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The microbial ecology of raw milk cheeses: an update of knowledge about the range of potential benefits.

La ecología microbiana de los quesos de leche cruda: qué sabemos hasta hoy sobre sus potenciales beneficios.

The microbial ecology of raw milk cheese : drivers, risks and potential benefits

Céline Delbès

UMR 545 Fromage, UCA, INRA, VetAgroSup, F-15000 Aurillac, France

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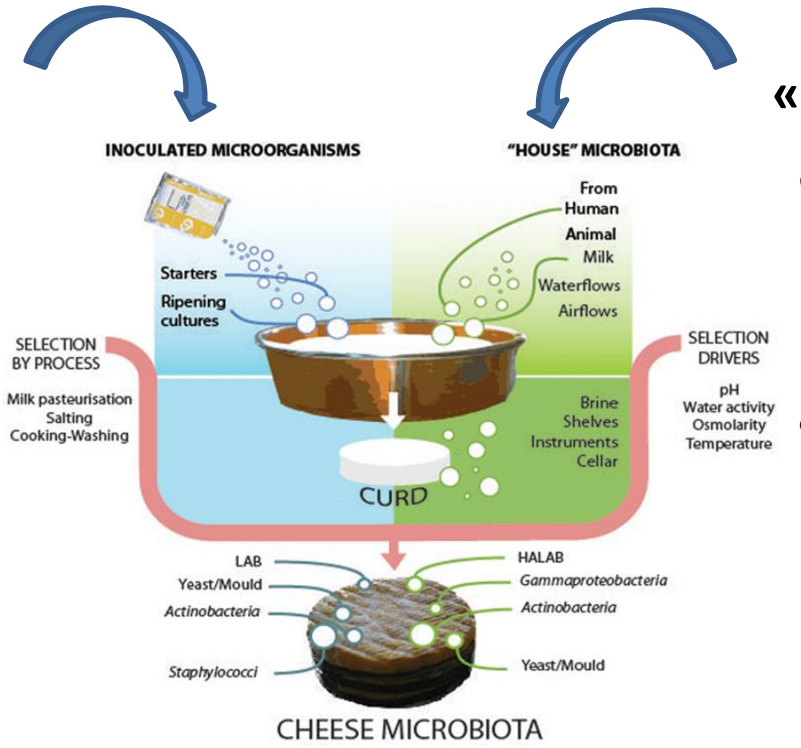
Cheese microbiota :
an evolving assembly of « house » and inoculated microbes

Change in microbial balance during ripening

Inoculated microorganisms

- **Commercial starters**
- **Autochtonous *consortia***
- **Traditional replication (back- slopping…)**

Acidification and ripening



Irlinger et al. 2015

« House » microbiota

- **Raw milk microbiota** under the influence of farming practices (dairy species, breed, milking practices…),
- **Cheese-making environment microbiota** (wood surfaces, brine, airflows…)

Essential for product typicity

Traditional cheeses = complex and specific microbiota
Deserves better understanding of diversity and ecological drivers

1/ Ecology of dairy products in the “meta-omics” era

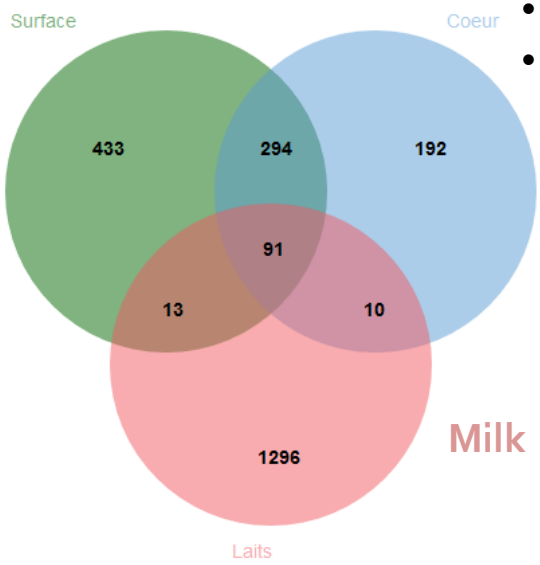
- “metabarcoding” approaches : rely on high-throughput sequencing (HTS) of target DNA (taxonomic marker)

New insights on milk and cheese microbial diversity using HTS metabarcoding

MétaPDOCheese
MÉTHODES OMIQUES

- 2400 cheese core and surface samples
- 400 milks
- 44 French PDO cheeses
- 7 cheese technological families
- 51 % farm-house productions
- 86 % raw milk

