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Role of lactic acid bacterial biofilms in wooden equipment and safety aspects of cheeses with traditional characteristics.

El papel de las bacterias ácido lácticas en los biofilms de la madera en la elaboración de quesos con características tradicionales.

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INTERNATIONAL SCIENTIFIC CONFERENCE ON RAW MILK

International Scientific Conference on Raw Milk and XI Annual Meeting of FACEnetwork

Complejo Cultural La Petxina, 23 – 25 October 2019

Role of lactic acid bacterial biofilms and safety aspects of cheeses with traditional characteristics made with wooden equipment

Luca Settanni

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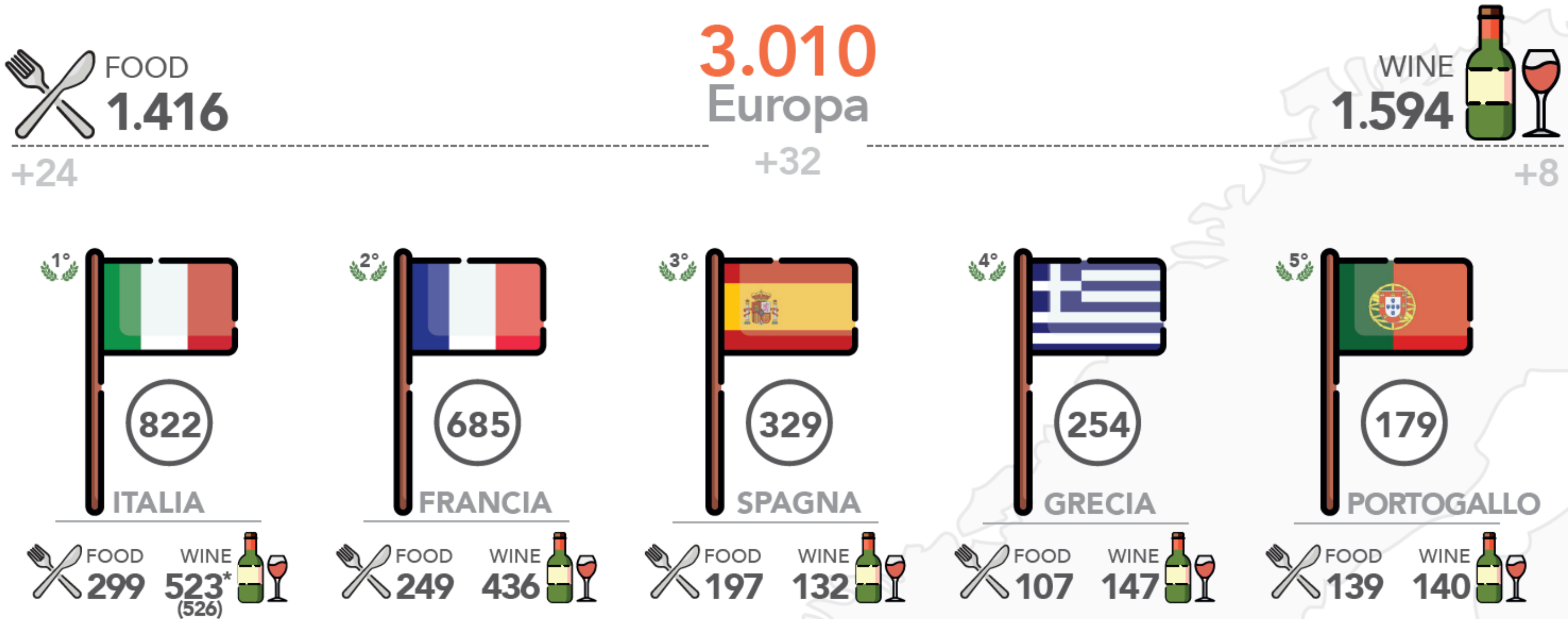
European food products enjoying a "recognition of quality" in 2018

protected designation of origin (PDO)

protected geographical indication (PGI)

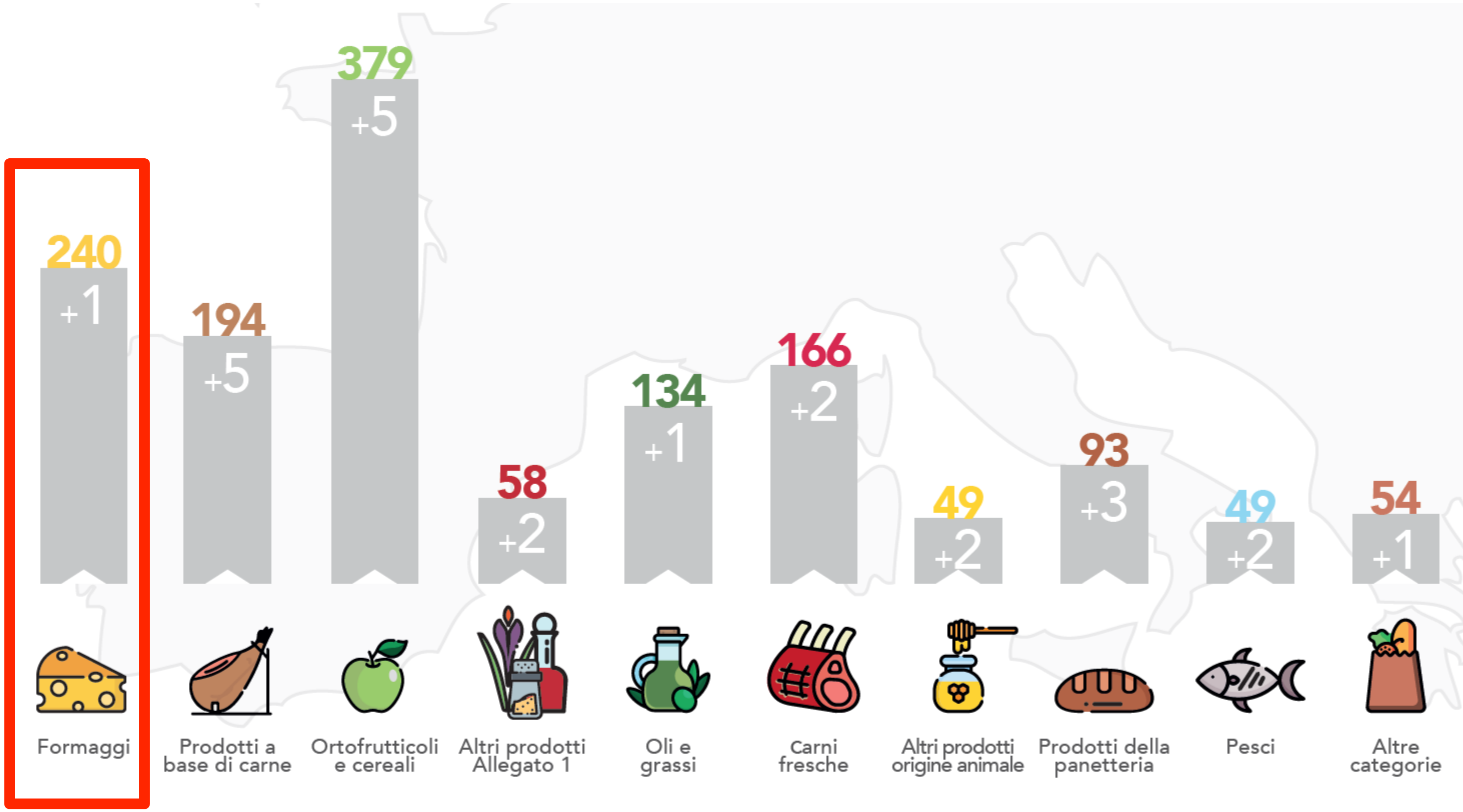
traditional specialty guaranteed (TSG)



Source: Rapporto ISMEA-Qualivita 2018

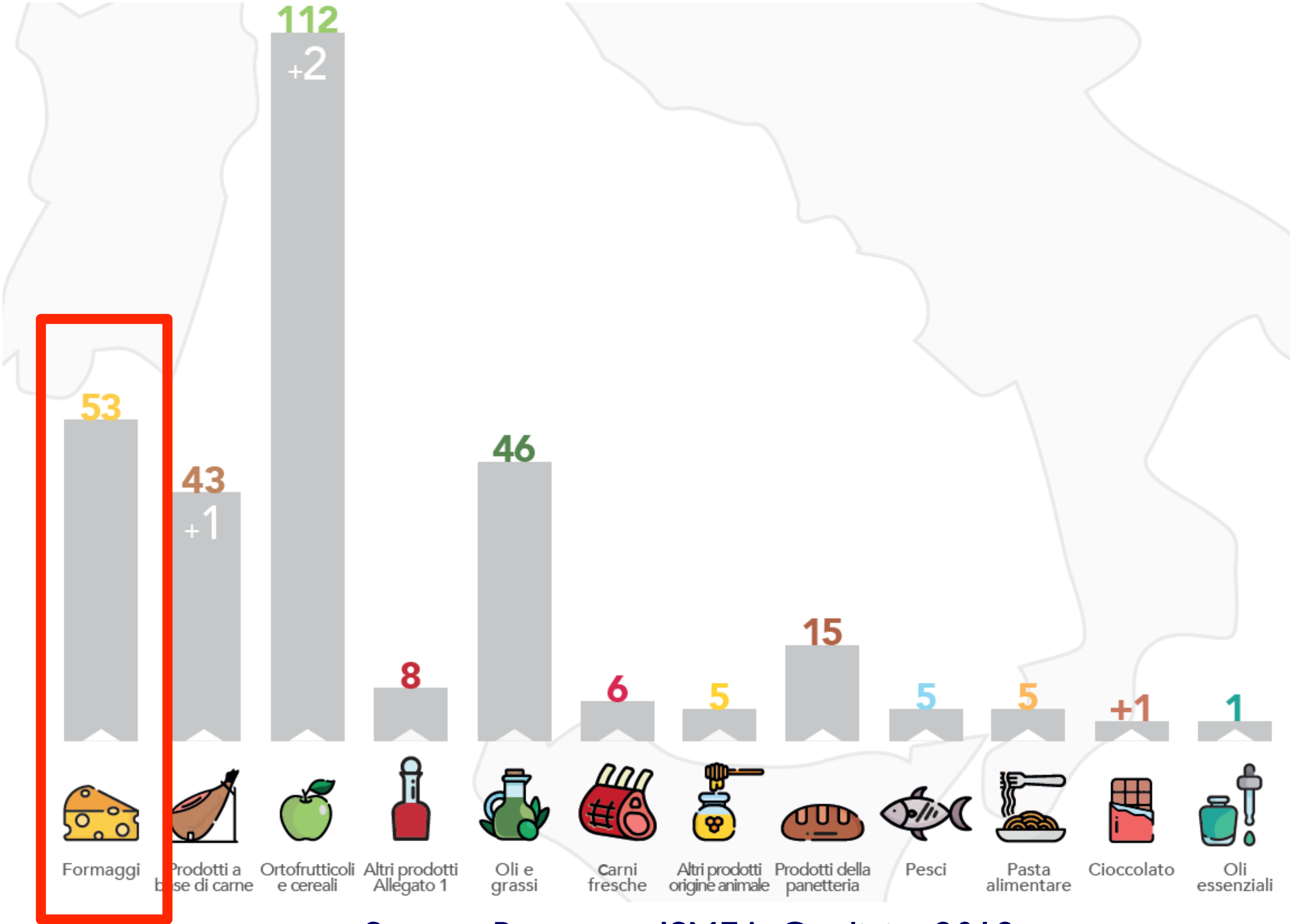
PDO, PGI and TSG foods in Europe (2018)

