Biopreservation of foodstuffs: Mechanisms and application

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● The actors
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Introduction
Biopreservation definition


“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, ...

Ressources
Microbiota
Environment
Food bacterial microbiota

- Pathogens
- Beneficial
- Spoilers

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

- Risk: Illness, Spoilage
- Benefit: Fermentation, Biopreservation
  - 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)

**To improve**
Organoleptic properties
Safety
Shelf life
Waste and losses

**CLEAN LABEL**

**GREEN LABEL**
Different targets/different strategies

Pathogens: one species / low number, specific tool (bacteriocin)

Spoilers: different species / high level, competitiveness

Knowing bacterial communities!

Hurdle technology

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Benefit</th>
<th>Application</th>
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<tbody>
<tr>
<td>HOLDBAC® YM-C</td>
<td>Growth control of yeasts and molds and some heterofermentative lactic</td>
<td>Fresh fermented foods</td>
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<tr>
<td>Plus</td>
<td>bacteria</td>
<td>White cheese</td>
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<tr>
<td>HOLDBAC® YM-B</td>
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<td>Plus</td>
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<tr>
<td>HOLDBAC® LC</td>
<td>Growth control of leuconostoc, heterofermentative lactobacilli and</td>
<td>Hard and semi-hard cheese</td>
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<td></td>
<td>enterococci</td>
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<tr>
<td>HOLDBAC® Listeria</td>
<td>Growth control of Listeria</td>
<td>Soft and smear cheese, dry and</td>
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<tr>
<td></td>
<td></td>
<td>semi-dry cured meats, cooked</td>
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<td></td>
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<td>and fresh ground meats</td>
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### 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.ingredientnetwork.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).


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
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 resources
- Hurdle
Lactic acid bacteria

*Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Carnobacterium, Enterococcus, Oenococcus, Vagococcus, Aerococcus, Weissella, Tetragenococcus*
Pathogens targeted by biopreservation

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

- Soilage: a complex phenomenon
- Many volatile organic compounds can be produced by spoilage microorganisms
- Many spoilage organisms involved
- Microbiota characterization
- Metagenomics/NGS
Natural flora of fresh food: a high diversity

Up to 150 OTU/product!
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
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$_2$O$_2$ production?

- Growth inhibition of *S. aureus* in milk, cheese
- *in vitro* inhibition modulated by level of aeration
- Inhibition higher when *L. garviae* produces H$_2$O$_2$
- Transcriptomic analysis
- Response of *L. garvieae* to aeration level differs according to the presence or absence of *S. aureus*.
- Higher concentration of H$_2$O$_2$ (with high aeration) not associated with a higher expression of *L. garvieae* H$_2$O$_2$ synthesis gene response but rather with a repression of *L. garvieae* H$_2$O$_2$ degradation genes (*trxB1*, *ahpC*, *ahpF*, and *gpx*).
- Original, previously undiscovered, H$_2$O$_2$ production regulation.
- Another extra cellular factor?

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*Loss of *S. aureus* SA15 (empty bars) and MW2 (full bars) cultivable cells under H$_2$O$_2$-stress condition. *p*-value < 0.05 according to Newman–Keuls test.*

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 thermosphacta* 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*. 
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?

*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

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

1. Selection of target strain to inhibit pathogenic or spoiling microorganisms

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

3. 1st selection of PC inhibition of target bacteria in model conditions

4. 2nd selection taxonomy, physiology of PC (growth at low temperature, acid production...)

5. 3rd selection safety (antibiogram, toxicity...), technology (resistance to lyophilization, production of strain...)

6. 4th selection absence of spoiling capacity in sterile food

7. 5th selection inhibition of target bacteria in sterile food (challenge tests)

8. 6th selection validation in naturally contaminated products

9. 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 thermosacta*, *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 improvement 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).

Leroy 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
Conclusion
• A complex phenomenon (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
• A french joint technical network
• Labelled by french agriculture ministry
• Food technology institutes (ACTALIA/AERIAL/ADIV/IFIP
• Research institutes (INRA/IFREMER)
• Education (AgroParistech/Oniris/University of Liège)

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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