Biopreservation of foodstuffs: Mechanisms and application

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European FooD-STA

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- Introduction
- The actors
- Their roles and plays
- Future trends

Introduction

Biopreservation definition

Michael E. Stiles Antonie van Leeuwenhoek 1996 Oct;70(2-4):331-45.

“Biopreservation refers to **extended storage life** and **enhanced safety** of foods using the **natural microflora** and (or) their **antibacterial products**. **Lactic acid bacteria** have a major potential for use in biopreservation because they are safe to consume and during storage they naturally dominate the microflora of many foods. In milk, brined vegetables, many cereal products and meats with added carbohydrate, the growth of lactic acid bacteria produces a **new food product**. In raw meats and fish that are **chill stored** under vacuum or in an environment with elevated carbon dioxide concentration, the lactic acid bacteria become the dominant population and preserve the meat with a **“hidden” fermentation**.

The food ecosystem

extended storage life

enhanced safety

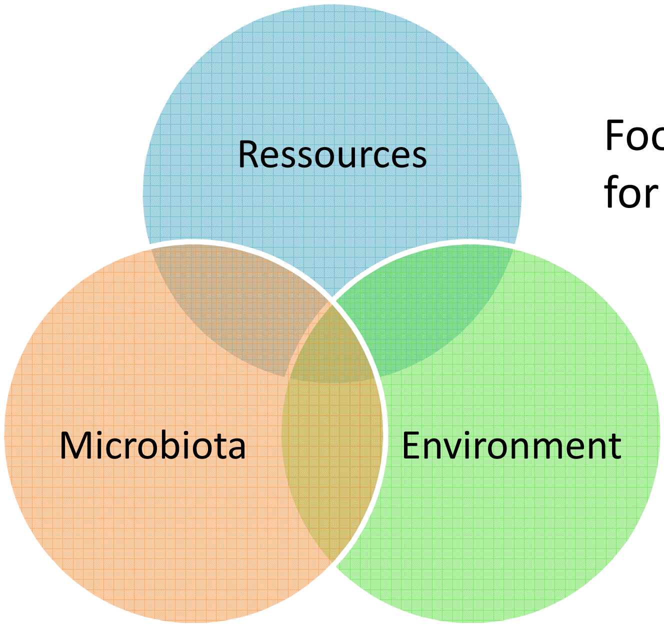
natural microflora

antibacterial products

Lactic Acid Bacteria

new food product

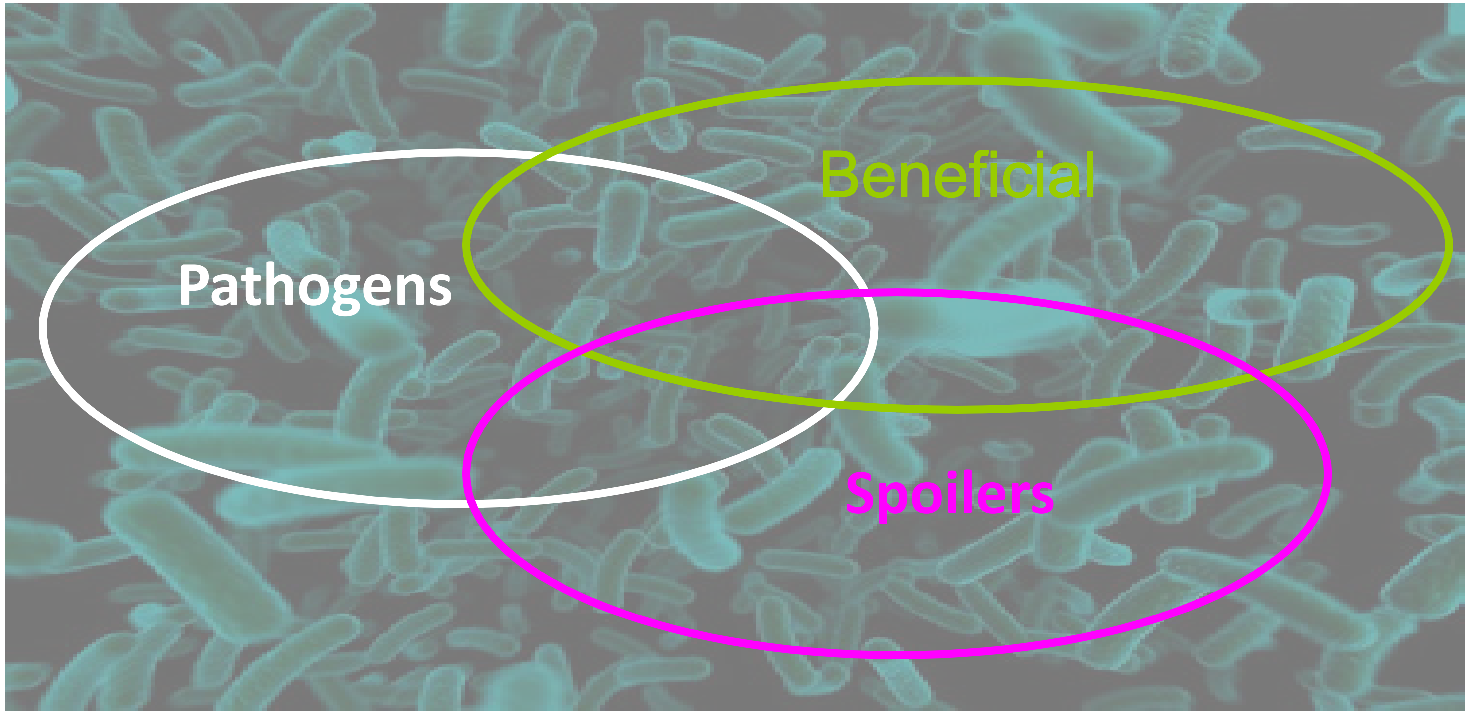
chill stored



Food matrix: nutrients for microorganisms

Process: temperature, pH, gaz, additives, …

Food bacterial microbiota



- Diversity
- Abundance
- Dynamics during storage and process
- Species status
- Beneficial or spoiler ?

Microorganisms in food

Microorganism communities

Risk

Benefit

Illness

Spoilage

Fermentation

New products

Biopreservation

Same product/chill stored

Biopreservation

Limiting growth and/or survival of unwanted microorganisms (pathogens/spoilers)
Limiting production or amounts of undesirable compounds

To lower

Thermical treatments
Preservatives (NaCl, nitrites)

CLEAN LABEL

To improve

Organoleptic properties
Safety
Shelf life
Waste and losses

GREEN LABEL

Different targets/different strategies