Cheeses Surface

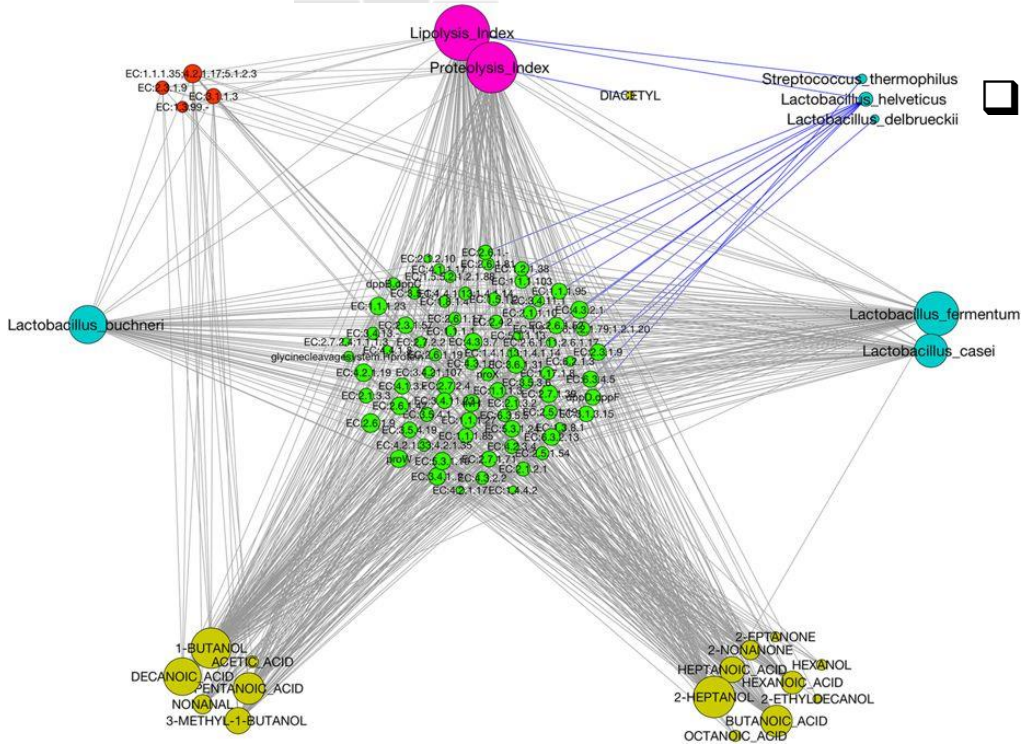


Cheese core

Milk

For the future…

foster dairy products in the “multi-omics” era



- Get deep insight into:
  - microbial diversity at strain level
  - interactions between microbiota composition, microbiota functional potentialities, and cheese biochemical properties

→ enrich scientific background for traditional cheeses specificities

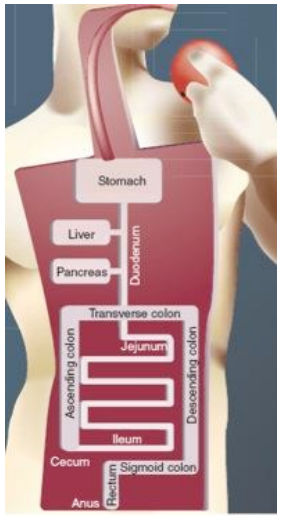
De Filippis, F. et al. Metatranscriptomics reveals temperature-driven functional changes in microbiome impacting cheese maturation rate. Sci. Rep. 6, 21871; doi: 10.1038/srep21871 (2016).

2/ Microbes in cheese and human health

Ingestion through the diet:

10^{9-} 10^{11} microorganisms per day
Solid matrix
Biodiversity
Potential presence of **food-borne pathogens**

O_2 pH



Saliva

10^4/g

Gut Microbiota

10^6/g

10^8 /g

10^{11}/g

Potential impact of cheese microbes on health:

- ✓ **Produce beneficial metabolites :** organic acids, vitamines K, B, C… essential amino-acids, conjugated linoleic acid…
- ✓ **Living microbes can act as probiotics**
- ✓ Modify cheese matrix via **microbial enzymes**
  - Bioactive peptides
  - **Digestibility**
  - **Bioavailability of minerals**
  - **Reduce metabolites with adverse effects** (lactose, mycotoxines, …),
  - **Reduce allergenicity ….**
- Cause **toxi-infections**

QUESTIONS

Interactions with the immune system ?
Interactions with the gut microbiota ?
Co-evolution over the millenary history of cheese production and consumption ?