17% cheeses



Source: Rapporto ISMEA-Qualivita 2018

PDO, PGI and TSG foods in Italy (2018)



Source: Rapporto ISMEA-Qualivita 2018

Producers of PDO, PGI and TSG foods in Italy (2018)

83.695* operatori Food IG

** Gli operatori che sono sia produttori che trasformatori sono conteggiati nel totale una sola volta*

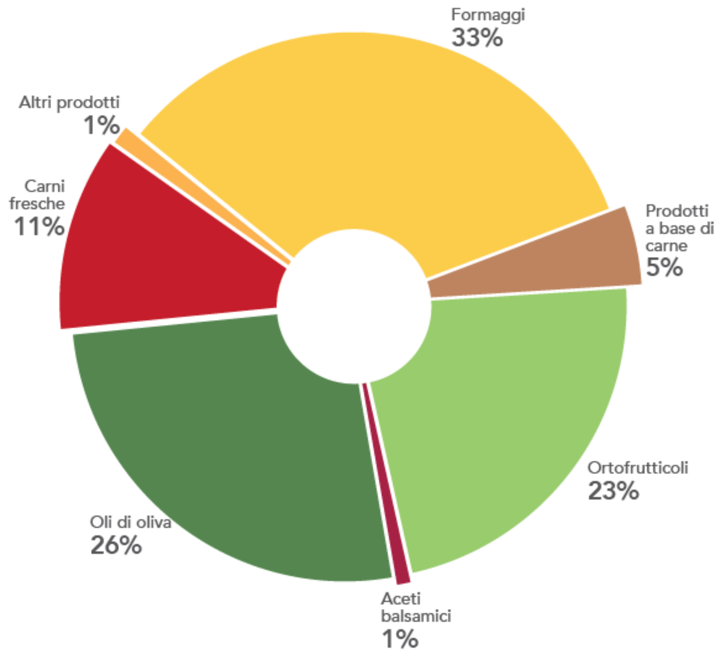
78.784 produttori

7.481 trasformatori

Infografica 2.1 operatori per categoria

Formaggi	27.933
Prod. a base di carne	4.014
Ortofrutticoli e cereali	18.829
Aceti Balsamici	650
Oli di oliva	21.881
Carni fresche	9.513
Altre categorie*	875

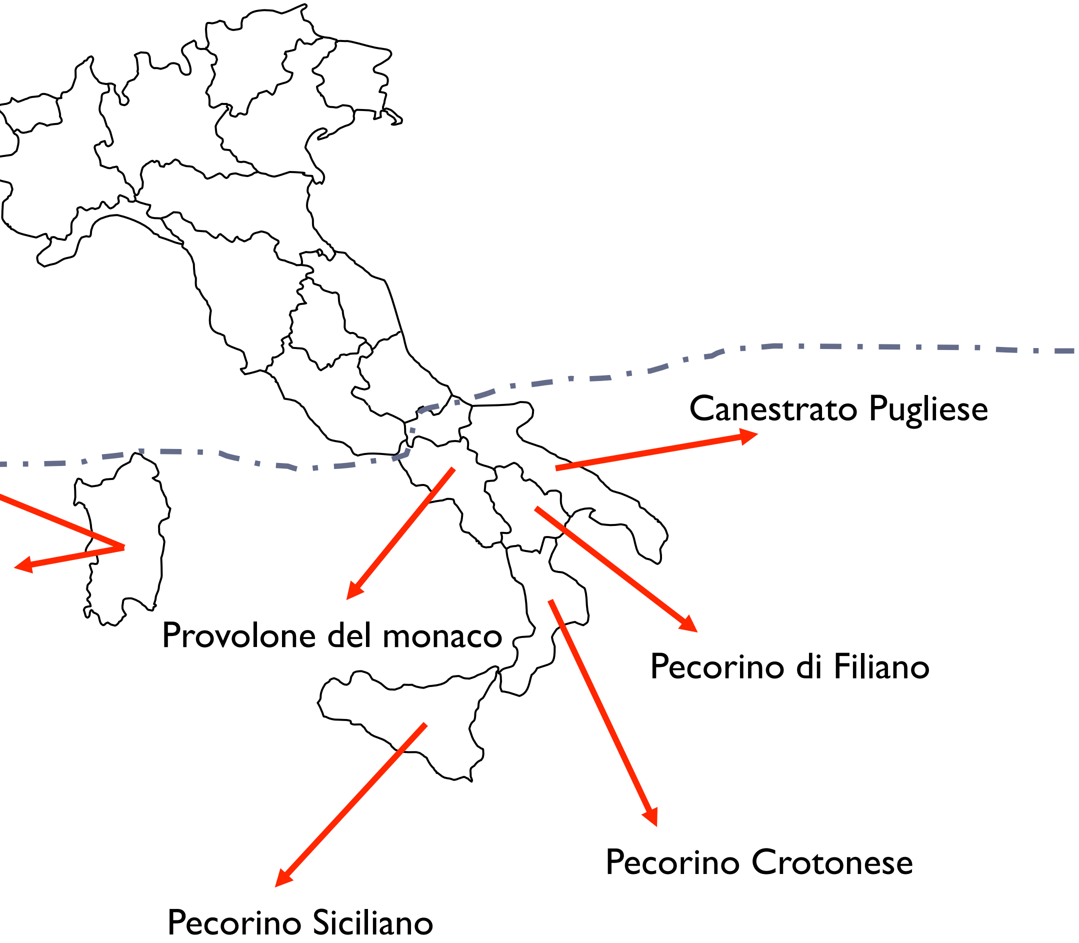
**Comprensivo del valore alla produzione Mozzarella STG*



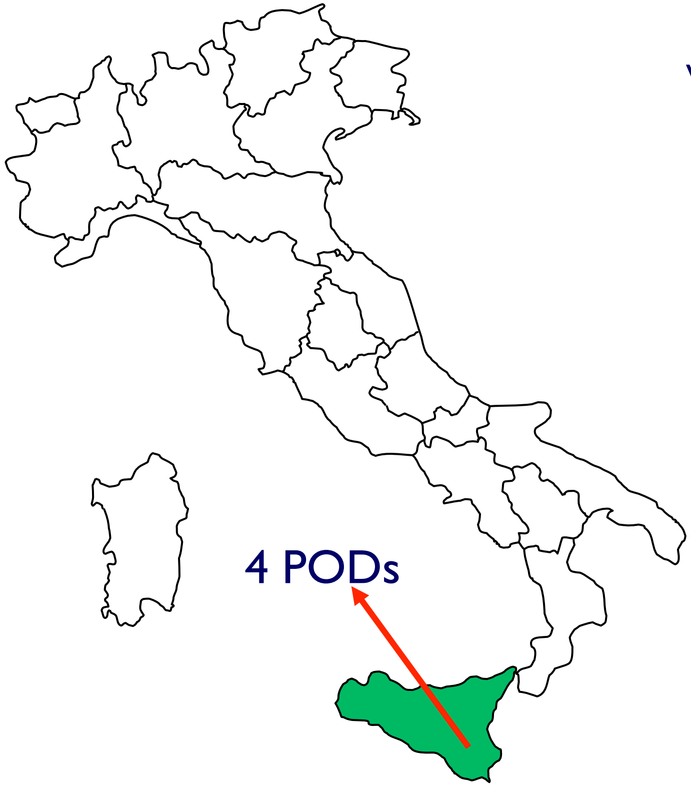
Source: Rapporto ISMEA-Qualivita 2018

Traditional PDO cheeses produced in South Italy

Only 13 PDO cheeses



Traditional PDO cheeses produced in Sicily



Vastedda della valle del Belìce

Ragusano

Pecorino Siciliano

Piacentinu Ennese

Traditional cheese making in Sicily

A food is considered traditional when an age-old process handed down from the past is applied (***Settanni and Moschetti*** *2014, Trends Food Sci Technol*)

raw milk

animal rennet paste

wooden vat

Without starter addition!

