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Microbial biodiversity in raw milk cheeses: a potential source of health.

Biodiversidad microbiana en quesos de leche cruda: una fuente potencial de salud.





INTERNATIONAL
SCIENTIFIC CONFERENCE
ON RAW MILK

MICROBIAL BIODIVERSITY IN RAW MILK CHEESES: A POTENTIAL SOURCE OF HEALTH

Milena Brasca

National Research Council

Institute of Sciences of Food Production

Milan, Italy

Italian PDO cheeses

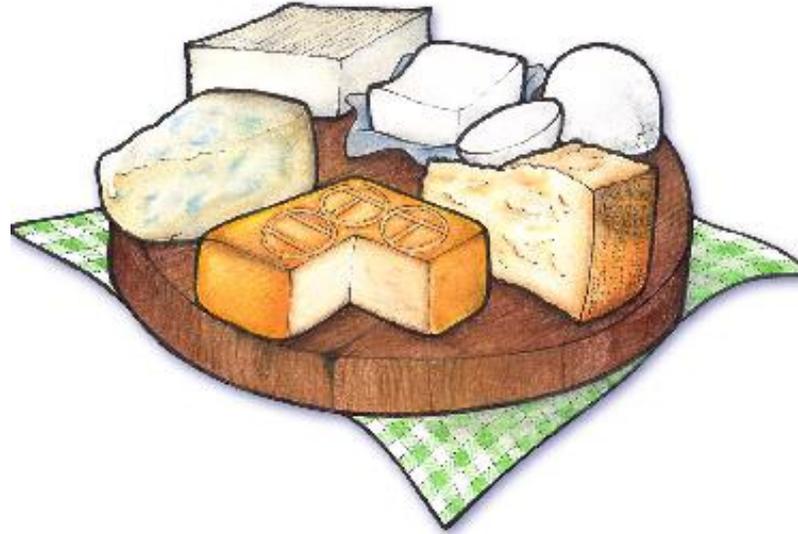


24/50

Raw milk

23/50

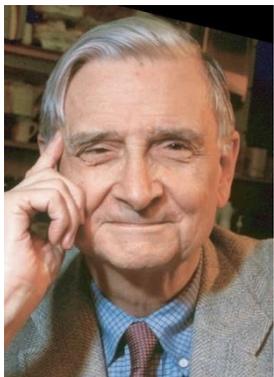
Raw or pasteurized milk



Specificity and peculiar sensory characteristics of traditional cheeses are strictly related to milk quality and to the traditional cheese making processes, but also to the **high level of biodiversity of the indigenous microbiota**

Over the centuries the technologies of these cheeses have selected complex bacterial ecosystems accounting for more than 400 of species

Microbial diversity in milk provides diversity in cheese sensory characteristics

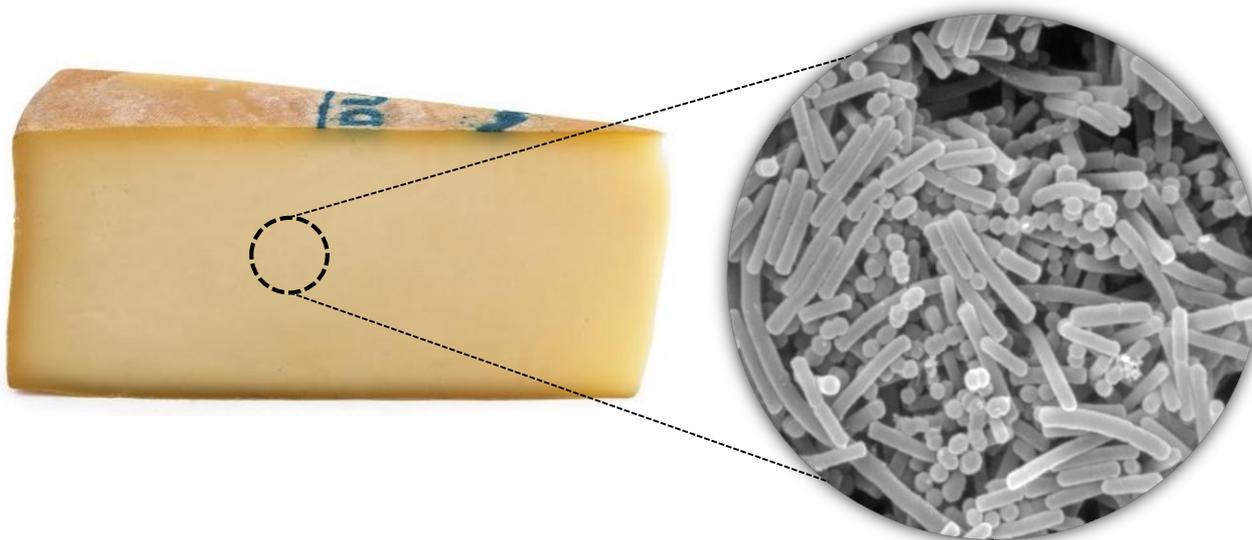


Biodiversity

“Variety of living organisms that coexist and interact within the same environment”.