Pathogens	one species /low number	specific tool (bacteriocin)
Spoilers	different species/high level	competitiveness

Hurdle technology

Knowing bacterial communities!

Ecological strategy

Evolution of biopreservation strategies

- Screening in lab medium
- Target: pathogens
- One species
- Single strain

- Global food microbiota characterization
- NGS
- Exploiting genomic diversity
- Fitness
- Ecology

Which products?

- Fresh products stored at low temperature (dairy, meat, fish products, vegetal,…)


- Fermented foods (dairy/meat)


- Processed foods (cooked/dried/ …




Examples of commercial applications

Dairy products

Product	Benefit	Application
HOLDBAC® YM-C Plus HOLDBAC® YM-B Plus	Growth control of yeasts and molds and some heterofermentative lactic bacteria	Fresh fermented foods White cheese
HOLDBAC® LC	Growth control of leuconostoc, heterofermentative lactobacilli and enterococci	Hard and semi-hard cheese
HOLDBAC® Listeria	Growth control of Listeria	Soft and smear cheese, dry and semi-dry cured meats, cooked and fresh ground meats

Fish products

The Sacco company has a protective culture for fish processing. **Lyoflora FP-18** is made of Carnobacterium producers of bacteriocins that inhibit the growth of L. monocytogenes in fish products.
The culture was developed by two French research institutes, Ifremer and Oniris, who hold the scientific documentation.

https://www.ingredientsnetwork.com/fish-cultures-prod958590.html

Examples of commercial applications

The protective lactic acid bacteria ferments can ensure or extend the shelf life by inhibiting the growth of spoilage bacteria (coliforms, Pseudomonas, other lactic acid bacteria, histamine-producing bacteria) or certain pathogens (Listeria monocytogenes).

http://www.bioceane.com/en/bio-preservation

Bactoferm™ B-LC-007 is a patented culture blend capable of acidification as well as preventing growth of *Listeria*. The culture produces pediocin and bavaricin (think of them like a kind of "antibiotics") that keeps *Listeria monocytogenes* at safe levels by the additional hurdle thrown at it