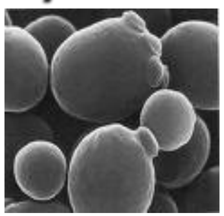
Cheese associated microorganisms

Aggregate of casein micelles forming a gel containing all solid components of milk

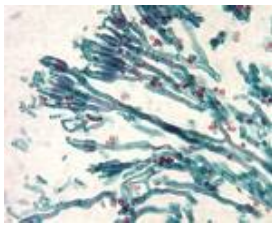
MICROORGANISMS are trapped

bacteria

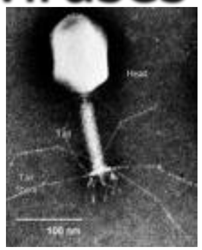
yeasts



moulds



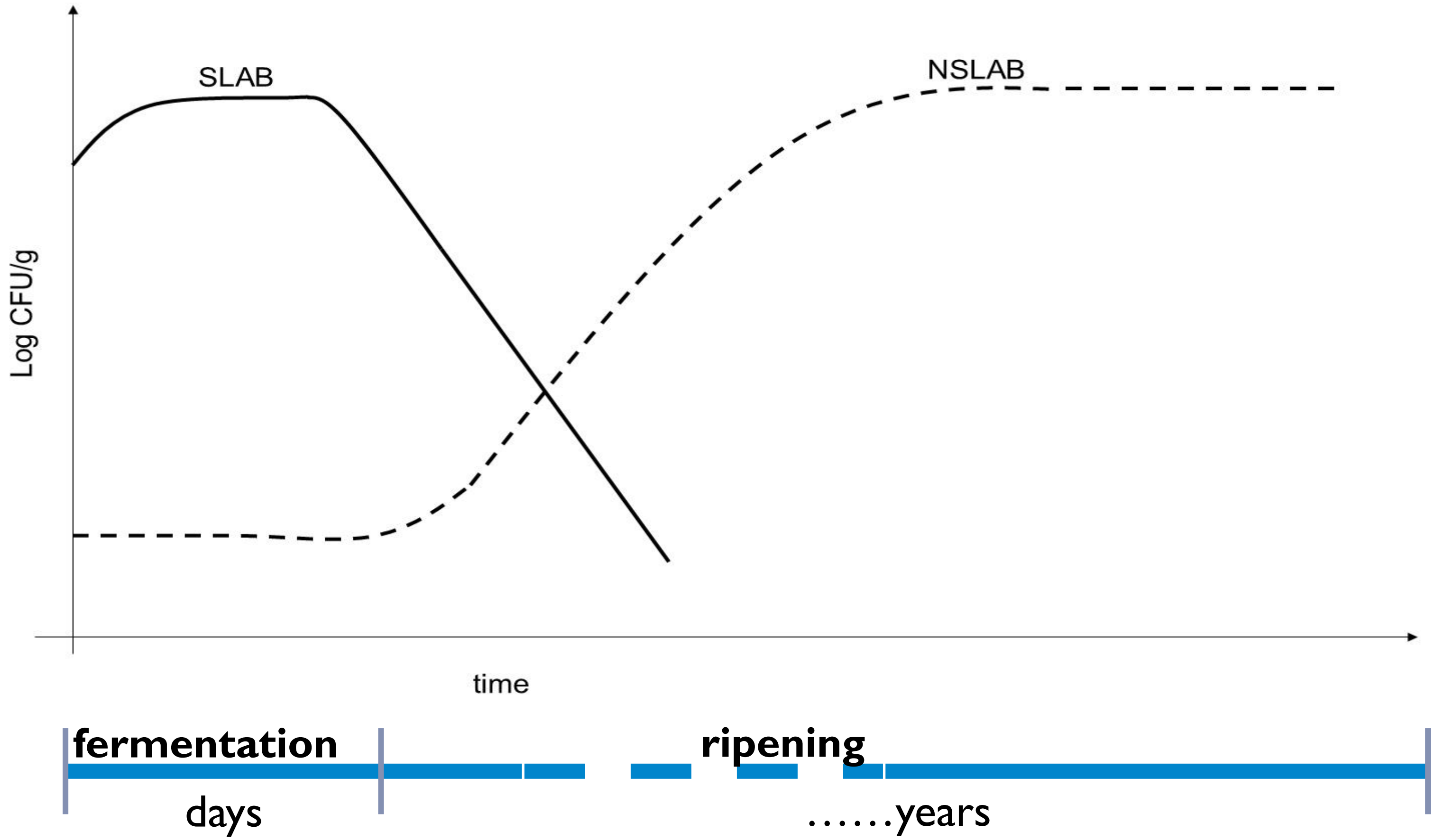
viruses



Evolution of LACTIC ACID BACTERIA during cheese production

LAB may play different roles in cheese-making:

- starter LAB (SLAB) perform the fermentation process
- non starter LAB (NSLAB) are implicated in the ripening (**Settanni and Moschetti**, 2010, Food Microbiol).



Sources of microbial contamination of traditional Sicilian cheeses

LACTIC ACID BACTERIA

Franciosi et al., 2009, Int Dairy J
Franciosi et al., 2011, World J Microbiol Biotechnol

Cruciata et al., 2014, Applied and Environmental Microbiology

Licitra et al., 2007, Appl Environ Microbiol
Lortal et al., 2009, Int J Food Microbiol
Settanni et al., 2012, International Journal of Food Microbiology
Scatassa et al., 2015, Food Microbiol.

Legislation regarding the use od wooden equipment in cheese making

- The Commission Regulation (EC) No 2074/2005 allows derogation from Regulation (EC) No 852/2004 for foods with traditional characteristics "as regards the type of materials of which the instruments and the equipment used specifically for the preparation, packaging and wrapping of these products are made"

- Regarding the materials in contact with cheese, the rule CE n. 1935/2004 reports the "principle of no contamination"

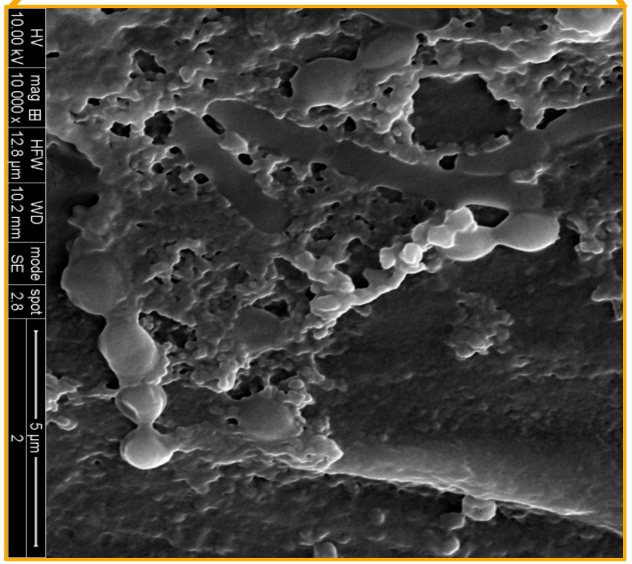
- However, no limitations to the use of wooden equipment are specifically indicated

- **Italy** (especially Sicily) and **France** take advantages of this derogation

Wooden equipment in traditional cheese making







A **BIOFILM** is an "aggregate of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS) adhere to each other and/or to a surface" (Vert et al., 2012 Pure Appl Chem)

Roles of wooden vat biofilms

- do they produce enough actic acid production and partecipate to the acidification of curd?
- inhibit most of the undesired microorganisms?
- ensure high aromatic complexity and almost constant organoleptic characteristics over time?

Microbiological characterization of wooden vats used in cheese making in Western Sicily

Characteristics of the wooden vats.

Wooden vat	City of dairy factory (province)[a]	Age of vat (years)	Type of wood	Cheese	Milk processed	Milk volume (L)	Type of washing
1	Godrano (PA)	28	chestnut	Caciocavallo Palermitano	Bovine	160	HDW (in winter); CW (in summer)
2	Godrano (PA)	10	chestnut	Caciocavallo Palermitano	Bovine	400	HDW
3	Santa Margherita del Belìce (AG)	5	douglas	Vastedda della valle del Belìce	Ovine	200	HDW
4	Menfi (AG)	5	douglas	Vastedda della valle del Belìce	Ovine	250	CW
5	Terrasini (PA)	10	chestnut	Caciocavallo Palermitano	Bovine	170	HDW (in winter); CW (in summer)
6	Cinisi (PA)	10	chestnut	Caciocavallo Palermitano	Bovine	300	HDW
7	Godrano (PA)	10	chestnut	Caciocavallo Palermitano	Bovine	250	HDW
8	Terrasini (PA)	10	chestnut	Caciocavallo Palermitano	Bovine	300	HDW (in winter); CW (in summer)
9	Salemi (TP)	5	douglas	Vastedda della valle del Belìce	Ovine	190	CW
10	Salemi (TP)	7	douglas	Vastedda della valle del Belìce	Ovine	190	CW
11	Partanna (TP)	5	douglas	Vastedda della valle del Belìce	Ovine	150	HDW
12	Godrano (PA)	20	chestnut	Caciocavallo Palermitano	Bovine	220	HDW (in winter); CW (in summer)