(1988 - Edward O. Wilson)

MILLIONS OF MICROORGANISMS IN EACH GRAM



Morelli, 2017

MICROBIAL BIODIVERSITY STARTS AT FARM LEVEL

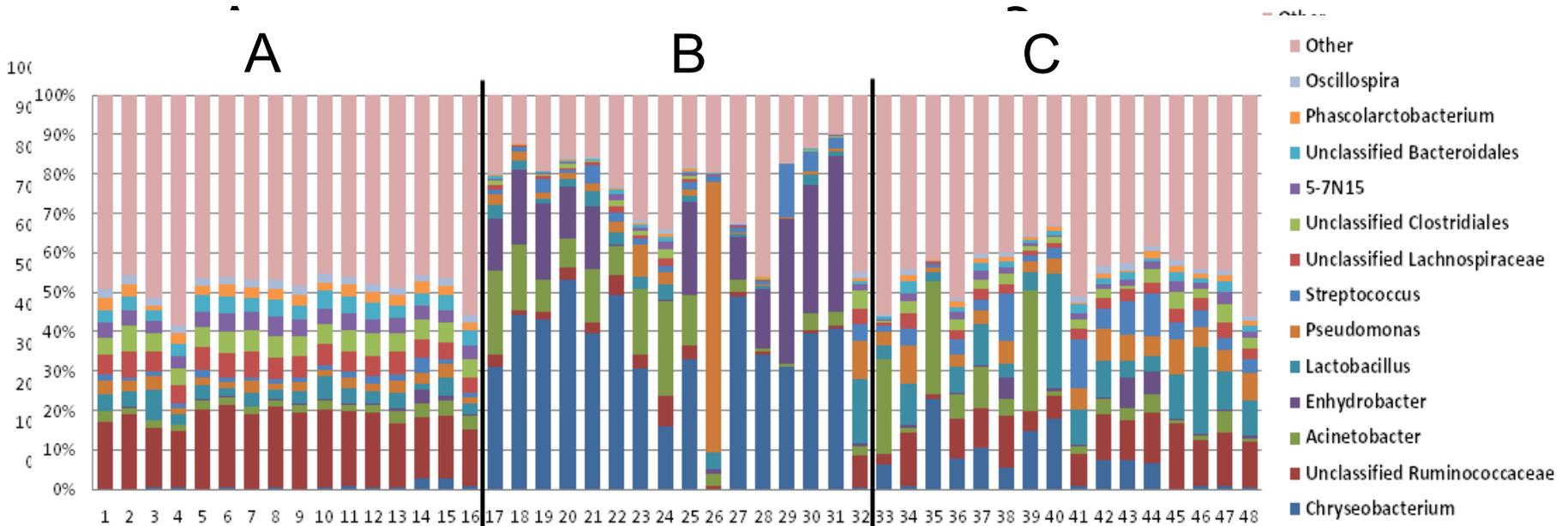


Different factors are involved in microbial quality of raw milk

- Environment (stable and the milking parlour)
- Teat skin
- Hygienic conditions of livestock
- Feed (corn silages and herbage silages, pasture, hay....)
- Milking practices
- Storage conditions

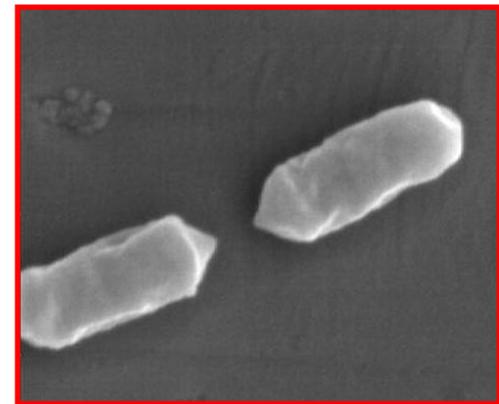
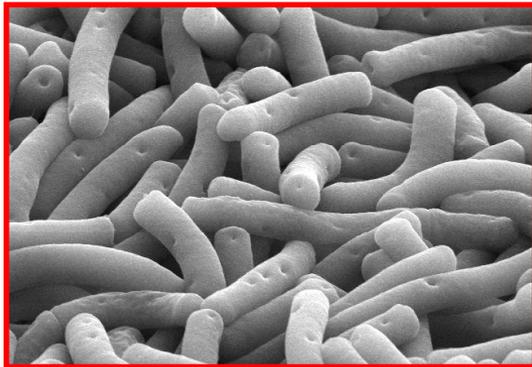
MICROBIAL BIODIVERSITY IN MILK

Microbial relative abundances at genus level for 16 milk samples from 3 different farms similar for herd composition and management



EACH FARM POSSESSES ITS OWN MICROBIAL BIODIVERSITY!

