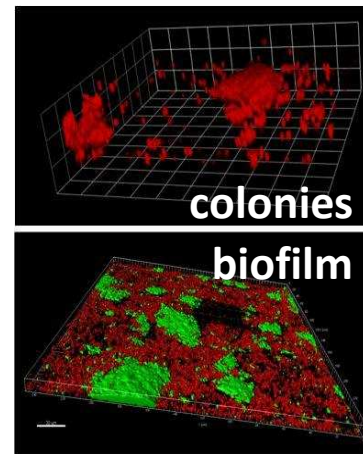
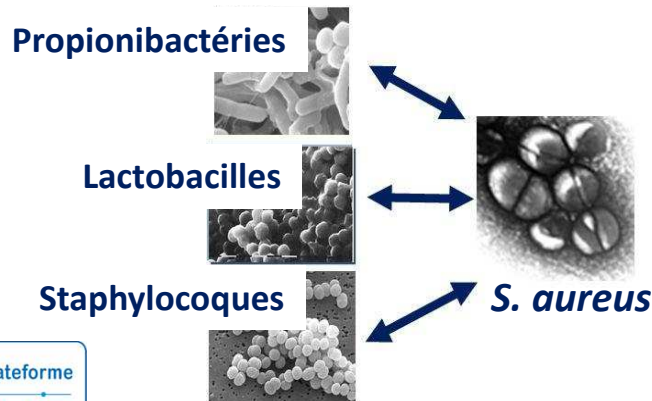
AEM, 2009 / ASM Highlights  
 AEM, 2009  
 EMIR, 2011



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



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



D2R



# To sum up...

$$\begin{aligned} \frac{\partial}{\partial t} f(x,0) &= -M \left( \frac{\partial}{\partial x} \ln M(x,0) \right) \cdot f(x,0) \\ \frac{\partial}{\partial t} \ln M(x,0) &= -M \left( \frac{\partial}{\partial x} \ln M(x,0) \right) \cdot f(x,0) \end{aligned}$$

**Modelling**  
(growth / expression)

**Physiology *Pf* / ripening**

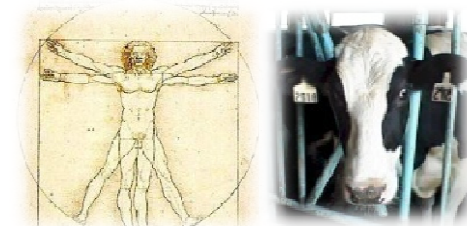
**Interactions *Sa* - LAB**

**Surface properties *Pf* /  
immunomodulation**

**Pathogenicity *Sa* / mastitis**



**Rational Selection**  
(technological / probiotic / inhibitory)



**Host response**  
(well-being/ prevention)