Scatassa et al., 2015. Food Microbiol 52:31–41

Biofilm collection

Didienne et al., 2012. Int J Food Microbiol 156:91–101

Microbiological counts of LAB

Sample	Bacterial counts TMC	Enterococci	Rod LAB MRS-30 °C	Rod LAB MRS-44 °C	Coccus LAB M17-30 °C	Coccus LAB M17-44 °C
Wooden vat:						
WV1	4.9 ± 0.2^{AB}	2.3 ± 0.2^{A}	4.7 ± 0.2^{B}	4.9 ± 0.2^{CD}	5.3 ± 0.3^{B}	5.9 ± 0.1^{C}
WV2	5.7 ± 0.2^{C}	3.2 ± 0.1^{BC}	3.9 ± 0.2^{A}	5.4 ± 0.2^{D}	5.4 ± 0.2^{BC}	5.7 ± 0.2^{C}
WV3	4.5 ± 0.3^{AB}	3.2 ± 0.2^{BC}	3.6 ± 0.1^{A}	3.0 ± 0.1^{A}	4.9 ± 0.1^{AB}	5.2 ± 0.1^{BC}
WV4	6.1 ± 0.2^{CD}	4.4 ± 0.1^{D}	5.8 ± 0.2^{C}	5.9 ± 0.1^{DE}	6.2 ± 0.2^{C}	6.3 ± 0.1^{CD}
WV5	6.1 ± 0.1^{CD}	2.9 ± 0.1^{B}	4.8 ± 0.2^{B}	4.7 ± 0.2^{C}	6.2 ± 0.2^{C}	5.8 ± 0.1^{C}
WV6	5.0 ± 0.1^{B}	4.2 ± 0.2^{D}	4.8 ± 0.2^{B}	4.9 ± 0.1^{CD}	5.8 ± 0.2^{C}	5.6 ± 0.1^{BC}
WV7	6.4 ± 0.1^{D}	4.0 ± 0.0^{CD}	4.1 ± 0.1^{A}	5.8 ± 0.2^{DE}	6.9 ± 0.1^{D}	6.5 ± 0.2^{D}
WV8	5.4 ± 0.1^{BC}	2.2 ± 0.2^{A}	5.1 ± 0.1^{B}	5.1 ± 0.1^{CD}	5.7 ± 0.1^{BC}	5.5 ± 0.1^{BC}
WV9	5.7 ± 0.1^{C}	3.5 ± 0.1^{C}	6.0 ± 0.1^{C}	6.1 ± 0.1^{E}	4.6 ± 0.2^{A}	5.3 ± 0.1^{BC}
WV10	4.3 ± 0.1^{A}	3.4 ± 0.1^{BC}	4.6 ± 0.1^{B}	4.9 ± 0.1^{CD}	4.5 ± 0.2^{A}	3.6 ± 0.1^{A}
WV11	5.2 ± 0.1^{BC}	2.5 ± 0.1^{AB}	3.8 ± 0.1^{A}	4.0 ± 0.1^{B}	4.9 ± 0.1^{AB}	5.1 ± 0.2^{B}
WV12	5.4 ± 0.1^{BC}	3.3 ± 0.1^{BC}	5.2 ± 0.1^{B}	5.0 ± 0.1^{CD}	5.4 ± 0.2^{BC}	5.5 ± 0.2^{BC}
Statistical significance	***	***	***	***	***	***
Milk before contact:						
MBC1	5.4 ± 0.2^{CD}	4.4 ± 0.1^{CD}	4.9 ± 0.1^{C}	4.8 ± 0.1^{B}	5.0 ± 0.2^{B}	5.1 ± 0.1^{BC}
MBC2	6.5 ± 0.2^{E}	6.8 ± 0.3^{F}	5.9 ± 0.2^{DE}	6.2 ± 0.2^{D}	6.5 ± 0.3^{DE}	6.5 ± 0.1^{DE}
MBC3	7.1 ± 0.1^{F}	4.1 ± 0.2^{C}	7.2 ± 0.1^{F}	7.1 ± 0.2^{E}	7.0 ± 0.1^{F}	7.1 ± 0.1^{F}
MBC4	6.2 ± 0.1^{DE}	5.1 ± 0.1^{DE}	5.5 ± 0.1^{D}	5.4 ± 0.2^{C}	6.1 ± 0.3^{CD}	6.1 ± 0.2^{D}
MBC5	5.4 ± 0.1^{C}	3.7 ± 0.3^{BC}	3.7 ± 0.2^{AB}	4.1 ± 0.1^{A}	5.6 ± 0.1^{C}	4.8 ± 0.2^{B}
MBC6	4.8 ± 0.1^{B}	4.7 ± 0.1^{D}	4.2 ± 0.1^{B}	4.3 ± 0.1^{AB}	4.5 ± 0.2^{B}	4.4 ± 0.1^{AB}
MBC7	5.8 ± 0.2^{CD}	5.6 ± 0.2^{E}	6.1 ± 0.2^{E}	6.1 ± 0.1^{D}	6.2 ± 0.1^{D}	6.0 ± 0.3^{D}
MBC8	7.1 ± 0.2^{F}	4.4 ± 0.1^{CD}	6.9 ± 0.2^{F}	6.9 ± 0.2^{E}	6.8 ± 0.1^{EF}	6.8 ± 0.1^{EF}
MBC9[b]	4.0 ± 0.2^{A}	3.4 ± 0.2^{AB}	3.6 ± 0.3^{A}	3.9 ± 0.2^{A}	3.7 ± 0.1^{A}	3.9 ± 0.2^{A}
MBC10[b]	4.0 ± 0.2^{A}	3.4 ± 0.2^{AB}	3.6 ± 0.3^{A}	3.9 ± 0.2^{A}	3.7 ± 0.1^{A}	3.9 ± 0.2^{A}
MBC11	5.0 ± 0.1^{BC}	3.5 ± 0.1^{B}	3.7 ± 0.2^{AB}	3.8 ± 0.2^{A}	4.9 ± 0.1^{B}	5.0 ± 0.2^{BC}
MBC12	5.6 ± 0.2^{C}	3.1 ± 0.1^{A}	5.4 ± 0.1^{CD}	5.6 ± 0.2^{C}	5.6 ± 0.1^{C}	5.4 ± 0.1^{C}
Statistical significance	***	***	***	***	***	***
Milk after contact:						
MAC1	5.5 ± 0.2^{B}	5.0 ± 0.2^{BC}	5.6 ± 0.2^{C}	5.6 ± 0.2^{C}	6.1 ± 0.3^{C}	6.2 ± 0.2^{BC}
MAC2	6.2 ± 0.1^{C}	6.5 ± 0.1^{D}	5.7 ± 0.1^{CD}	6.2 ± 0.3^{D}	6.5 ± 0.2^{CD}	6.5 ± 0.1^{C}
MAC3	7.4 ± 0.1^{E}	5.2 ± 0.3^{BC}	6.2 ± 0.2^{D}	6.8 ± 0.1^{E}	7.5 ± 0.2^{E}	7.5 ± 0.2^{D}
MAC4	7.1 ± 0.3^{DE}	5.3 ± 0.3^{C}	7.2 ± 0.2^{E}	6.1 ± 0.1^{CD}	7.3 ± 0.2^{E}	7.2 ± 0.3^{D}
MAC5	5.7 ± 0.1^{BC}	4.7 ± 0.2^{B}	4.5 ± 0.3^{AB}	4.3 ± 0.2^{B}	5.4 ± 0.1^{BC}	4.8 ± 0.2^{A}
MAC6	5.8 ± 0.2^{BC}	5.0 ± 0.3^{BC}	4.1 ± 0.1^{A}	3.7 ± 0.1^{A}	5.4 ± 0.2^{BC}	5.2 ± 0.2^{AB}
MAC7	6.8 ± 0.2^{D}	6.2 ± 0.2^{D}	6.4 ± 0.1^{D}	6.3 ± 0.2^{DE}	6.3 ± 0.3^{CD}	6.4 ± 0.1^{C}
MAC8	7.0 ± 0.2^{DE}	5.2 ± 0.1^{BC}	6.7 ± 0.3^{DE}	6.6 ± 0.2^{DE}	6.7 ± 0.2^{D}	6.7 ± 0.2^{CD}
MAC9	4.5 ± 0.2^{A}	4.1 ± 0.2^{A}	5.9 ± 0.1^{CD}	5.6 ± 0.2^{C}	4.7 ± 0.3^{A}	5.1 ± 0.1^{A}
MAC10	4.9 ± 0.1^{A}	4.3 ± 0.1^{AB}	4.6 ± 0.3^{AB}	4.5 ± 0.1^{B}	5.3 ± 0.3^{B}	5.7 ± 0.1^{B}
MAC11	5.5 ± 0.2^{B}	4.5 ± 0.2^{AB}	4.8 ± 0.1^{B}	4.6 ± 0.2^{B}	5.5 ± 0.2^{BC}	6.6 ± 0.2^{C}
MAC12	6.3 ± 0.2^{CD}	4.1 ± 0.3^{A}	5.9 ± 0.2^{CD}	5.9 ± 0.1^{CD}	5.9 ± 0.2^{C}	6.0 ± 0.1^{BC}
Statistical significance	***	***	***	***	***	***

Wooden vat biofilms

Bulk milk at arrival in dairy farms

Bulk milk after 5 min contact

Effects of wooden vat biofilms on milk microbiology

The levels of milk lactic acid bacteria are consistently influenced by wooden vat biofilms when their levels at arrival to the dairy farms are lower than 6 Log CFU/mL