MICROBIAL BIODIVERSITY



ORIGIN OF CHEESE MICROBIAL BIODIVERSITY

MILK



STARTER



ENVIRONMENT

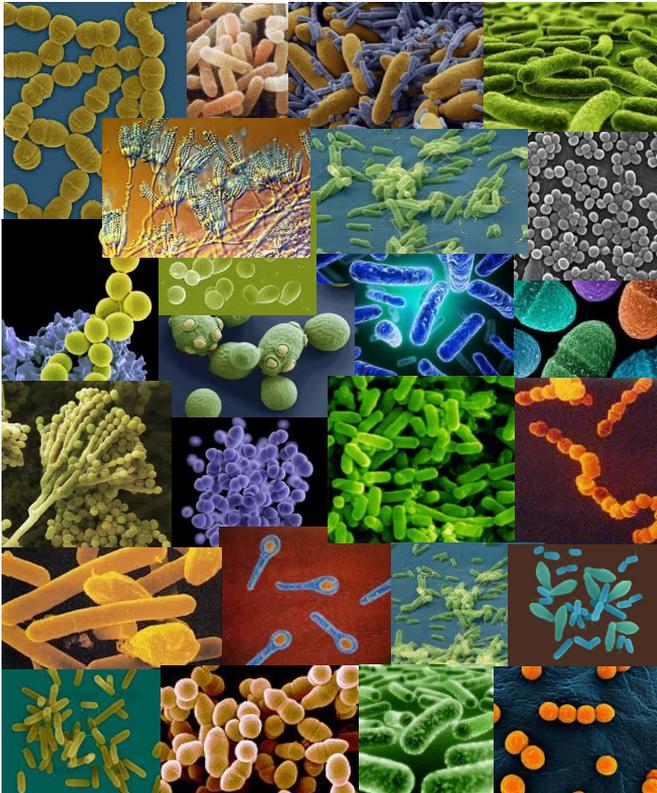


Technology

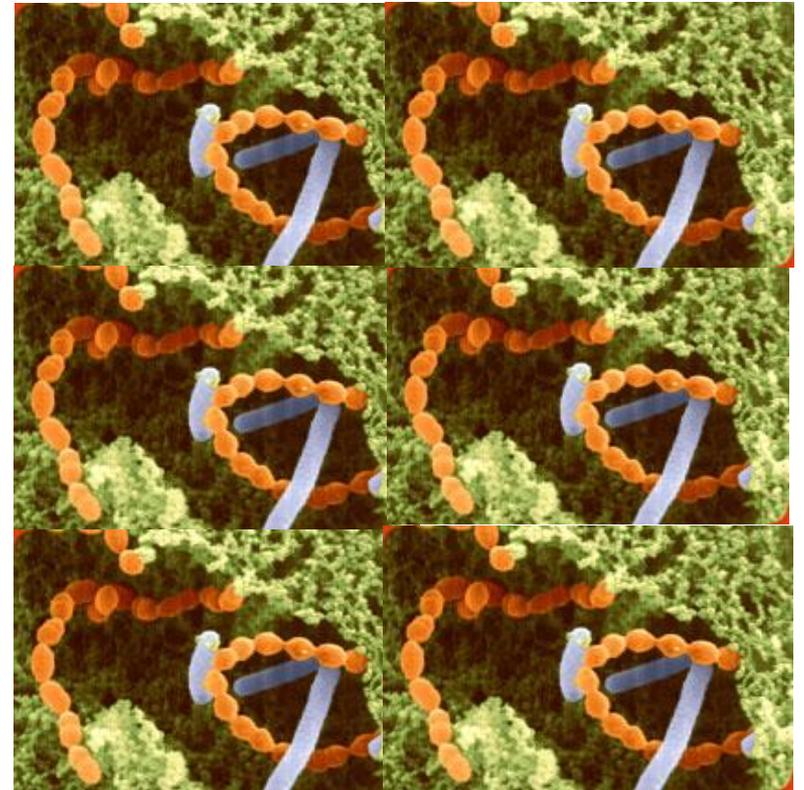


BIODIVERSITY OF THE MICROBIOTA

Raw milk cheese



Pasteurized milk cheese



THE LEADING ACTOR: LACTIC ACID BACTERIA

UP TO NOW evaluated on technological basis

- LAB provide the **fast acidification** of the milk through the production of organic acids mainly lactic acid
- They produce also acetic acid, ethanol, and **aromatic compounds**
- They can have and **proteolytic** and **lipolytic** activity
- **Exopolysaccharides** (EPS) produced naturally by LAB have the ability to bind water, interact with proteins, and to increase the viscosity of the medium
- They can produce **CO₂** contributing to eyes formation

More recently....

- These bacteria possess inherent, functional properties, aiming at improving the quality of the end product by offering **technological, sensory, safety, nutritional and health advantages**
- **Multifunctional strains** are present in complex microbial populations and in particular in raw milk fermented products

Lactic Acid Bacteria collection (2000 wild strains)



Bitto



**Valtellina
Casera**



**Toma
Piemontese**



Taleggio



Scimudin



**Grasso
d'Alpe**



**Formaggella
del Luinese**



**Formai
de Mut**



Fontina



Gorgonzola



Asiago



**Formagèla
Valseriana**



Raschera



Fatulì



**Grana
Padano**



Strachitunt



**Quartiolo
Lombardo**



**Parmigiano
Reggiano**



Silter



**Formaggella
Valle di Scalve**



Semuda

TECHNOLOGICAL CHARACTERIZATION

Acidifying activity

Redox activity

Proteolytic activity

Lipolytic activity

CO₂ production

Aroma production

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Redox potential to discriminate among species of lactic acid bacteria

M. Brasca¹, S. Morandi¹, R. Lodi¹ and A. Tamburini²

¹ CNR – Istituto di Scienze delle Produzioni Alimentari, Milan, Italy

² Istituto di Zootecnia Generale Università degli Studi di Milano, Milan, Italy



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International Dairy Journal 16 (2006) 867–875

INTERNATIONAL
DAIRY
JOURNAL

www.elsevier.com/locate/jdairyj

Technological and molecular characterisation of enterococci isolated from north–west Italian dairy products

Stefano Morandi^a, Milena Brasca^{a,*}, Christian Andrighetto^b,
Angiolella Lombardi^b, Roberta Lodi^a

Journal of Dairy Research (2013) 80 457–466. © Proprietors of *Journal of Dairy Research* 2013
doi:10.1017/S0022029913000447

457

Technological characterisation, antibiotic susceptibility and antimicrobial activity of wild-type *Leuconostoc* strains isolated from north Italian traditional cheeses

Stefano Morandi¹, Paola Cremonesi², Tiziana Silveti¹ and Milena Brasca^{1*}

¹ Institute of Sciences of Food Production, Italian National Research Council, Milan, Italy

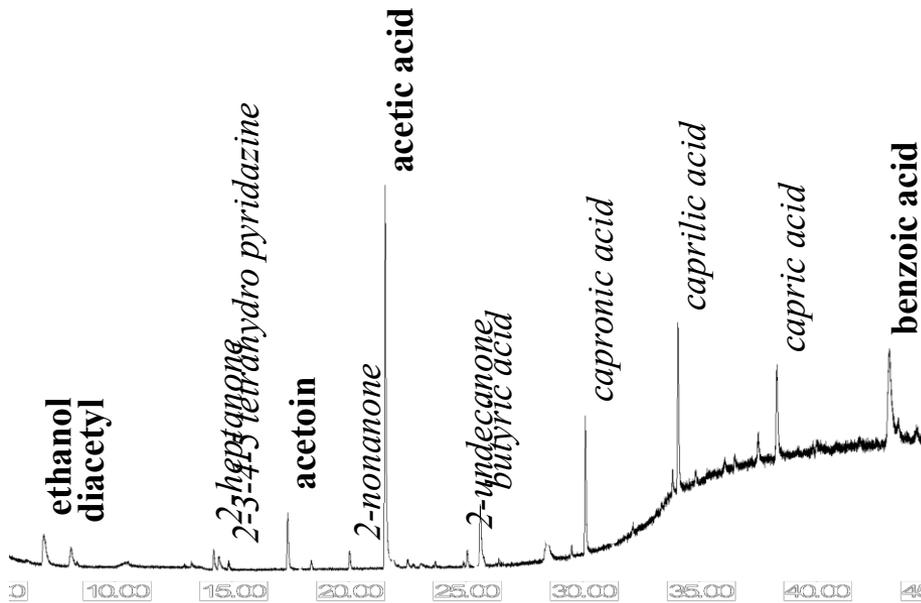
² Institute of Agricultural Biology and Biotechnology, Italian National Research Council, Milan, Italy