2.1. Risk associated with pathogens

SCIENTIFIC REPORT

EFSA Journal

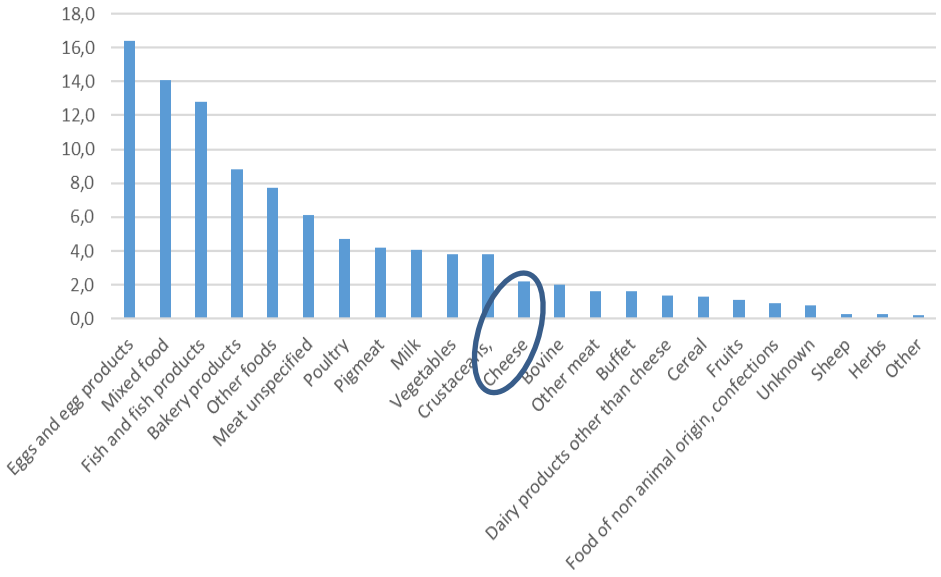
APPROVED: 19 November 2018

doi: 10.2903/j.efsa.2018.5500

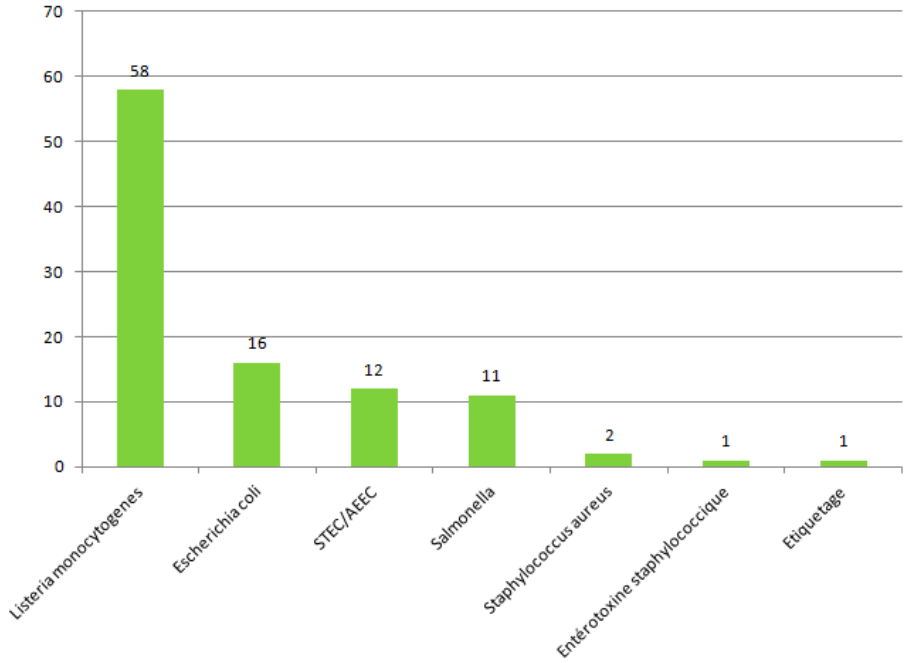
The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017

European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC)

Reported food-borne outbreaks in EU in 2017 per food type



Pathogens responsible for outbreaks associated with raw milk cheeses in France in 2017 (N=101) (DGAL/MUS/2019-86, 01/02/2019)



Cheese represents **2.2 %** of total food-borne outbreaks reported in EU for 2017

The most frequent pathogens in outbreaks associated with raw milk cheeses are *L. monocytogenes*, shigatoxin-producing *E. coli* and *Salmonella*

2. 2. Cheese and modulation of the human gut microbiota

Camembert : increase in *Enterococcus* populations, colonization by *Geotrichum candidum* in human fecal samples


Contents lists available at ScienceDirect
International Journal of Food Microbiology
journal homepage: www.elsevier.com/locate/ijfoodmicro

Fate and effects of Camembert cheese micro-organisms in the human colonic microbiota of healthy volunteers after regular Camembert consumption

Olivier Firmesse [a], Elise Alvaro [a], Agnès Mogenet [b], Jean-Louis Bresson [b], Riwanon Lemée [c], Pascale Le Ruyet [c], Cécile Bonhomme [c], Denis Lambert [c], Claude Andrieux [a], Joël Doré [a], Gérard Corthier [a], Jean-Pierre Furet [a,*], Lionel Rigottier-Gois [a]

Consumption of Camembert cheese stimulates commensal enterococci in healthy human intestinal microbiota

Olivier Firmesse, Sylvie Rabot, Luis G. Bermúdez-Humarán, Gérard Corthier & Jean-Pierre Furet

Unité d'Ecologie et Physiologie du Système Digestif, INRA, Jouy-en-Josas, France

Cooked pressed cheese : reduction of the level of amoxicilline-resistant *Enterococcus* after antibiotic treatment (amoxicilline + clavulanic acid)

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Effect of cheese consumption on emergence of antimicrobial resistance in the intestinal microflora induced by a short course of amoxicillin–clavulanic acid

X. Bertrand[1], V. Dufour[2], L. Millon[2], E. Beuvier[3], H. Gbaguidi-Haore[1], R. Piarroux[2], D.A. Vuitton[2] and D. Talon[1]

1 Service d'Hygiène Hospitalière, Centre Hospitalier Universitaire de Besançon, Besançon, France
2 Unité Santé et Environnement Rural, Université de Franche-Comté, Besançon, France
3 Unité de Recherches en Technologie et Analyses Laitières, Institut National de Recherche Agronomique, Poligny, France

- Only a few studies yet
- Show the **survival** of technological flora to the digestion process (protection by the cheese matrix) **without colonisation**
- **Modulation of the gut microbiota** after cheese consumption
- **Different responses depending on the cheese type**

↳ Microbial drivers associated with these responses ?

LAB, other bacteria ? Yeasts ? Microbial associations ?