https://www.butcherspantry.com/starter-cultures/bactoferm-b-lc-007

The actors

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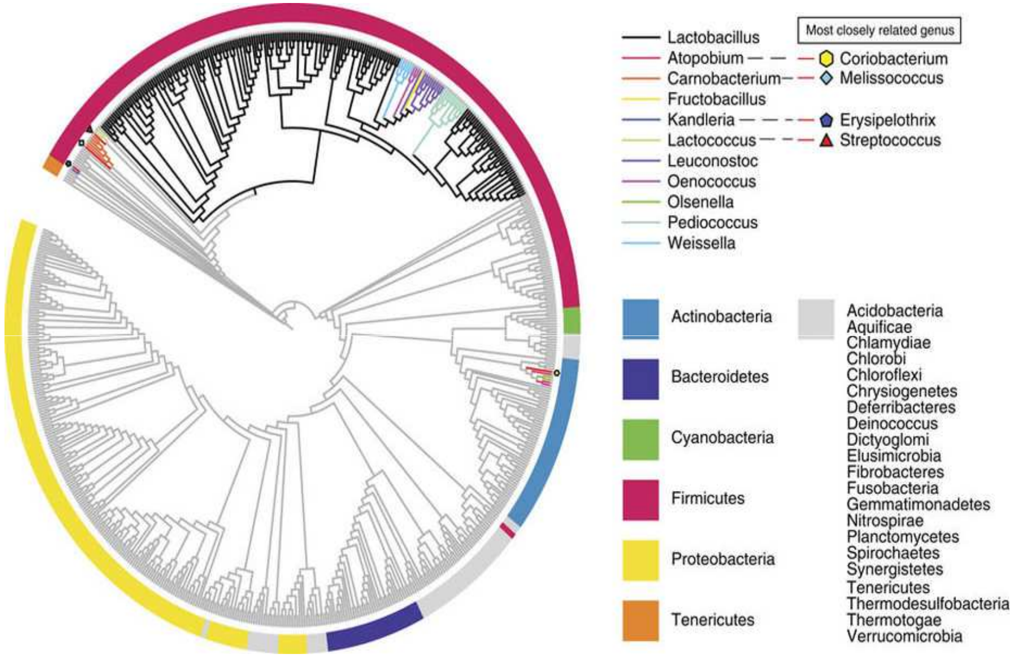
The biopreservers/bioprotective cultures

- Lactic acid bacteria: good candidates
- Natural flora
- Nutrient rich niches (animals, plants, humans, food)
- Fermented foods
- QPS (Qualified Presumption of Safety), GRAS (Generally Recognized as Safe)
- Genomic diversity
- Metabolic diversity
- Metabolite production: organic acid, hydrogen peroxide, anti microbial compounds (bacteriocins)
- Competition for ressources
- Hurdle

Lactic acid bacteria

***Lactobacillus*, *Lactococcus*,**
Leuconostoc, Pediococcus,
Streptococcus, ***Carnobacterium***,
Enterococcus, Oenococcus,
Vagococcus, Aerococcus,
Weissella, Tetragenococcus



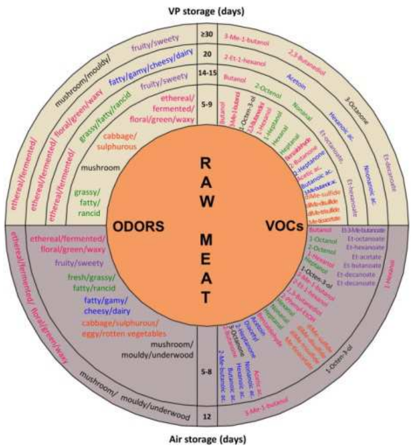
Sun et al, Nature Communications,2014

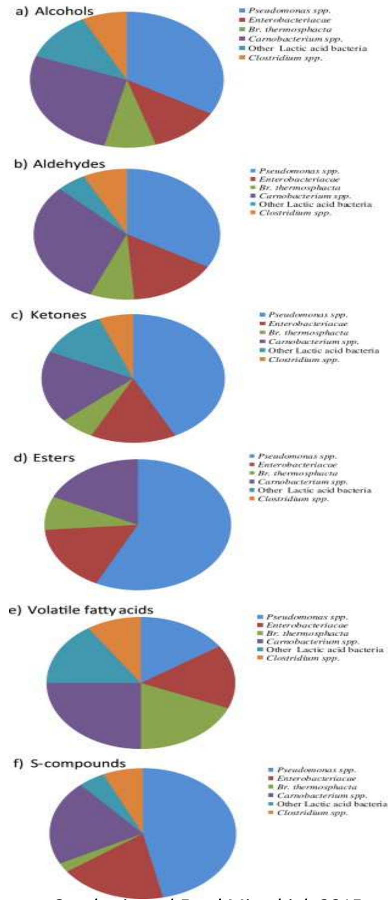
Pathogens targeted by biopreservation

- A few species
- ***Listeria monocytogenes***
- *Escherichia coli (O157:H7)*
- *Salmonella*
- *Staphylococcus aureus*