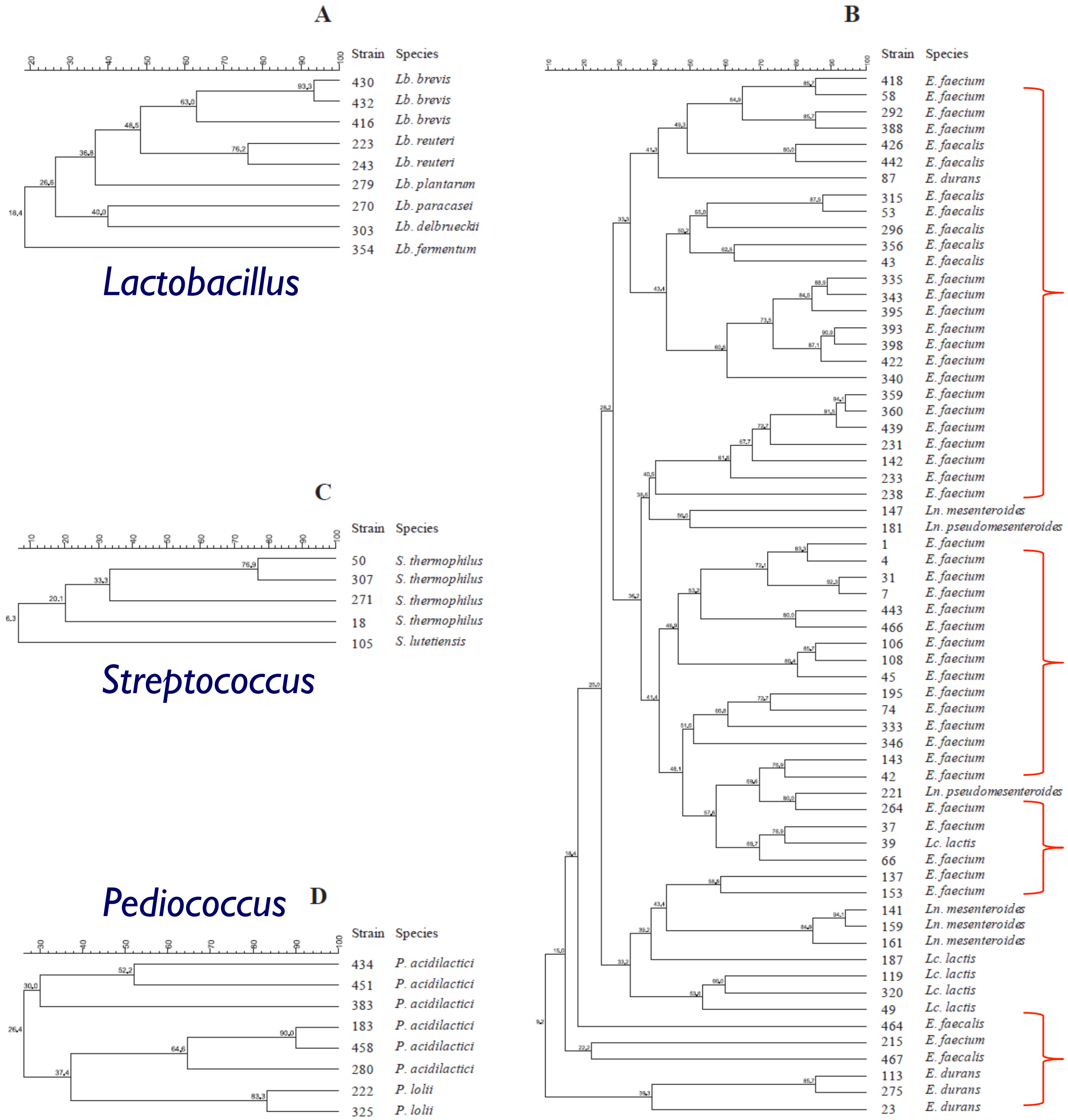
Microbiological counts of undesired groups

Sample	Bacterial counts		
	TMC	Total coliforms	*E. coli*
Wooden vat:			
WV1	4.9 ± 0.2^{AB}	0.3 ± 0.0^{A}	$<1^{A}$
WV2	5.7 ± 0.2^{C}	2.3 ± 0.1^{CD}	$<1^{A}$
WV3	4.5 ± 0.3^{AB}	0.9 ± 0.1^{B}	$<1^{A}$
WV4	6.1 ± 0.2^{CD}	6.0 ± 0.1^{E}	3.2 ± 0.2^{C}
WV5	6.1 ± 0.1^{CD}	3.2 ± 0.1^{D}	2.5 ± 0.2^{B}
WV6	5.0 ± 0.1^{B}	0.7 ± 0.0^{AB}	$<1^{A}$
WV7	6.4 ± 0.1^{D}	0.7 ± 0.1^{AB}	$<1^{A}$
WV8	5.4 ± 0.1^{BC}	2.0 ± 0.2^{C}	$<1^{A}$
WV9	5.7 ± 0.1^{C}	1.8 ± 0.1^{C}	$<1^{A}$
WV10	4.3 ± 0.1^{A}	0.6 ± 0.1^{AB}	$<1^{A}$
WV11	5.2 ± 0.1^{BC}	1.4 ± 0.1^{BC}	$<1^{A}$
WV12	5.4 ± 0.1^{BC}	2.8 ± 0.2^{D}	2.0 ± 0.1^{B}
Statistical significance	***	***	***

Staphylococcus aureus, *L. monocytogenes* and *Salmonella* spp.

not detected

LAB biodiversity



85 strains

16 species

6 genera

High biodiversity of *Enterococcus*

Role of wooden vat starter LAB

versus

Traditional production without commercial STARTERS

Standard production with commercial STARTERS

The same RAW bulk milk in both productions

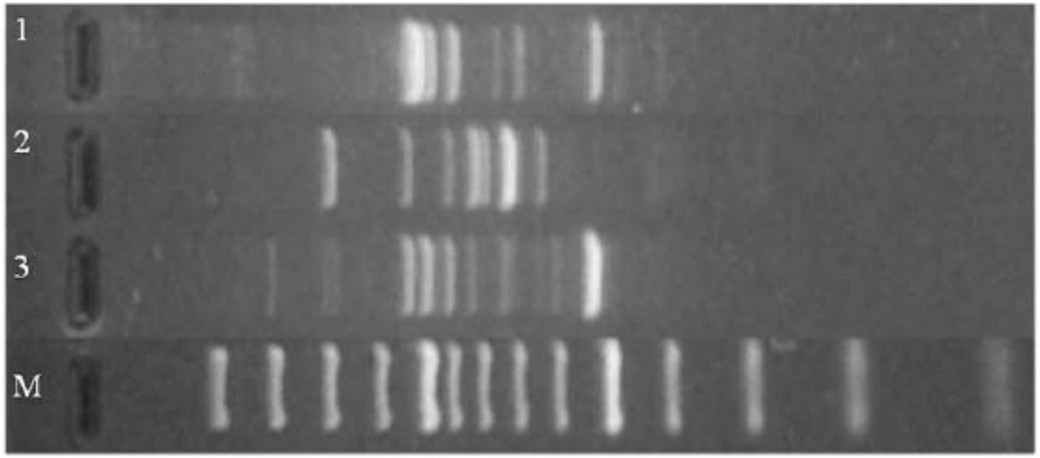
Persistence and dominance of wooden vat SLAB

Identification and distribution of LAB strains through traditional Caciocavallo Palermitano cheese production.

Strain	Phenotypic group	Acc. No.	Species	Matrices								
				Bulk milk	Wooden vat surfaces	Bulk milk after resting	Curd	Whey	Cooked curd	Acidified curd	Stretched curd	Whey after stretching
FMA108	III	HQ721253	*E. casseliflavus*		■	■						
FMA8	III	HQ721252	*E. durans*	■		■						
FMA444	III	HQ721272	*E. faecalis*	■		■	■	■	■	■	■	
FMA463	III	HQ721256	*E. faecalis*	■		■	■				■	
FMA604	III	HQ721258	*E. faecalis*	■		■	■		■	■	■	
FMA713	III	HQ721273	*E. faecalis*	■		■	■	■	■	■		
FMA721	III	HQ721277	*E. faecalis*		■	■	■		■		■	
FMA797	III	HQ721260	*E. faecalis*	■		■	■	■	■	■	■	
MOB6	III	n.d.	*E. faecalis*		■	■	■	■	■	■		
FMA505	III	HQ721257	*E. faecium*		■	■						
FMA288	III	HQ721269	*E. gallinarum*		■	■						
FMA192	III	HQ721268	*E. italicus*		■	■						
FMA295	III	HQ721276	*E. italicus*		■							
FMA224	V	HQ721246	*Lb. alimentarius*	■								
FMA205	VI	HQ721262	*Lb. parabuchneri*	■								
FMA395	II	HQ721245	*Lc. garvieae*	■		■	■		■			
FMA401	II	HQ721279	*Lc. garvieae*	■		■	■			■	■	
FMA809	II	HQ721265	*Lc. garvieae*	■		■	■		■	■		
FMA187	II	HQ721254	*Lc. lactis spp. lactis*	■		■	■	■				
FMA558	II	HQ721275	*Lc. lactis spp. lactis*	■								
MOB4	II	HQ721267	*Lc. lactis spp. lactis*	■								
FMA6	I	n.d.	*Leuconostoc* spp.	■		■			■			
FMA766	IV	HQ721264	*S. bovis*	■								
FMA830	IV	HQ721250	*S. macedonicus*						■			■
FMA196	IV	HQ721271	*S. thermophilus*		■	■	■	■	■	■	■	■
FMA327	IV	HQ721274	*S. thermophilus*		■	■		■	■	■	■	■
FMA617	IV	HQ721278	*S. thermophilus*		■	■	■	■		■	■	
FMA701	IV	HQ721263	*S. thermophilus*		■	■	■	■	■			
FMA808	IV	HQ721249	*S. thermophilus*		■	■	■	■	■			
FMA854	IV	HQ721261	*S. thermophilus*		■	■	■	■	■	■	■	■
FMA204	VII	HQ721255	*W. paramesenteroides*	■		■	■		■			
FMA246	VII	HQ721270	*W. paramesenteroides*		■	■	■					
FMA539	VII	HQ721247	*W. paramesenteroides*	■		■	■					

Settanni et al., 2012. Int. J. Food Microbiol 155: 73–81

Role of wooden vat non starter LAB



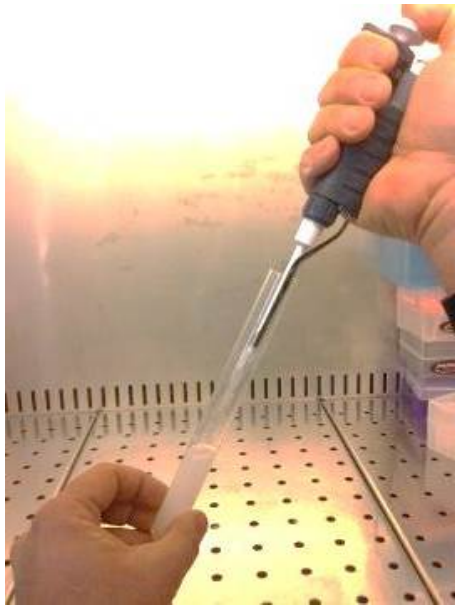
30 d	60 d	120 d
TA, TB	TA, TB	TA, TB
TA, TB	TB	n.f.
TA, TB	n.f.	n.f.