2. 3. Raw milk, cheeses and immunomodulation: epidemiological studies

The protective effect of farm milk consumption on childhood asthma and atopy: The GABRIELA study

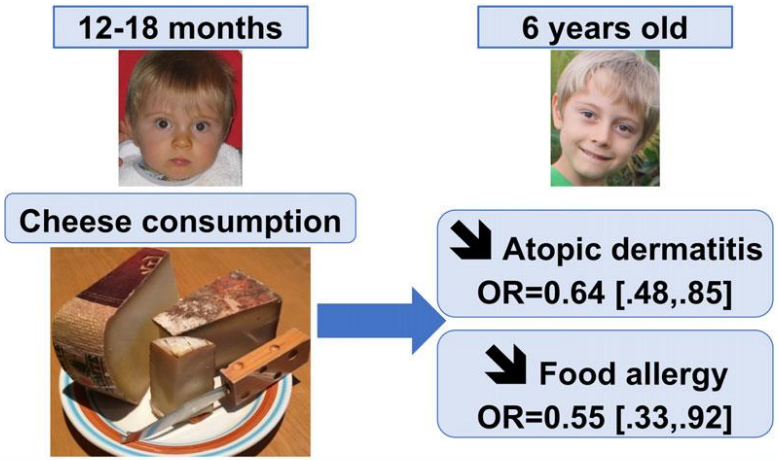
Georg Loss, MSc,[a,b] Silvia Apprich, PhD,[c] Marco Waser, PhD,[a,b] Wolfgang Kneifel, PhD,[c] Jon Genuneit, MD,[d] Gisela Büchele, PhD,[d] Juliane Weber, MD,[e] Barbara Sozanska, MD,[f] Hanna Danielewicz, MD,[f] Elisabeth Horak, MD,[g] R. J. Joost van Neerven, PhD,[h] Dick Heederik, PhD,[i] Peter C. Lorenzen, PhD,[i] Erika von Mutius, MD,[e] Charlotte Braun-Fahrländer, MD,[a,b] and the GABRIELA study group* *Basel, Switzerland, Vienna and Innsbruck, Austria, Ulm, Munich, and Kiel, Germany, Wroclaw, Poland, and Deventer and Utrecht, The Netherlands*

Consumption of unprocessed cow's milk protects infants from common respiratory infections

Georg Loss, PhD,[a,b,c] Martin Depner, PhD,[a] Laurien H. Ulfman, PhD,[d] R. J. Joost van Neerven, PhD,[d,e] Alexander J. Hose, MPH,[a] Jon Genuneit, MD,[f] Anne M. Karvonen, PhD,[g] Anne Hyvärinen, PhD,[g] Vincent Kaulek, PhD,[h] Caro
Juha
Char
Muni
Kuop

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY

Nicklaus S, Divaret-Chauveau A, Chardon M-L, et al. ; Pasture Study Group. The protective effect of cheese consumption at 18 months on allergic diseases in the first 6 years. *Allergy*. 2018;00:1–11. https://doi.org/10.1111/all.13650



- **Raw milk consumption** would contribute to the child protection against :
  - Asthma
  - Allergies
  - Respiratory infections

- **Cheese consumption** would contribute to the child protection against :
  - Atopic dermatitis
  - Food allergies

Relations with whey proteins ?
Microbiota ?

2. 4. Overview of potential risk and benefits for health

Cheese matrix :

+ :

proteins (caseins), lipids (PUFA), minerals (Ca, P), vitamins

– :

lipids (SFA), salt

Am J Clin Nutr 2018;108:1–8. Printed in USA. © 2018 American Society for Nutrition.

Dairy matrix effects: response to consumption of dairy fat differs when eaten within the cheese matrix—a randomized controlled trial

Emma L Feeney,[1,2] Rebecca Barron,[1,2] Victoria Dible,[1,2] Zita Hamilton,[1,2] Yvonne Power,[2] Linda Tanner,[2] Cal Flynn,[2] Paul Bouchier,[2] Tom Beresford,[2,3] Nessa Noronha,[1,2] and Eileen R Gibney[1,2]

Fermentation :

+ :

bioactive peptides, vitamines

Microbiota :

+ :

Living microorganisms, potential interactions with the immune and digestive systems

Raw milk cheeses :

+ :

Unprocessed raw material

→ preservation of proteins, lipids, vitamins

–:

pathogens

+ :