Spoilers and spoilage

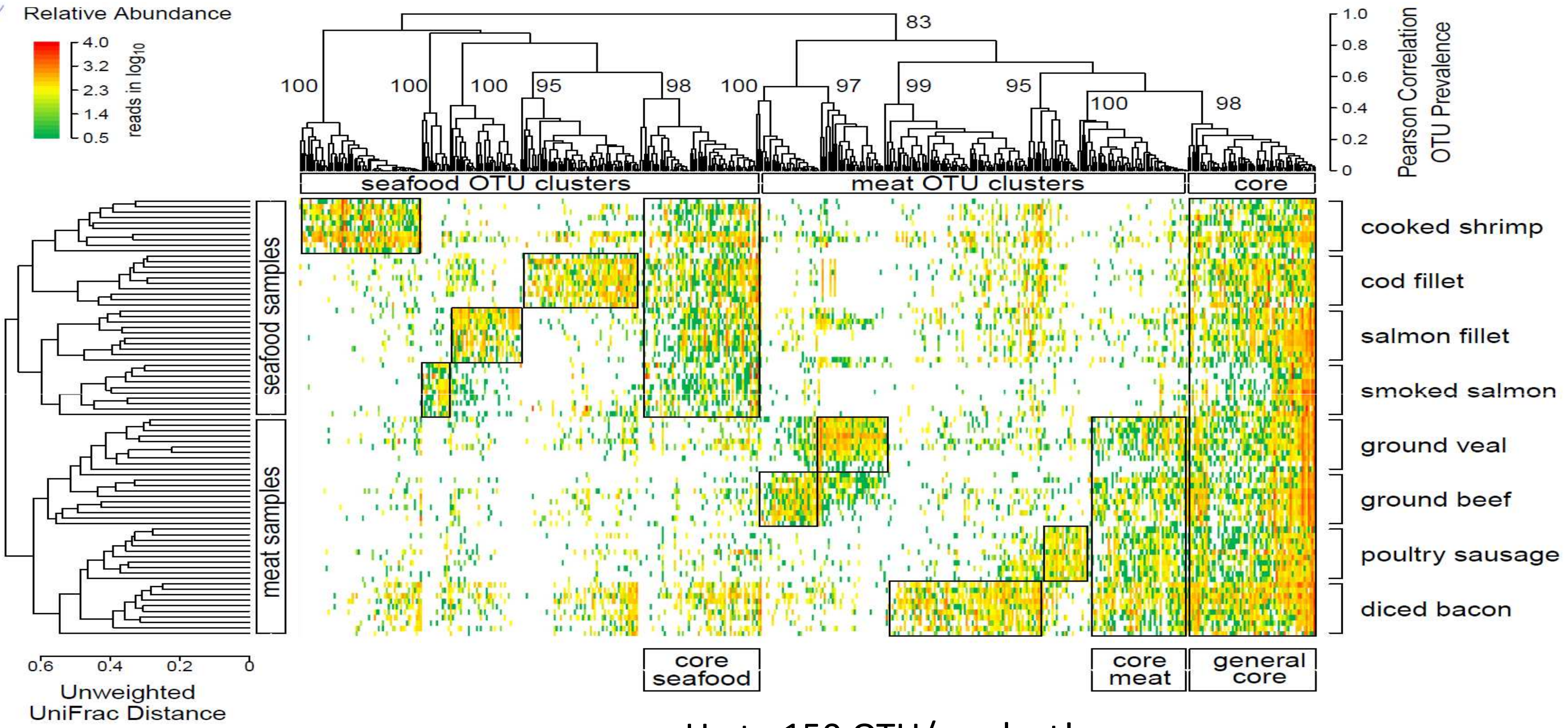
- Soilage: a complex phenomenum
- Many volatile organic compounds can be produced by spoilage microorganisms
- Many spoilage organisms involved
- Microbiota characterization
- Metagenomics/NGS





Casaburi et al Food Microbiol. 2015

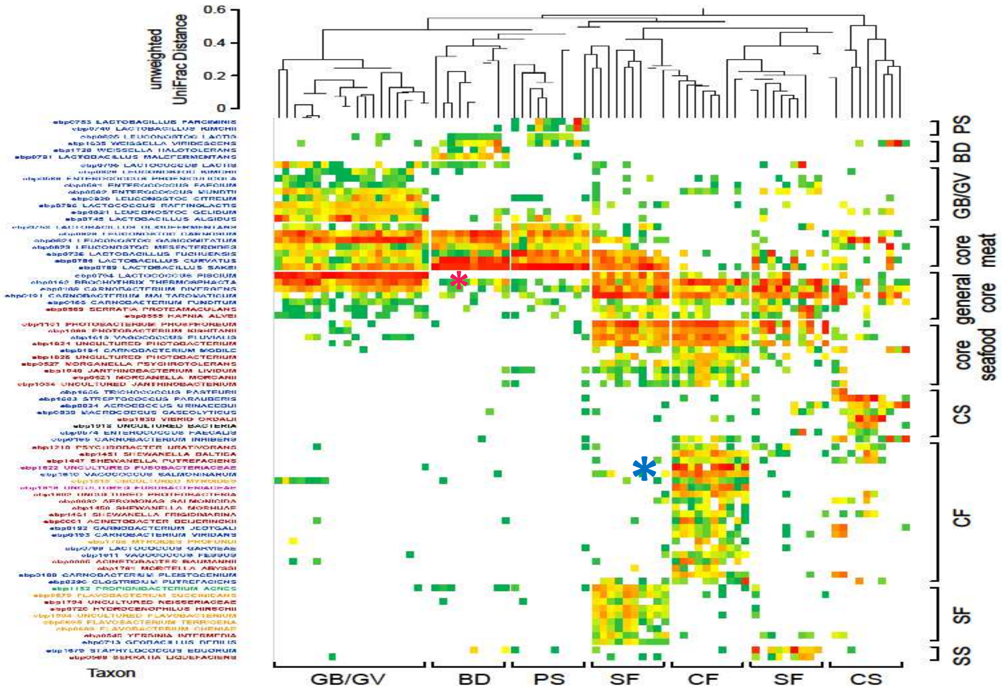
Natural flora of fresh food: a high diversity



Up to 150 OTU/product!