FIRST EVIDENCE

METABOLIC ACTIVITIES ARE SPECIES DEPENDENT

METABOLIC ACTIVITIES ARE STRAIN DEPENDENT

PRODUCTION OF AROMATIC COMPOUNDS

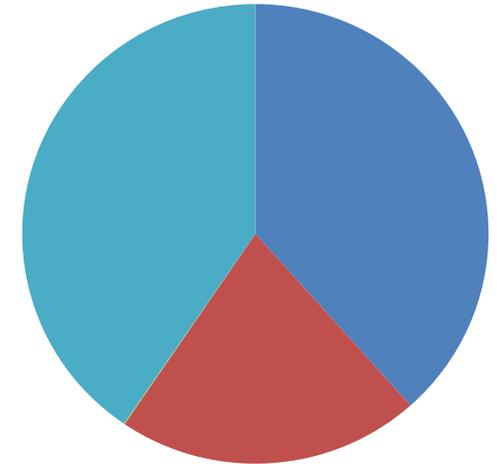
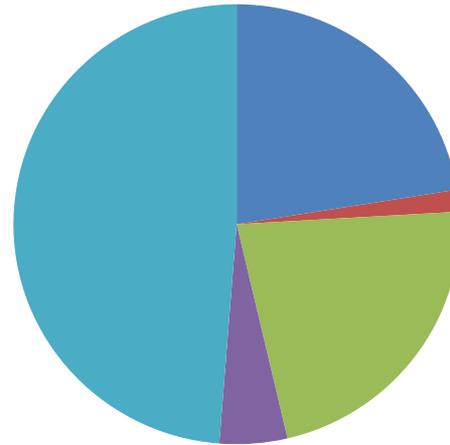
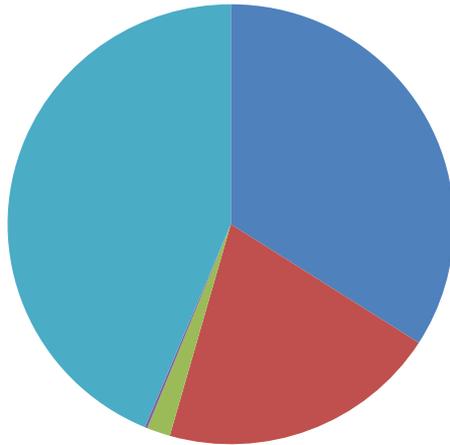


SPME extraction



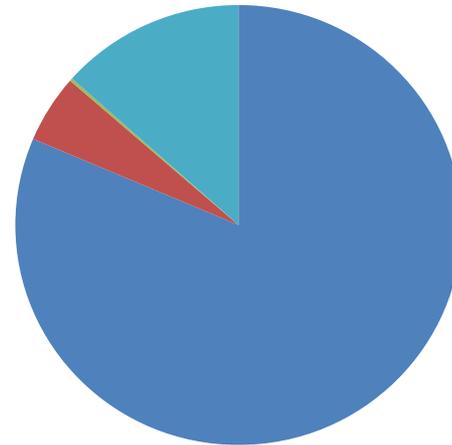
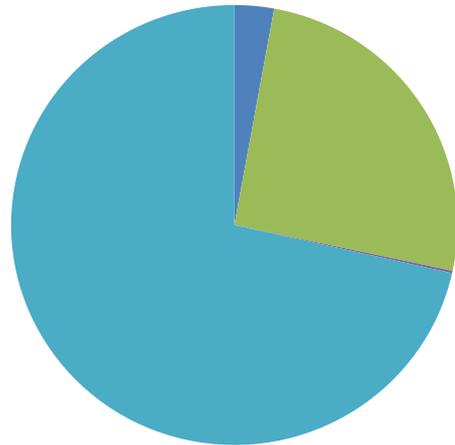
Enterococcus Lactococcus