Microbial diversity

Sensory richness of the product

Increased potential of interactions with immune and digestive systems

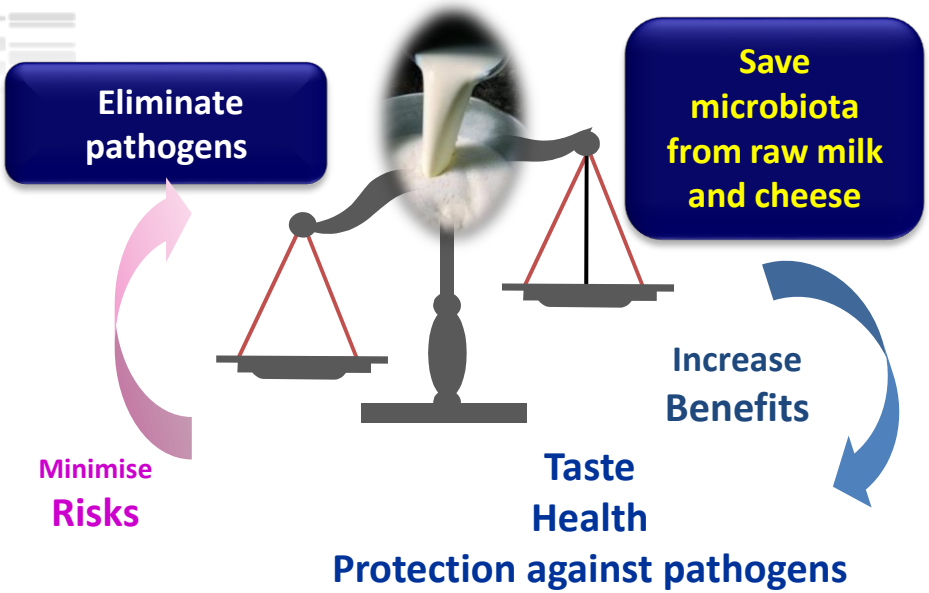
Protection against cardiovascular affections ?

Reducing blood pressure ?

Anti-microbial/anti-inflammatory properties ?

Anti oxydative activities ?

3/ Risks and benefits management : a daily challenge for raw milk cheese productions



- Find a trade-off between safety, microbial diversity and sensory richness

International Journal of Food Microbiology

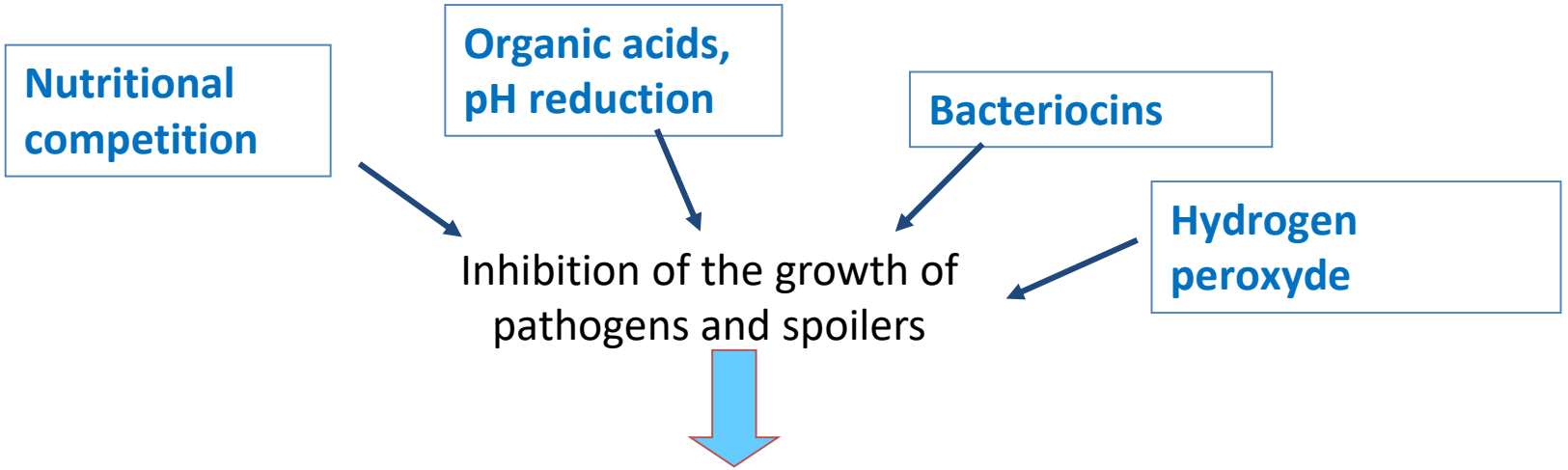
Traditional cheeses: Rich and diverse microbiota with associated benefits

Marie-Christine Montel [a,*], Solange Buchin [b], Adrien Mallet [c,d], Céline Delbes-Paus [a], Dominique A. Vuitton [d,e], Nathalie Desmasures [c,d], Françoise Berthier [b]

[a] INRA, Unité Recherches Fromagères, 20 Côte de Reyne, F-15000 Aurillac, France
[b] INRA, UR342 Technologie et Analyses Laitières, F-39801 Poligny, France
[c] Normandie Univ, France
[d] UNICAEN, ABTE, F-14032 Caen, France
[e] EA3181/Université de Franche-Comté, 25030, Besançon, France

3. 1. Targeted approaches : Biopreservation = Relying on microbial diversity in milk and cheese as a barrier against pathogens

Antagonistic properties of microbes:

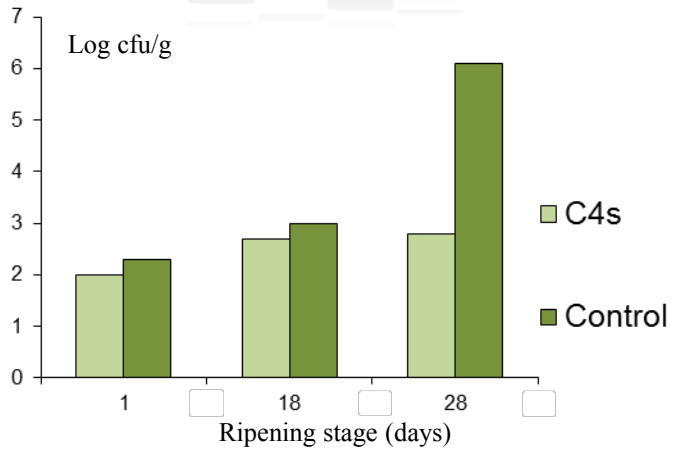


Improve microbial quality and safety of fermented and non-fermented foods

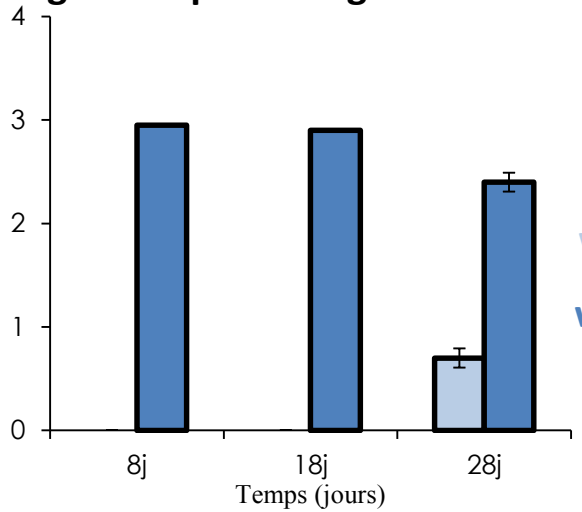
Preserve sensory and nutritional properties

Inhibition of pathogens in raw milk cheeses by microbial consortia

Listeria monocytogenes



Shigatoxin-producing *Escherichia coli* (STEC)



- **Inhibition is determined by specific species**

  32 strains = 14 strains = 4 strains

- **Cooperation between species is required :**

  individual strains are not antagonistic as themeselves

- Several **individual** strains can inhibit STEC
- **Synergistic effects of strains associations**
- Inhibition depends on cheese technology


International Journal of Food Microbiology 174 (2014) 98–109

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Microbial biodiversity in cheese consortia and comparative *Listeria* growth on surfaces of uncooked pressed cheeses

Cécile Callon *, Emilie Retureau, Robert Didienne, Marie-Christine Montel

INRA URF 545 Fromagères, 20 Côte de Reyne, 15000 Aurillac, France


Food Control 96 (2019) 499–507

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Can lactic acid bacteria be an efficient tool for controlling *Listeria monocytogenes* contamination on cheese surface? The case of Gorgonzola cheese

Stefano Morandi, Tiziana Silvetti*, Giovanna Battelli, Milena Brasca

Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Celoria 2, 20133, Milan, Italy


Food Microbiology

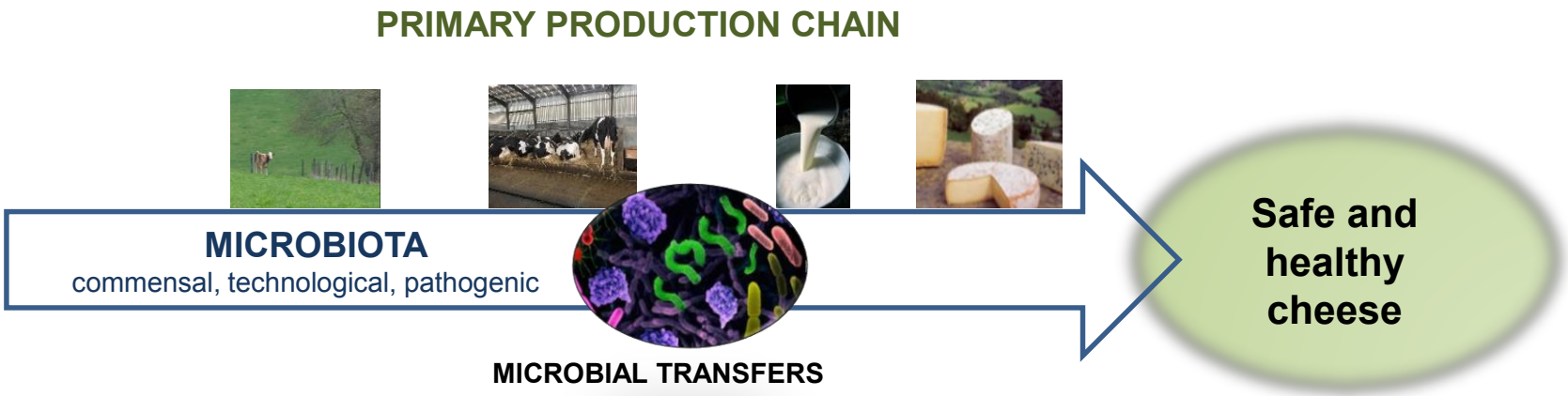
journal homepage: www.elsevier.com/locate/fm

Control of Shigatoxin-producing *Escherichia coli* in cheese by dairy bacterial strains

Cécile Callon*, Céline Arliguie, Marie-Christine Montel

INRA, UR545 Fromagères, 20 Côte de Reyne, 15000 Aurillac, France

3. 2. An alternative: management of microbial resources from the primary production environment across the cheese process chain



Objectives:

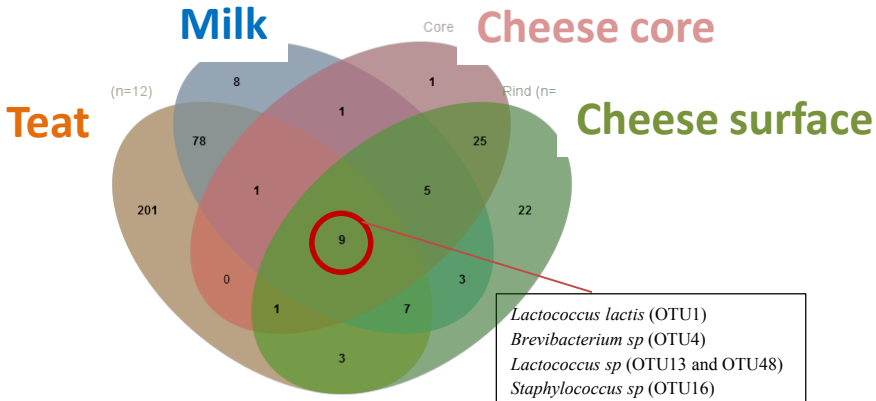
Understanding the drivers of microbiota for safe and healthy raw milk cheese

- Influence of the overall farm management system ?
- Several running projects under the network “RMT Terroir”
  (Amont Saint-Nectaire, IFEP, Phyllos…)

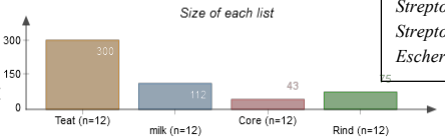
réseau Fromages de Terroirs

Microbial transfers and interactions from dairy farm environment to the human gut

Shared bacterial species between cow teat, milk and cheese



Total number of detected species per environnement



85% of the species in milk are also present on teat

27% of species in cheese (core and surface) are also present on teat, especially species potentially involved in ripening *(B. linens, Staph. equorum)*

SCIENTIFIC REPORTS

OPEN **Bacterial community assembly from cow teat skin to ripened cheeses is influenced by grazing systems**

Received: 15 September 2017
Accepted: 11 December 2017
Published online: 09 January 2018

Marie Frétin[1,2], Bruno Martin[2], Etienne Rifa[1], Verdier-Metz Isabelle[2], Dominique Pomiès[2], Anne Ferlay[2], Marie-Christine Montel[2] & Céline Delbès[1]