Chaillou et al ISME J. 2015

A revisited version of spoilage?



- 15 to 60 species
- Unsuspected species *
- A putative new dominant spoiling species for fish?
- Yet uncultured! *

Chaillou et al ISME J. 2015

The roles and plays
some examples of mechanisms

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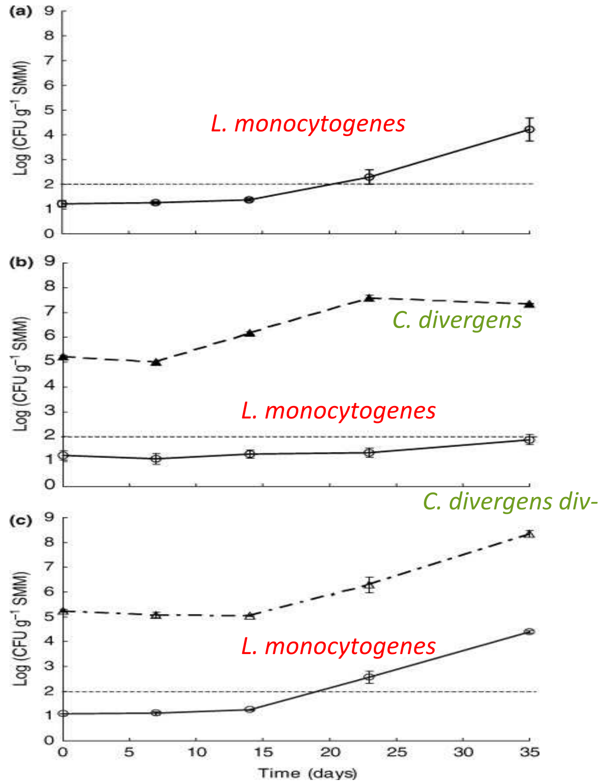
Inhibition of *L. monocytogenes*: a bacteriocin producing *Carnobacterium*

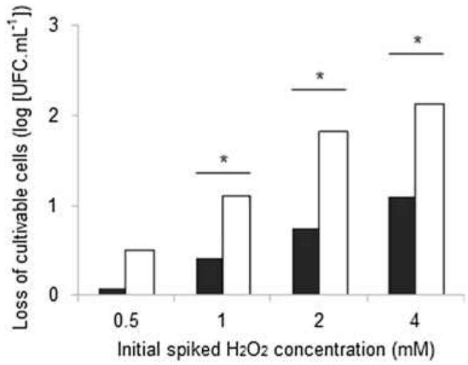
- *Carnobacterium divergens* V41
- Bacteriocin: divercin
- Mutant *div* -
- Smoked salmon
- Challenge tests



Richard et al 2003, Lett Appl Microbiol

Inhibition of *S. aureus* by *Lactococcus garviae*: H_2O_2 production?

- Growth inhibition of *S. aureus* in milk, cheese
- *in vitro* inhibition modulated by level of aeration
- Inhibition higher when *L. garviae* produces H_2O_2
- Transcriptomic analysis
- Response of *L. garvieae* to aeration level differs according to the presence or absence of *S. aureus*.
- Higher concentration of H_2O_2 (with high aeration) not associated with a higher expression of *L. garvieae* H_2O_2 synthesis gene response but rather with a repression of *L. garvieae* H_2O_2 degradation genes (*trxB1*, *ahpC*, *ahpF*, and *gpx*).
- Original, previously undiscovered, H_2O_2 production regulation.
- Another extra cellular factor ?

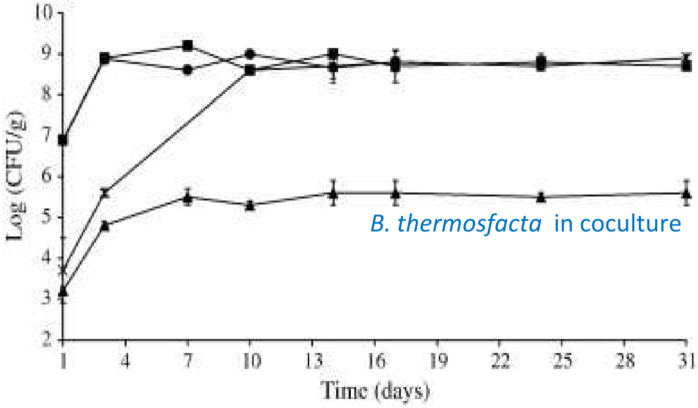


Loss of *S. aureus* SA15 (empty bars) and MW2 (full bars) cultivable cells [a]under H_2O_2-stress condition. [[a]difference between the cellular concentration in culture with no H_2O_2 and cultures with H_2O_2 spiking (0.5, 1, 2 or 4 mM) after two hours of incubation. *significant difference between the two *S. aureus* strains (p-value < 0.05 according to Newman–Keuls test)].