Persistence of *Enterococcus faecalis* of wooden vat origin in cheese until 120 days of ripening

Di Grigoli et al., 2015. Food Microbiol 46: 81–91

Contribution of wooden vat biofilms to cheese aroma

Wooden vat LAB in cheese based medium and their correlation with ripened cheese



Sgarbi, et al. 2013. J Dairy Sci 96: 4223-4234

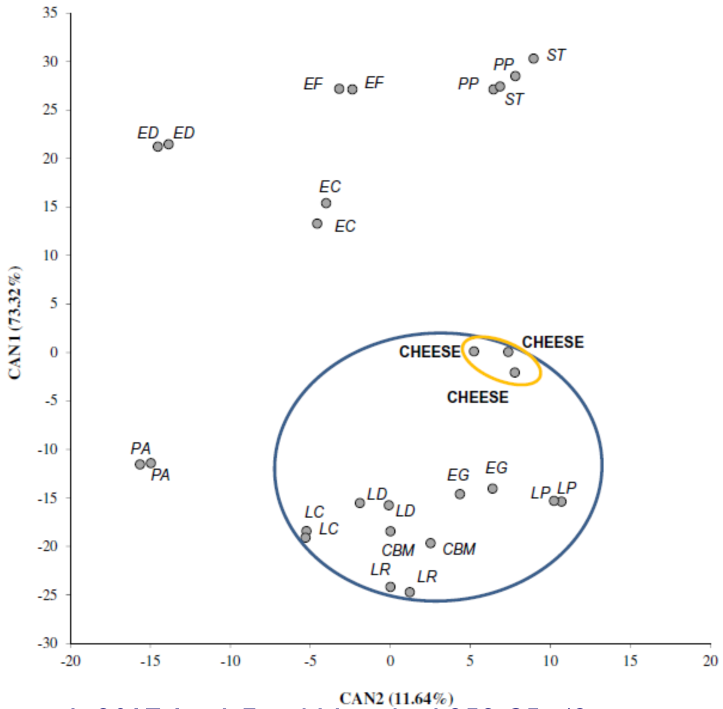
Contribution of wooden vat biofilms to cheese aroma

Microbial loads of individual LAB in CBM (log CFU/mL).

Species	Strain	Inoculation level	Growth level
E. casselliflavus	FMAC163	6.73 ± 0.04	7.44 ± 0.03
E. faecalis	FMA721	5.56 ± 0.01	7.67 ± 0.14
E. durans	FMAC134B	5.66 ± 0.06	7.56 ± 0.01
E. gallinarum	FMA288	6.20 ± 0.05	7.71 ± 0.03
P. acidilactici	FMAC31	6.22 ± 0.10	7.59 ± 0.05
P. pentosaceus	FMAC67	5.53 ± 0.06	7.41 ± 0.57
L. casei	FMAC16	6.23 ± 0.32	7.43 ± 0.01
L. paracasei	FMAC21	6.39 ± 0.07	7.34 ± 0.01
L. rhamnosus	FMAC240	5.24 ± 0.04	7.86 ± 0.18
L. delbrueckii	FMAC8	6.66 ± 0.22	7.11 ± 0.14
S. thermophilus	FMA854	6.36 ± 0.03	7.05 ± 0.02
Negative control		0	0

Canonical discriminant analysis: correlation coefficients for VOCs in common between Caciocavallo Palermitano cheese and CBM fermented by LAB strains with the canonical variables 1 and 2.

Compound[a]	Relative abundance % in cheese	Canonical V1	Canonical V2
		Variance %: 73.32	Variance %: 11.64
Ethanol	2.01	0.218364	−0.108000
2-Butanol	2.45	0.207920	−0.308471
1-Butanol	0.19	0.274967	−0.047738
2-Heptanol	0.52	0.381865	−0.330127
1-Hexanol	0.57	0.560646	0.078972
Hexanal	0.35	0.333622	−0.511534
2-Butanone	1.25	−0.621729	−0.020801
2-Nonanone	2.59	0.294526	−0.392939
Hexanoic acid, ethyl ester	2.75	0.120034	0.468177
Ethyl acetate	0.56	−0.899220	0.184209
o-Xylene	0.17	0.859064	0.049584
m-Xylene	0.51	0.814294	0.203808
Butanoic acid	51.59	−0.373260	0.247021
Hexanoic acid	32.07	−0.285883	0.191259
Octanoic acid	2.43	−0.222745	−0.304033



Guarrasi et al., 2017. Int. J. Food Microbiol 259: 35–42

Characterization of dairy enterococci isolated along the entire cheese making chains

Enterococci are responsible of several typicality traits of traditional cheeses (Folquié-Moreno et al., 2006)

Enterococci are also responsible for some health concerns due to their virulence. Furthermore, they can transfer antibiotic resistance genes

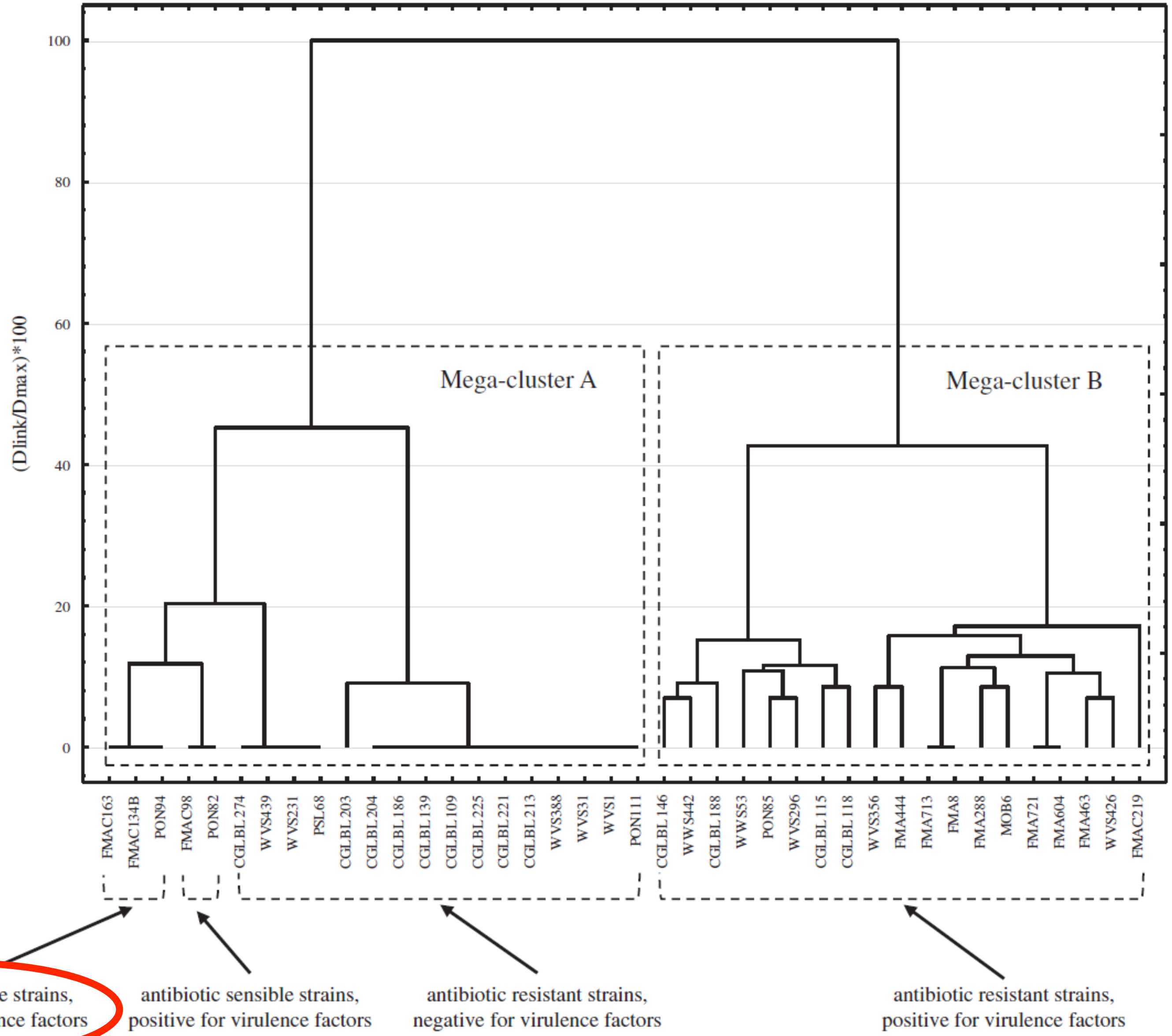
No nosocomial infection due to the consumption of dairy products containing enterococci has been registered