Lactobacillus



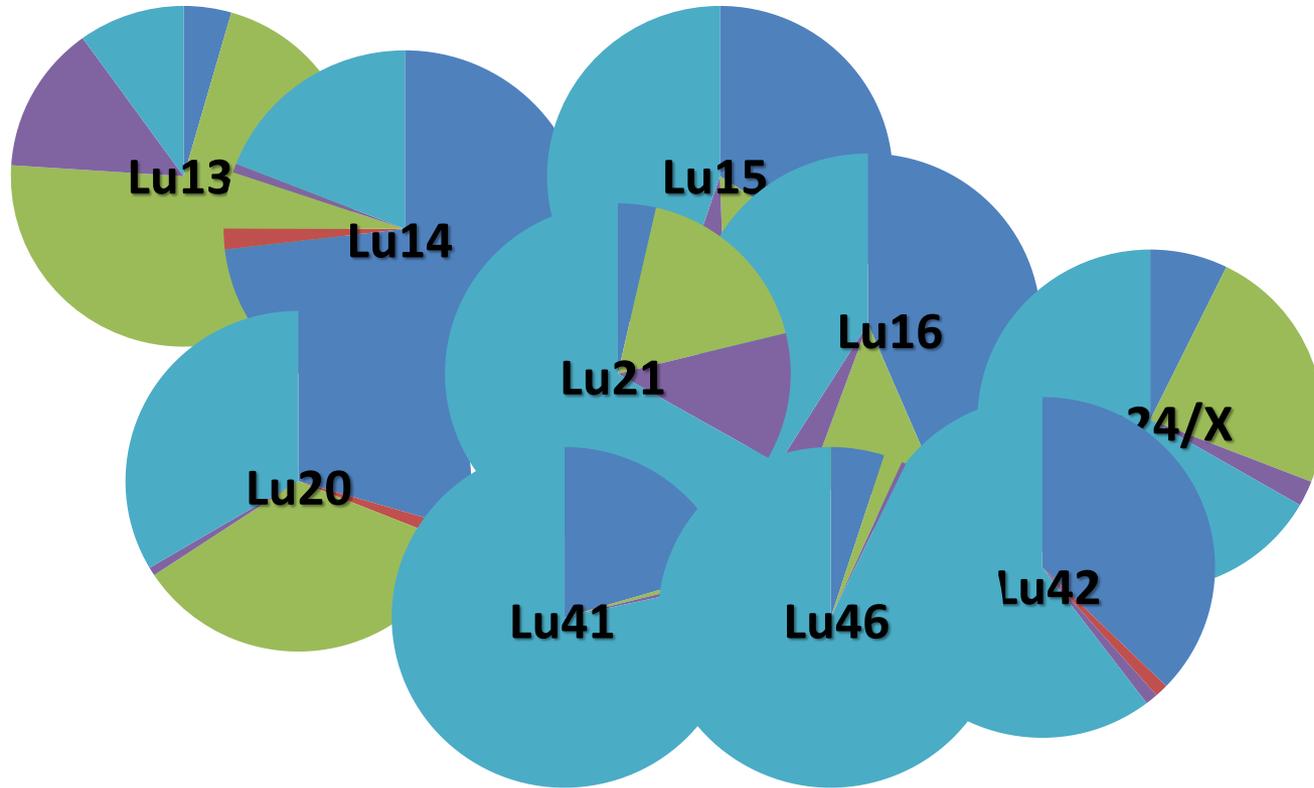
Leuconostoc

Streptococcus



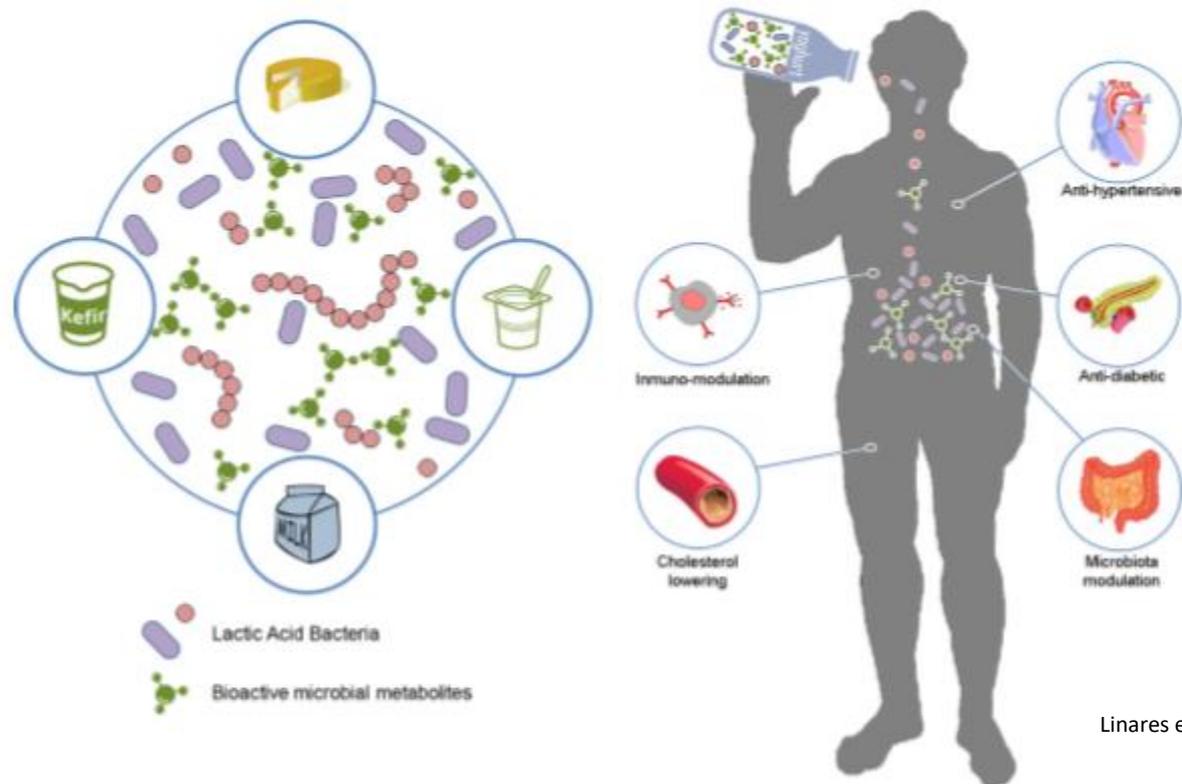
■ diacetyl ■ acetoin ■ 3-methyl-1-butanol ■ 3-methylbutanal ■ acetic acid

Lactococcus lactis subsp. *lactis*



■ diacetyl ■ acetoin ■ 3-methyl-1-butanol ■ 3-methylbutanal ■ acetic acid

Ingestion of fermented dairy products can exert health promoting activities



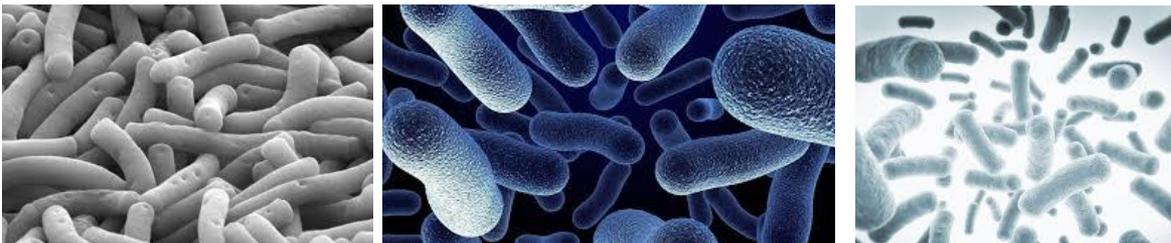
Linares et al. (2017).