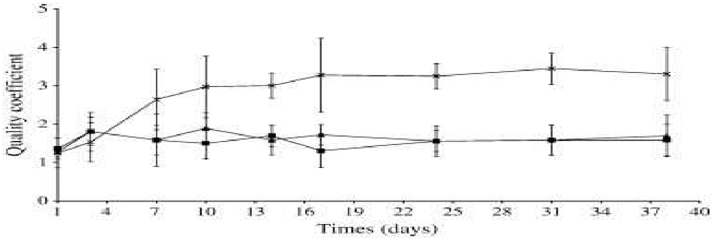
Delbes Paus et al Food Microbiol 2010, Delpech et al Food Microbiol 2015, Delpech P et al Front Microbiol. 2017

Inhibition of spoilage by *Lactococcus piscium*

- Shrimp spoilage prevention (sensory quality)
- Strain dependent
- *In vitro* inhibitory activity against *Brochothrix*, *Carnobacterium*, *Lactobacillus*, *Vagococcus*, *Enterococcus, Psychrobacter, Schewanella, Pseudomonas,...*
- Inhibition of *Brochothrix thermosfacta* in food matrixes (salmon, shrimps)
- Difficult to reproduce *in vitro*??
- Mechanism?



Growth of *Lactococcus piscium* CNCM I-4031 and *Brochothrix thermosphacta* CD340 in peeled and cooked shrimp packed under modified atmosphere and stored at 8 °C. (■) *L. piscium* alone; ● *L. piscium* in co-inoculation; (x) *B. thermosphacta* alone; (▲) *B. thermosphacta* in co-inoculation.

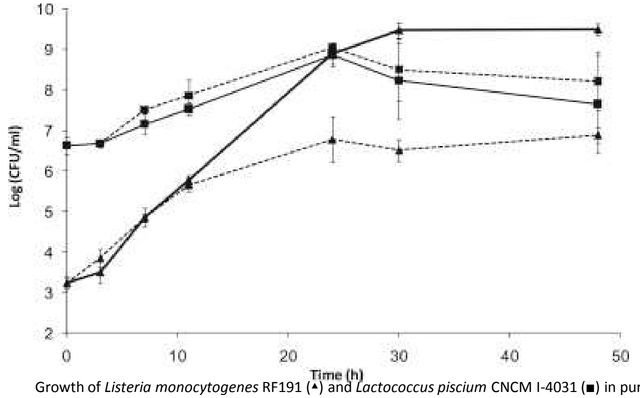


Quality coefficient of inoculated cooked peeled MAP shrimp stored at 8 °C. (■) scores of batches inoculated with *L. piscium* CNCM I-4031 alone; (x) *B. thermosphacta* CD340 alone; (▲) *L. piscium and B. thermosphacta*.

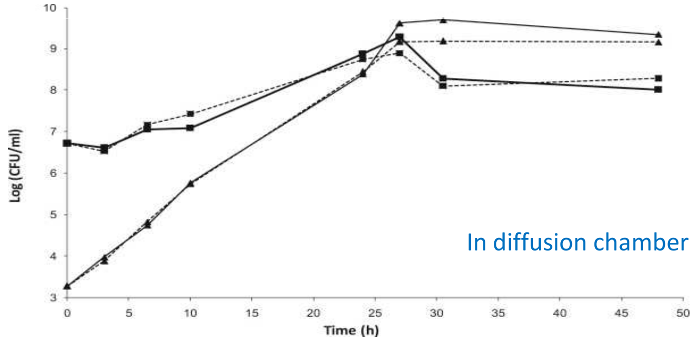
Fall et al , IJFM, 2012, Saraoui et al, J.Appl Microbiol, 2016, Leroi et al 2015

Inhibition of *Listeria monocytogenes* by *Lactococcus piscium*: a contact dependent mechanism

- *Lactococcus piscium CNCM1-4031*
- Inhibition of *L. monocytogenes* in shrimps
- *In vitro, a* chemically defined medium
- a contact dependent mechanism
- cell /cell communication mechanism?



Growth of *Listeria monocytogenes* RF191 (▲) and *Lactococcus piscium* CNCM I-4031 (■) in pure culture (full line) and in co-culture (dotted line) in MSMA at 26 °C.