Origin of dairy Sicilian enterococci

Strain	Species	Origin	Reference
PON82	*E. gallinarum*	PDO Vastedda della valle del Belìce cheese[a]	Gaglio et al. (2014a)
PON85	*E. faecalis*	PDO Vastedda della valle del Belìce cheese[a]	Gaglio et al. (2014a)
PON94	*E. faecium*	PDO Vastedda della valle del Belìce cheese[a]	Gaglio et al. (2014a)
PON111	*E. faecium*	PDO Vastedda della valle del Belìce cheese[a]	Gaglio et al. (2014a)
PSL68	*E. faecium*	PDO Pecorino Siciliano cheese[b]	Todaro et al. (2011)
MOB6	*E. faecalis*	Wooden vat surfaces	Settanni et al. (2012)
FMA8	*E. faecalis*	Bovine bulk milk	Settanni et al. (2012)
FMA288	*E. gallinarum*	Wooden vat surfaces	Settanni et al. (2012)
FMA444	*E. faecalis*	Bovine bulk milk	Settanni et al. (2012)
FMA463	*E. faecalis*	Bovine bulk milk	Settanni et al. (2012)
FMA604	*E. faecalis*	Bovine bulk milk	Settanni et al. (2012)
FMA713	*E. faecalis*	Bovine bulk milk	Settanni et al. (2012)
FMA721	*E. faecalis*	Wooden vat surfaces	Settanni et al. (2012)
CGLBL109	*E. faecium*	Animal rennet	Cruciata et al. (2014)
CGLBL115	*E. faecalis*	Animal rennet	Cruciata et al. (2014)
CGLBL118	*E. faecium*	Animal rennet	Cruciata et al. (2014)
CGLBL139	*E. faecium*	Animal rennet	Cruciata et al. (2014)
CGLBL146	*E. faecalis*	Animal rennet	Cruciata et al. (2014)
CGLBL186	*E. faecium*	Animal rennet	Cruciata et al. (2014)
CGLBL188	*E. faecalis*	Animal rennet	Cruciata et al. (2014)
CGLBL203	*E. faecium*	Animal rennet	Cruciata et al. (2014)
CGLBL204	*E. faecium*	Animal rennet	Cruciata et al. (2014)
CGLBL213	*E. faecium*	Animal rennet	Cruciata et al. (2014)
CGLBL221	*E. faecium*	Animal rennet	Cruciata et al. (2014)
CGLBL225	*E. faecium*	Animal rennet	Cruciata et al. (2014)
CGLBL274	*E. faecium*	Animal rennet	Cruciata et al. (2014)
FMAC98	*E. casseliflavus*	Caciocavallo Palermitano cheese[c]	Di Grigoli et al. (2015)
FMAC134B	*E. durans*	Caciocavallo Palermitano cheese[c]	Di Grigoli et al. (2015)
FMAC163	*E. casseliflavus*	Caciocavallo Palermitano cheese[c]	Di Grigoli et al. (2015)
FMAC219	*E. faecalis*	Caciocavallo Palermitano cheese[c]	Di Grigoli et al. (2015)
WVS1	*E. faecium*	Wooden vat surfaces (cows' cheese)	Scatassa et al. (2015)
WVS31	*E. faecium*	Wooden vat surfaces (cows' cheese)	Scatassa et al. (2015)
WVS53	*E. faecalis*	Wooden vat surfaces (cows' cheese)	Scatassa et al. (2015)
WVS231	*E. faecium*	Wooden vat surfaces (cows' cheese)	Scatassa et al. (2015)
WVS296	*E. faecalis*	Wooden vat surfaces (cows' cheese)	Scatassa et al. (2015)
WVS356	*E. faecalis*	Wooden vat surfaces (ewes' cheese)	Scatassa et al. (2015)
WVS388	*E. faecium*	Wooden vat surfaces (ewes' cheese)	Scatassa et al. (2015)
WVS426	*E. faecalis*	Wooden vat surfaces (ewes' cheese)	Scatassa et al. (2015)
WVS439	*E. faecium*	Wooden vat surfaces (ewes' cheese)	Scatassa et al. (2015)
WVS442	*E. faecalis*	Wooden vat surfaces (cows' cheese)	Scatassa et al. (2015)

Fresh and ripened cheeses, raw milk, rennet pastes, wooden vat surfaces

Grouping based on the antibiotic resistance and virulence



Gaglio et al. 2016. Int. J. Food Microbiol 236: 107–114

Ad hoc biofilm activation

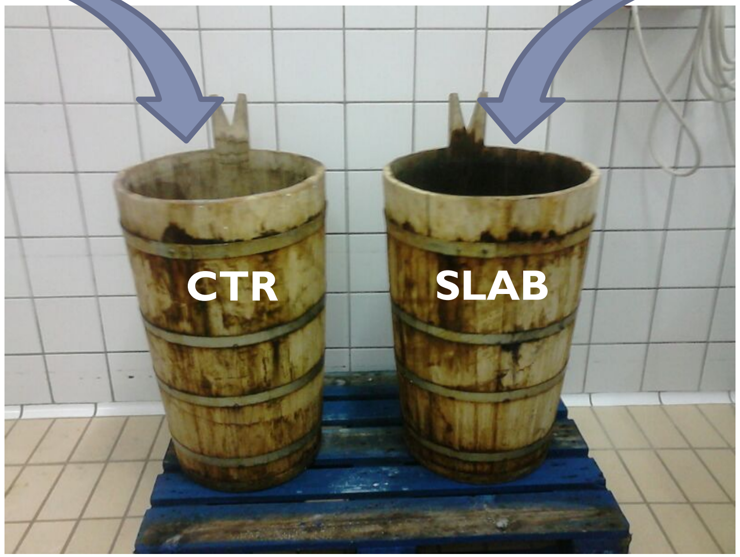
Pre-Activation

all four vats were treated daily with hot water (75 to 80°C) for 30 days, in order to remove the tannin components

Activation

- **Control vat**: addition of previous day (acidified) whey
- **Experimental vat**: addition of a natural whey starter culture prepared with 3 selected *Lactococcus lactis* subsp. *cremoris*

Previous day acidifed whey (60 L)



NWSC (60 L)

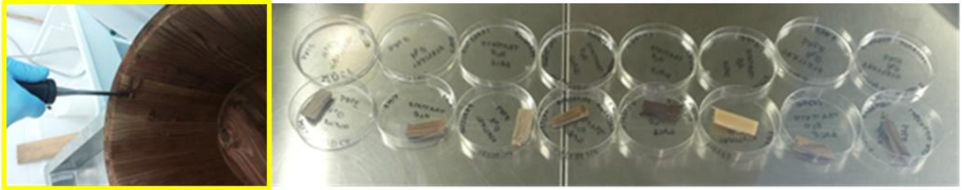
Controlled conditions: Istituto Zooprofilattico

PILOT PLANT

Cheese factory conditions:

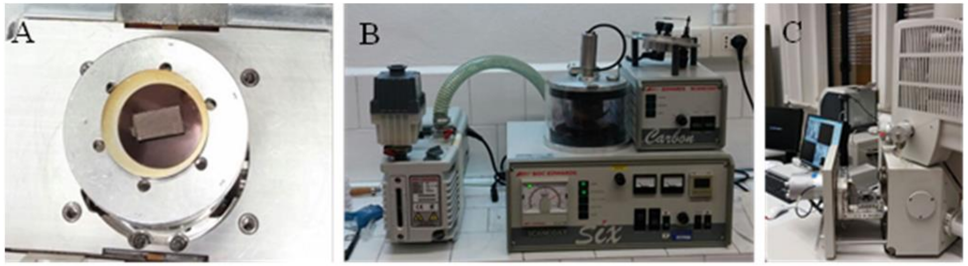
DAIRY FARM

Analysis of biofilms - Scanning Electron Microscopy



Samples collection of vat splinters

SEM: rectangular (50 × 35 mm) wood splinters (1- to 2-mm thickness)



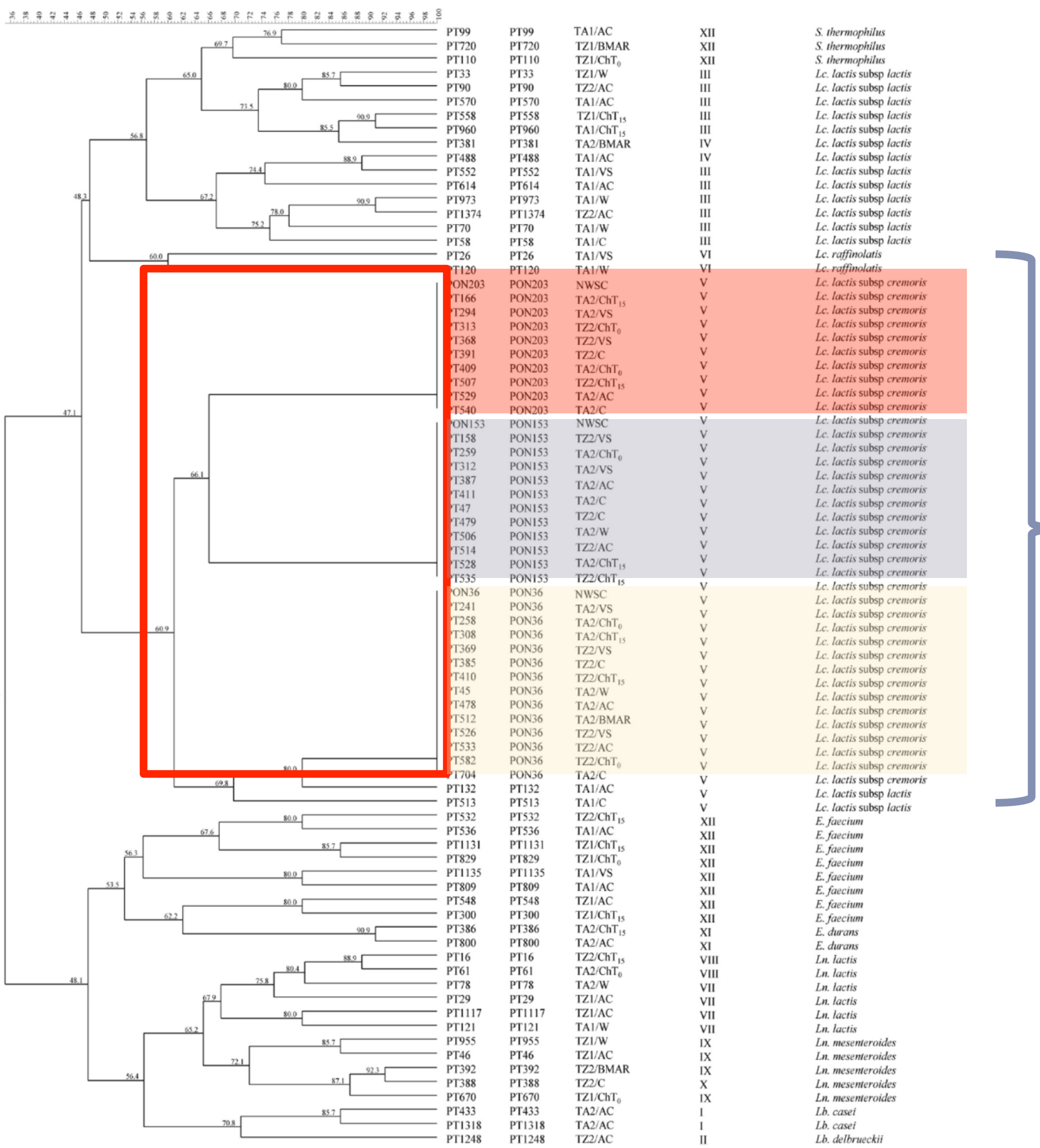
Steps of observation to scanning electron microscopy