Beneficial bacteria present in cheese can exert beneficial effects

PROBIOTICS

According to the Food and Agricultural Organization/World Health Organization probiotics are

- **live microorganisms** that, when supplied in adequate amounts, may confer a health benefit on the host (FAO/WHO, 2006).
- the **vitality and viability** at high cell densities (at least **10^7 cfu g⁻¹** of end product) is an essential requirement for probiotics (EFSA 2010; Health Canada 2009)
- Adhesion to the intestinal surface and the subsequent colonization of the human gastro-intestinal tract are further, important prerequisite for probiotic action



FUNCTIONAL

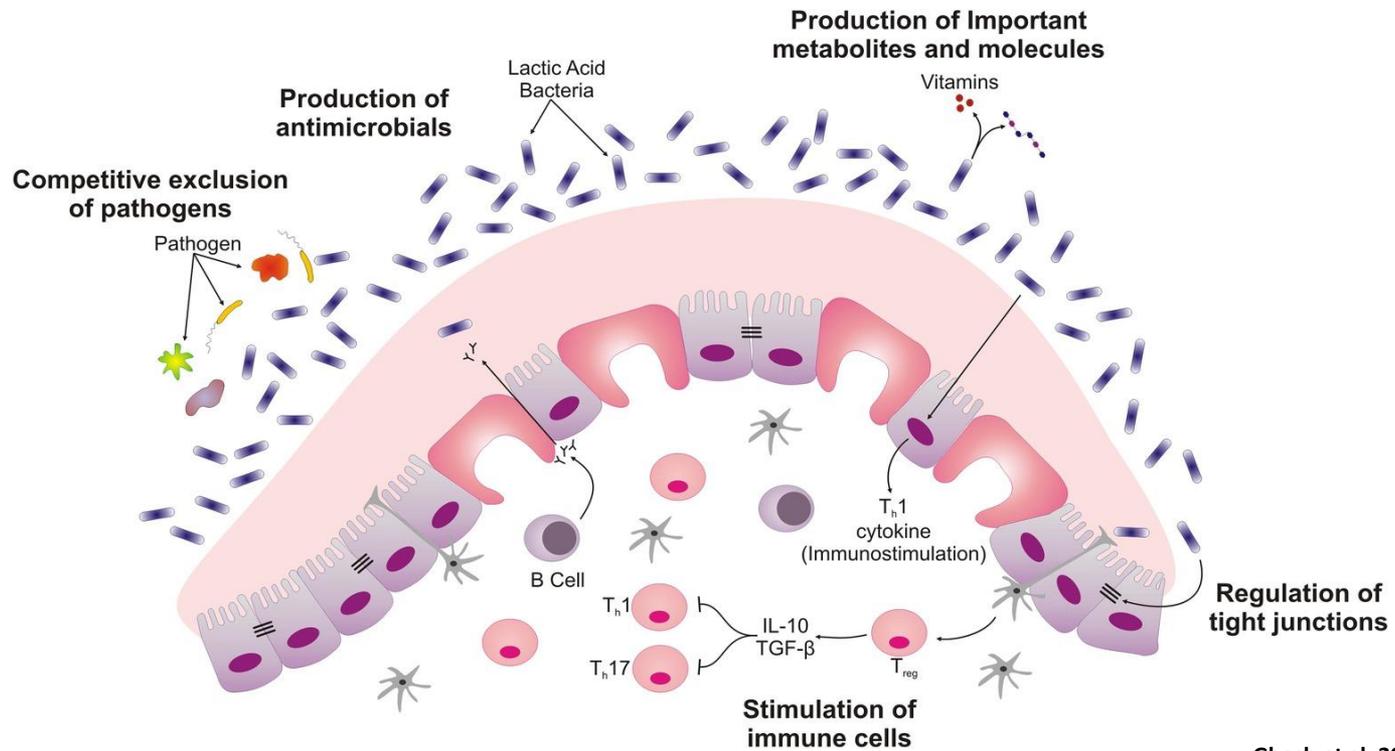
Functional Foods: whole foods and fortified, enriched, or enhanced food including dietary components, that may reduce the risk of chronic disease and provide a health and physiological benefit

Functional microorganisms and health benefits:

- direct interactions of ingested **live microorganisms** with the host (probiotic effect)
- ingestion of microbial **metabolites** synthesized during fermentation (biogenic effect)

There is consistent evidence to support the immunoregulatory activity of **dead probiotic cells**, intact or broken.

FUNCTIONAL PROPERTIES



Ghosh et al. 2019

MICROBIAL DIVERSITY TO COMBAT PATHOGENS

Against...

Pathogenic bacteria

Listeria monocytogenes

Staphylococcus aureus

Bacillus cereus

Escherichia coli

Spoilage bacteria

Clostridium spp.

Pseudomonas spp.