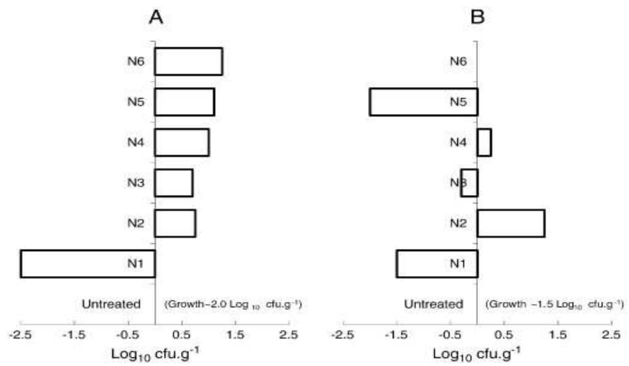
Growth of *Listeria monocytogenes* RF191 (▲) and *Lactococcus piscium* CNCM I-4031 (■) alone (against sterile MSMA) (full line) and in co-culture (dotted line) in MSMA at 26 °C using a diffusion chamber.

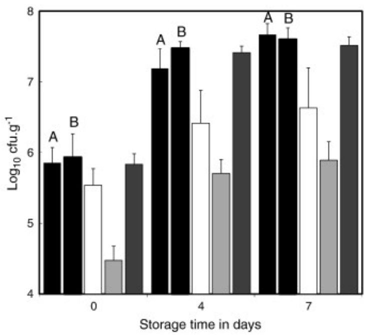
Saraoui et al, 2015, Food Microbiol

Inhibition of *E. coli/Salmonella* in meat by a *Lactobacillus sakei* cocktail

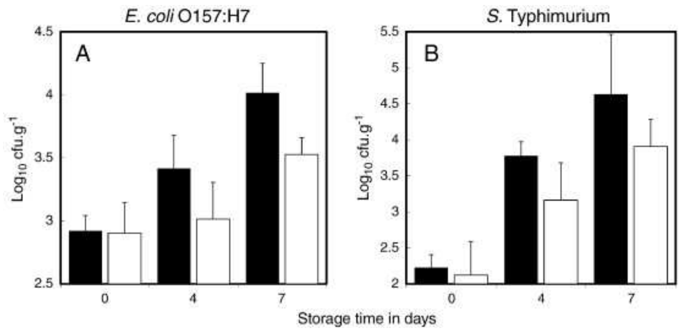
- Ground beef
- *E. coli/Salmonella*
- Challenge tests
- *L. sakei* cocktail (3 strains/genomic diversity)
- Effects on growth of pathogens
- Strain quantification
- Strain complementarity?
- Traceability

E. coli reduction — *Salmonella* reduction





Quantification by q-RT-PCR of cocktail N1 *L. sakei* strains at different storage time. Black bars: species-level probes with either *katA* gene-QMF01 (A) or sum of the three strain-specific probes (B). White bar: strain 112 with probe QMF02; light gray bar: strain 18 with probe QMF16; dark gray bar: strain 156 with probe QMF07.



Chaillou et al 2013, Meat Science

Strategy for the selection of protective cultures to improve food quality and safety

Selection of target strain to inhibit
pathogenic or spoiling microorganisms

↓

Collection of presumptive protective culture (PC)
commercial culture, private collection...

↓

1st selection of PC
inhibition of target bacteria in model conditions

↓

2nd selection
taxonomy, physiology of PC (growth at low temperature, acid production...)

↓

3rd selection
safety (antibiogram, toxicity...), technology (resistance to lyophilization, production of strain...)

↓

4th selection
absence of spoiling capacity in sterile food

↓

5th selection
inhibition of target bacteria in sterile food (challenge tests)