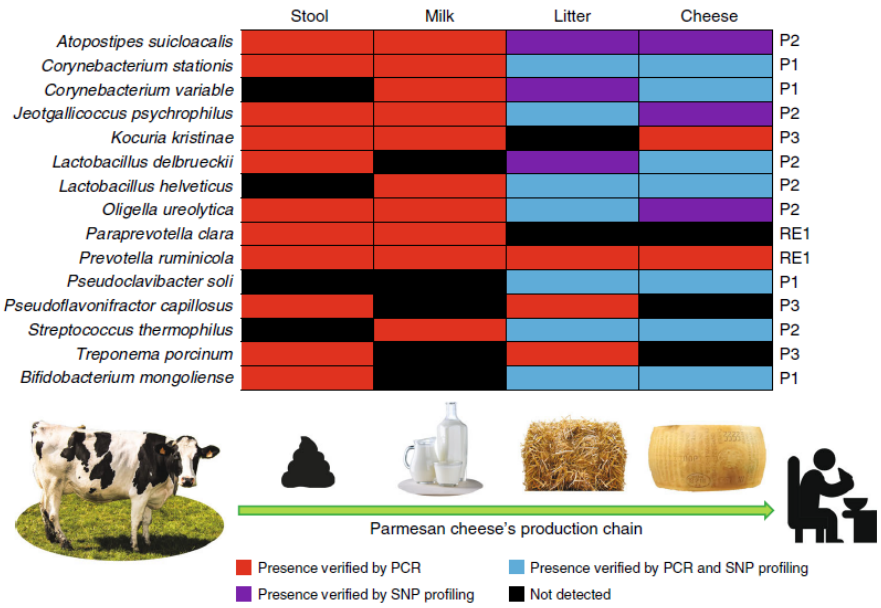
nature COMMUNICATIONS

ARTICLE

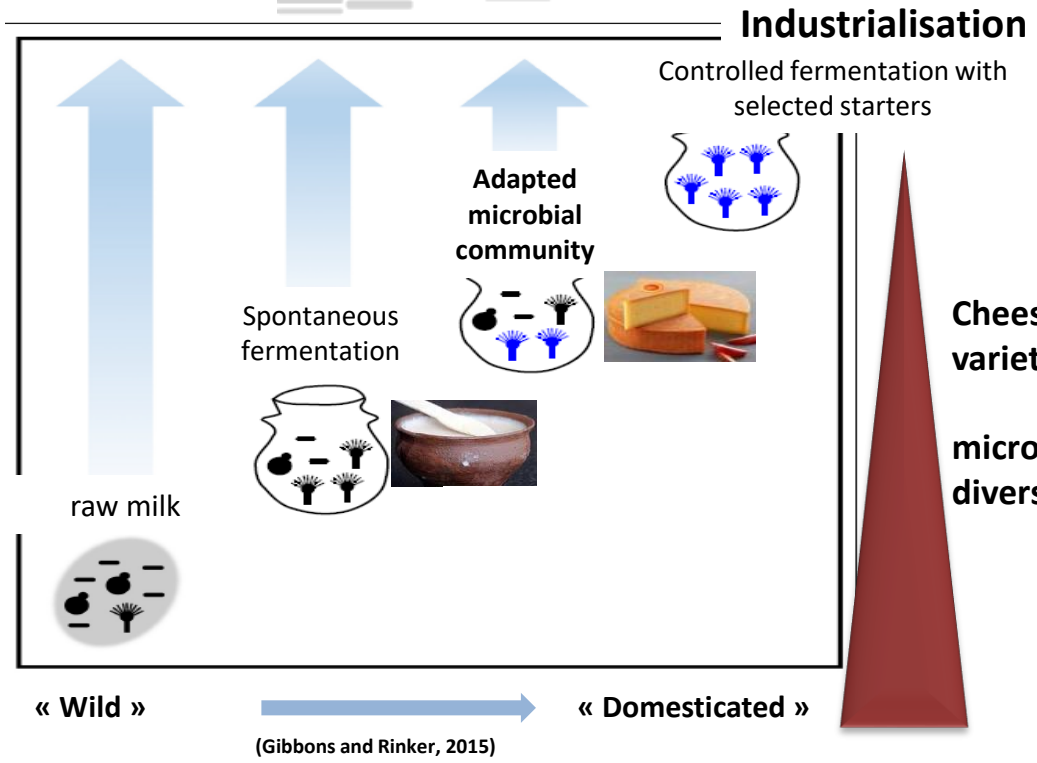
https://doi.org/10.1038/s41467-019-09303-w OPEN

Colonization of the human gut by bovine bacteria present in Parmesan cheese

Christian Milani[1], Sabrina Duranti[1], Stefania Napoli[2], Giulia Alessandri[3], Leonardo Mancabelli[2], Rosaria Anzalone[2], Giulia Longhi[2], Alice Viappiani[2], Marta Mangifesta[1,2], Gabriele Andrea Lugli[1], Sergio Bernasconi[4], Maria Cristina Ossiprandi[3], Douwe van Sinderen[3,5,6], Marco Ventura[1,4] & Francesca Turroni[1,4]



3. 3. Hygienism vs hygiene : microbial diversity endangered … and health too ?



- **hygiene** = « Principles and practices for health conservation »

- **Increased sanitary pressure** (risk for pathogens)
  - ➢ **drastic hygiene practices** along the production chain from farm to cheese
  - ➢ Low microbial levels in milk
- **Intensification of production practices :**
  - ➢ Changes in microbial balance in farm, milk and cheese
- **Urbanisation, highly transformed food**
  - ➢ Associated with an increase in **auto-immune affections**

Ecological and health issue :

- ✓ Loss of microbial diversity
- ✓ Lower exposition to food-borne and environmental microorganisms
  - ↳ Relation with the gut microbiota and auto-immune affections ?

C. Villeneuve et al. / Microbes and Infection 20 (2018) ,https://doi.org/10.1016/j.micinf.2017.11.001
Evolution of the hygiene hypothesis into biota alteration theory: what are the paradigms and where are the clinical applications?

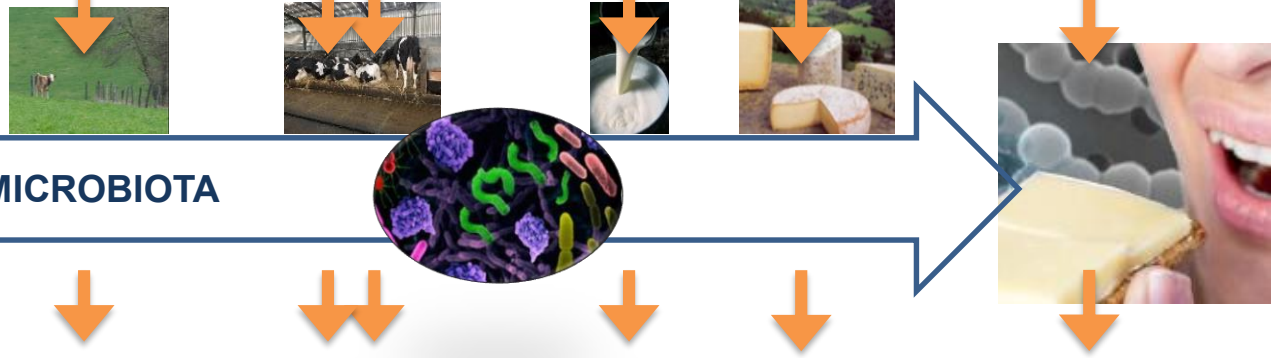
Perspectives

Find solutions through systemic and trans-disciplinary approaches ?

→ PROCESSING

PRIMARY PRODUCTION CHAIN

→ PUBLIC HEALTH



MICROBIAL INPUTS vs. HYGIENISM

MICROBIOTA

(Micro)biodiversity in natural and anthropogenic environments

Animal health and human health

FARMER → CHEESE-MAKER → STAKEHOLDERS → CONSUMER

Representation of Nature and microbes

Know-how

FEEDBACK

Integrative, transdisciplinary approaches (agronomy, animal science, microbiology, immunology, sociology, anthropology…)

Thanks for your attention

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RMT Fromages de Terroirs