Yeasts

Moulds



Journal of Food Safety

Journal of Food Safety ISSN 1745-4565

ANTIMICROBIAL ACTIVITY, ANTIBIOTIC RESISTANCE AND THE SAFETY OF LACTIC ACID BACTERIA IN RAW MILK VALTELLINA CASERA CHEESE

S. MORANDI¹, T. SILVETTI¹, J.M. MIRANDA LOPEZ² and M. BRASCA^{1,3}

¹Institute of Sciences of Food Production, Italian National Research Council, Via Celoria 2, Milan 20133, Italy
²Department of Analytical Chemistry, Nutrition and Bromatology, Veterinary Faculty, University of Santiago de Compostela, Lugo, Spain

CyTA Journal of Food, 2013
<http://dx.doi.org/10.1080/19476337.2013.825327>

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Biopreservation potential of *Enterococcus faecalis* isolated from Italian traditional raw milk cheeses

Potencial de biopreservación de cepas de *Enterococcus faecalis* aisladas de quesos tradicionales Italianos de leche cruda

Tiziana Silvetti*, Stefano Morandi and Milena Brasca

Food Control 96 (2019) 499–507

Contents lists available at ScienceDirect

Food Control

ELSEVIER 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

Check for updates

INTERNATIONAL SCIENTIFIC CONFERENCE ON RAW MILK

LAB CAN ENRICH CHEESE WITH BIOACTIVE COMPOUNDS PROMOTING HUMAN HEALTH

GABA (γ -Aminobutyric acid)

neurotransmission,
induction of hypotension,
regulation of depression, sleeplessness,
autonomic disorders
prevention of diabetic conditions,
stimulation of immune cells

Folate

protective properties against:
megaloblastic anaemia, neurological
disturbances, neural tube defects, and
other congenital malformations
cardiovascular diseases
several types of cancer

Functional peptides

Antihypertensive peptides
Antioxidative peptides
Immune system affecting peptides

CLA (conjugated linoleic acid)

immune function with antilipogenic,
antidiabetic,
antiatherosclerotic
anticarcinogenic effects

EPS (exopolysaccharides)

Prebiotics
antitumour effects
cholesterol level,
blood pressure,
blood glucose
Immunostimulating activity

Oligosaccharides

Prebiotic effect: on calcium absorption and bone
health,
modulation of the human gut microbiota,
Immunomodulatory effect

BIOACTIVE COMPOUNDS ARE PRODUCED DURING RIPENING

Cheese is a biologically and biochemically dynamic system in which **bioactive compounds are being constantly released**

Several studies have reported that the level of bioactive compounds in cheese is **dependent on ripening time**

Aged cheeses are richer in bioactive molecules

Production of γ -aminobutyric acid by **wild strains** of *St. thermophilus* in milk

Positive strains: 20 out of 191

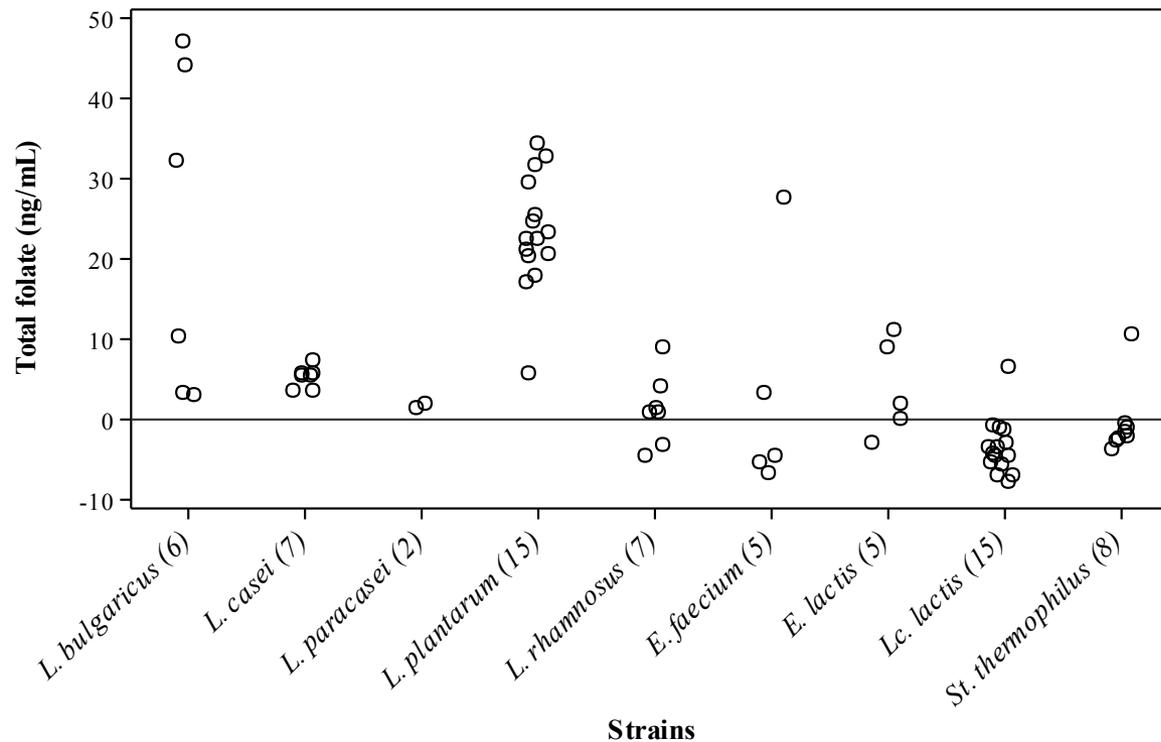


Brasca, M., et al. (2016). Proteolytic activity and production of γ -aminobutyric acid by *Streptococcus thermophilus* cultivated in microfiltered pasteurized milk.

Journal of agricultural and food chemistry, 64(45), 8604-8614.