↓

6th selection
validation in naturally contaminated products

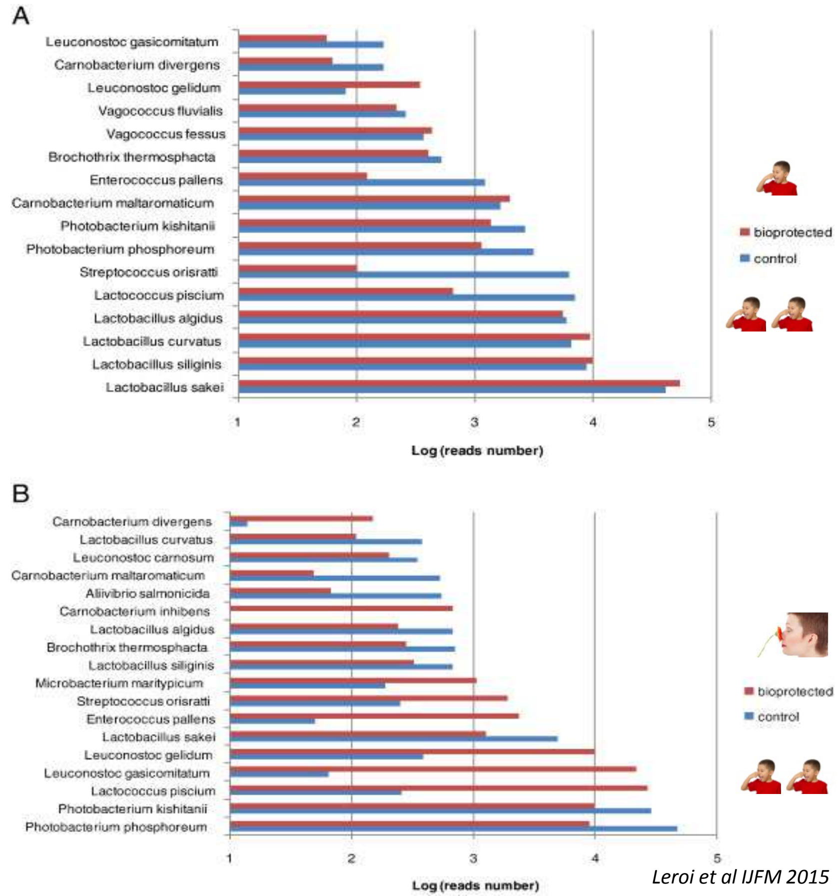
↓

validation in industry

Leroi et al IJFM 2015

Microbiota/sensory quality/protective cultures

- Cold smoked salmon
- Four specific spoilage bacteria (dominant)
- *Photobacterium phosphoreum*, *Serratia proteomaculans, Brochothrix thermosfacta, Carnobacterium divergens*
- Six protective cultures
- Different effect depending on target strains
- In sterile food matrixes
- *L. piscium* prevents spoilage by two strains
- In the natural ecosystem, contrasted results
- No correlation between sensory improvment and microbial ecosystem

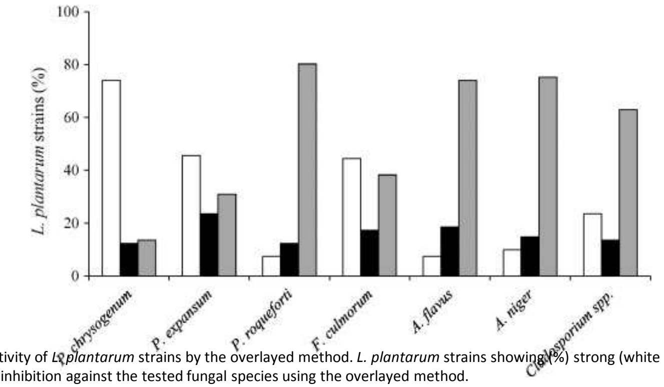


Abundance of dominant species in (a) batch A (control or bioprotected with *L. piscium* EU2241) and (b) batch C (control or bioprotected with *L. piscium* EU2241), after 3 weeks of storage (1 week at 4 °C and 2 weeks at 8 °C).

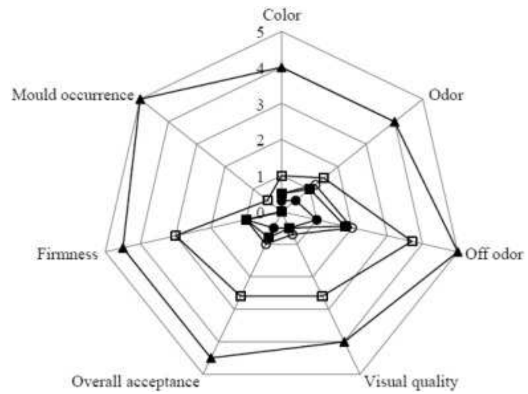
Leroi et al IJFM 2015

Antifungal properties of *Lactobacillus plantarum*

- Screening antifungal properties
- Lactic acid and phenyllactic acid
- Oat based beverages by fermentation
- Strain UFG 121 strongest antifungal properties



Antifungal activity of *L. plantarum* strains by the overlayed method. *L. plantarum* strains showing (%) strong (white bar), mild (black bar) or no (gray bar) inhibition against the tested fungal species using the overlayed method.



Russo et al IJFM et al 2017

Conclusion

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- A complex phenomenum (multiple factors and actors)
- Interactions
- No general mechanism
- No universal solution
- Specific development for each product?
- Ecological strategy
- Spoilage black box
- Traceability
- From lab to food

Future trends

- Combining hurdles (HHP and biopreservation, ANR project)
- Combining strains
- Dynamics of bacterial communities
- Pathogens and ecosystem
- Interactions
- Targetting expressed functions
- Measuring metabolites: metabolomics
- The question of european reglementation
- Network researchers/industry

RÉSEAU MIXTE TECHNOLOGIQUE

RMT FLOREPRO

LES FLORES PROTECTRICES
POUR LA CONSERVATION DES ALIMENTS

ACTIA

- A french joint technical network
- Labelled by french agriculture ministery
- Food technology institutes (ACTALIA/AERIAL/ADIV/IFIP
- Research institutes (INRA/IFREMER)
- Education (AgroParistech/Oniris/University of Liège)

http://www.actia-asso.eu/fiche/rmt-28-florepro.html
Network coordination: ADIV Souad Christieans

Actions

• Setting up R&D programmes that are coherent with professional expectations
• Contribution to european reglementation for bioprotective cultures
• Communication and dissemination of all of the knowledge acquired on bioprotective cultures: information and training days, colloquiums, publications in technical and scientific journals.

Thank you for your attention

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