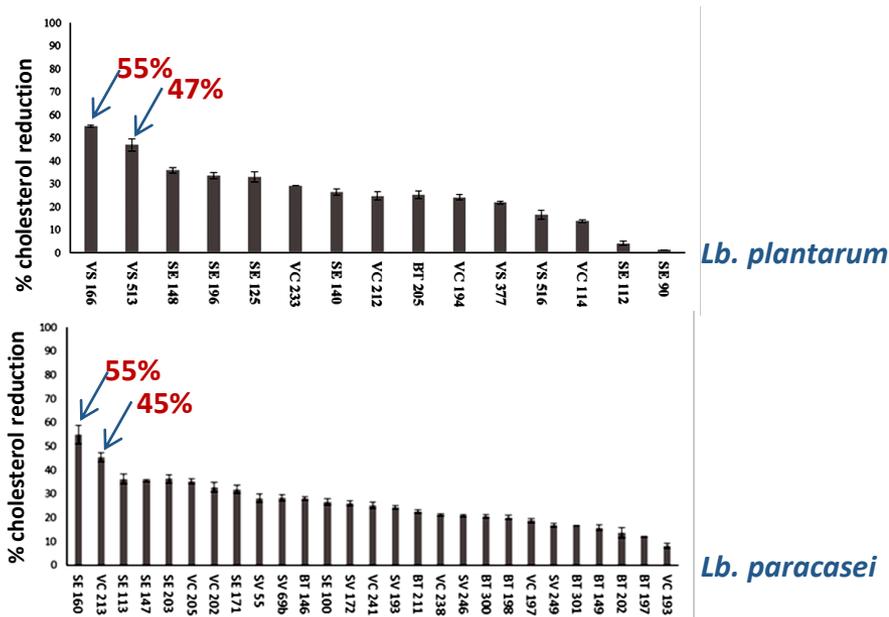
Production of folate by 70 wild LAB strains in cultural medium

Positive strains: 47 out of 70



Cholesterol reduction

Positive strains: 26 out of 58 provided a reduction



J. Dairy Sci. 101:10807–10818
<https://doi.org/10.3168/jds.2018-15096>
 © American Dairy Science Association®, 2018.

Lactic acid bacteria with cholesterol-lowering properties for dairy applications: In vitro and in situ activity

C. Albano,* S. Morandi,* T. Silveti,* M. C. Casiraghi,† F. Manini,* and M. Brasca*¹
^{*}Institute of Sciences of Food Production, National Research Council, Via Celoria 2, 20133 Milan, Italy
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Cholesterol content in cheeses produced with cholesterol lowering LAB strains during ripening



	30 days	60 days	P
	mg /100g fat	mg /100g fat	
Control_1	191.2 ± 1.7 ^b	180.3 ± 2.2 ^a	ns
<i>Lb. plantarum</i> VS 166	168.4 ± 0.5 ^a (12%)	170.7 ± 1.1 ^a (5%)	ns
Control_2	230.1 ± 13.1 ^c	209.0 ± 12.0 ^c	**
<i>Lb. plantarum</i> VS 513	182.8 ± 0.8 ^a (21%)	190.4 ± 0.1 ^b (9%)	ns
<i>Lb. casei</i> VC 199	193.2 ± 1.1 ^a (16%)	187.3 ± 0.3 ^b (10%)	ns
<i>Lb. paracasei</i> subsp. <i>paracasei</i> SE 160	210.2 ± 0.8 ^b (9%)	186.5 ± 1.2 ^b (11%)	***
<i>Lb. paracasei</i> subsp. <i>paracasei</i> VC 213	187.9 ± 0.6 ^a (18%)	174.7 ± 2.9 ^a (16%)	ns
Control_3	310.2 ± 2.7 ^c	258.2 ± 8.1 ^c	***
<i>E. lactis</i> BT 161	269.7 ± 0.3 ^b (13%)	199.8 ± 1.0 ^a (23%)	**
<i>E. faecium</i> VC 223	256.7 ± 2.4 ^a (17%)	225.6 ± 0.6 ^b (13%)	***



Strains able to reduce cholesterol in cheese were present in different cheeses

Health effects associated with consumption of cheese



<http://informahealthcare.com/ijf>
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Int J Food Sci Nutr, Early Online: 1-8
© 2015 Informa UK Ltd. DOI: 10.3109/09637486.2015.1024205

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healthcare

RESEARCH ARTICLE

Activity of 30 different cheeses on cholesterol plasma levels and Oxidative Balance Risk Index (OBRI) in a rat model

Umberto Cornelli¹, Gianpietro Bondiolotti², Giovanna Battelli³, Giuseppe Zanon⁴, Annarosa Finco⁵, and Martino Recchia⁶

¹Loyola University School of Medicine, Chicago, IL, USA, ²Pharmacology Department, University of Milan, Milan, Italy, ³CNR-ISPA, Milan, Italy, ⁴Organic Chemistry Department, University of Pavia, Pavia, Italy, ⁵Oxidation Research Department, Cor Con International Srl, Parma, Italy, and ⁶Biometrics Department, University of Lugano, Lugano, Switzerland

CI/AC/CM II



The model shows that some cheese can **reduce significantly CH levels** and **improve the antioxidant capacity**

Health effects associated with consumption of ripened cheese



J. Dairy Sci. 101:3742–3757
<https://doi.org/10.3168/jds.2017-13465>
© American Dairy Science Association®, 2018.

***Invited review:* Bioactive compounds produced during cheese ripening and health effects associated with aged cheese consumption**

Lourdes Santiago-López,^{*1} Jose E. Aguilar-Toalá,^{*1} Adrián Hernández-Mendoza,^{*} Belinda Vallejo-Cordoba,^{*} Andrea M. Liceaga,[†] and Aarón F. González-Córdova^{*2}

^{*}Laboratorio de Química y Biotecnología de Productos Lácteos, Coordinación de Tecnología de Alimentos de Origen Animal, Centro de Investigación en Alimentación y Desarrollo, A. C. (CIAD), Hermosillo, Sonora 83304, México

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The use of animal and clinical studies indicates that **consumption of cheese may contribute to reduced incidence of cardiovascular risk factors and other diseases**

Take home message



- Cheese represents an excellent natural and clean-labelled delivery system for functional microorganisms inclusion in the diet
- Raw milk cheeses are a **natural source of wide microbial biodiversity**
- LAB strains with functional properties are **naturally present** in raw milk cheeses
- **Potential human health benefits** associated with the consumption of traditional cheeses have been reported
- The results invite **new studies** on raw milk cheeses microbiome and the beneficial impact of cheese consumption on health