............after overnight contact

Virgin vat | Control vat | Experimental vat

Cruciata et al. 2016. Appl. Environ. Microbiol 82: 585–595

Genotypic diversity of cheese LAB



44 strains

Lactococcus lactis subsp. *cremoris*

Absence of pathogenic bacteria in wooden vat biofilms

***Salmonella* spp., *L. monocytogenes*, *E. coli* e CPS**

…………probably because the vats were new?

In order to verify the inhibitory action exerted by compounds released by wood, the water extracts collected during the treatment with hot water were tested against the four pathogens

↓

NEGATIVE

Artificial contamination tests

- Four new (virgin) chestnut wooden vats were activated with previous day whey
- All vats were filled with pasteurised milk
- After 15 min, two vats were artificially contaminated with a cocktail of the four main dairy pathogenic bacteria (10^3 CFU/ml for *E. coli* O157 ATCC 35150 and *S. aureus* ATCC 33862, and 30 CFU/ml for *L. monocytogenes* ATCC 7644 and *S. enteritidis* ATCC 13076)

control experimental

Artificial contamination tests

Pathogenic bacteria	First production				Second production			
	Milk CP[a]	Milk EP[b]	Vat CP	Vat EP	Milk CP	Milk EP	Vat CP	Vat EP
E. coli	<1	2.96 ± 0.26	<1	<1	<1	3.06 ± 0.20	<1	<1
L. monocytogenes	<1	1.59 ± 0.19	<1	<1	<1	1.49 ± 0.15	<1	<1
S. enteritidis	<1	1.65 ± 0.21	<1	<1	<1	1.45 ± 0.24	<1	<1
CPS	1.82 ± 0.21	3.03 ± 0.22	<1	<1	1.75 ± 0.19	3.09 ± 0.23	<1	<1

[a]CP, control production.
[b]EP, experimental production.

LAB biofilms were able to inhibit the adhesion of the 4 pathogenic bacteria

Gaglio et al. 2018. Appl. Environ. Microbiol 84: e02107-17

Influence of different woods on cheese

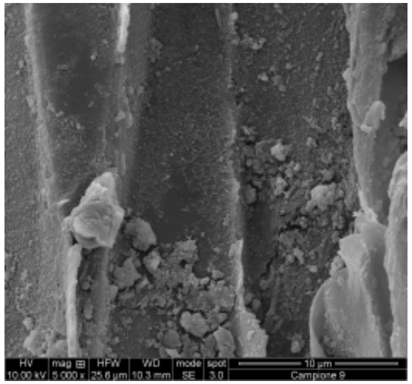
TABLE 5 Typology and origin of the woods used for wooden vat production

Trial	Wood type	Species	Origin
W1	Calabrian chestnut	*Castanea sativa* Miller	Cosenza, CS, Calabria
W2	Sicilian chestnut	*Castanea sativa* Miller	Petralia Sottana, PA, Sicily
W3	Cedar	*Cedrus libani* A. Rich.	Polizzi Generosa, PA, Sicily
W4	Cherry	*Prunus avium* L.	Castelbuono, PA, Sicily
W5	Ash	*Fraxinus ornus* L.	Castelbuono, PA, Sicily
W6	Walnut	*Juglans regia* L.	Castelbuono, PA, Sicily
W7	Black pine	*Pinus nigra* J.F. Arnold	Polizzi Generosa, PA, Sicily
W8	Poplar	*Populus nigra* L.	Castelbuono, PA, Sicily

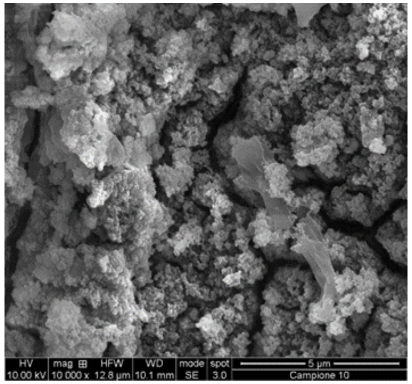
Influence of different woods on cheese

Before activation (No conctact with WHEY)

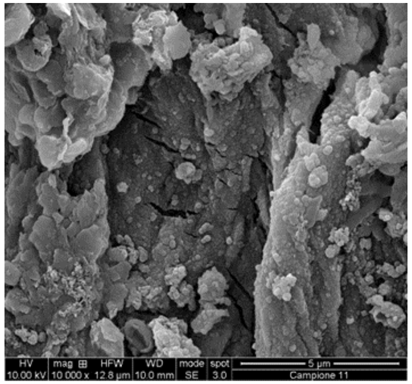
1. Calabrian chestnut



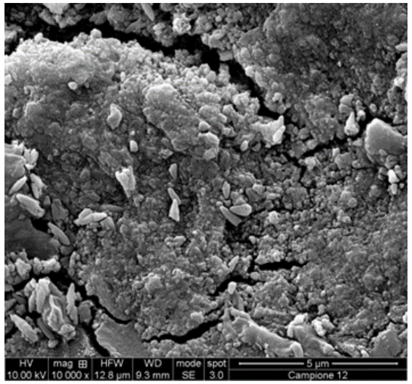
2. Sicilian chestnut



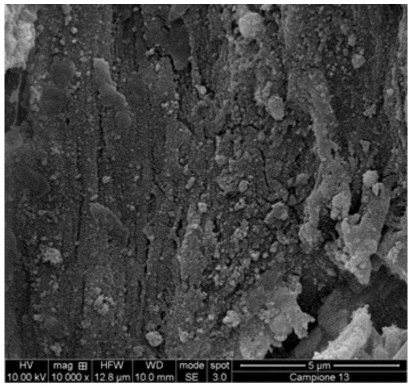
3. Cedar



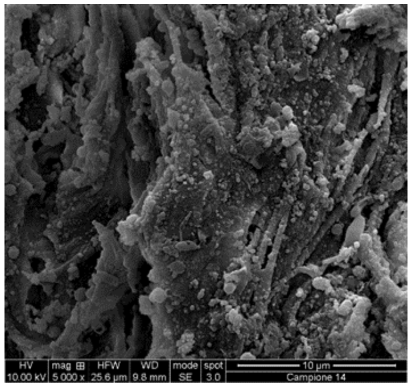
4. Cherry



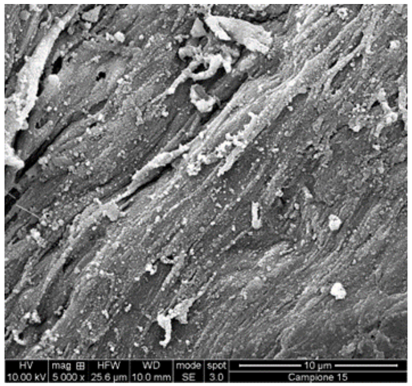
5. Ash



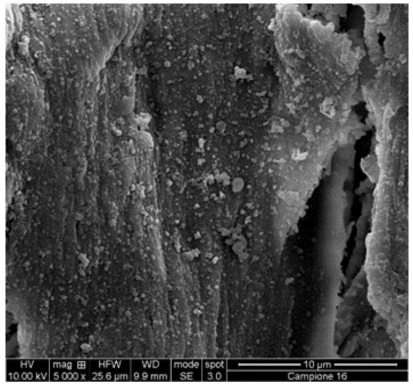
6. Walnut



7. Black Pine



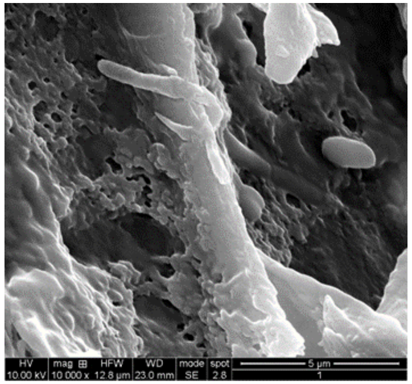
8. Poplar



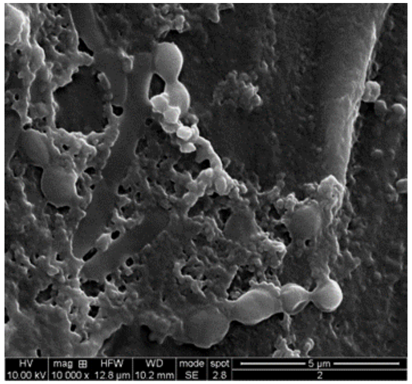
Influence of different woods on cheese

After activation (conctact with WHEY)

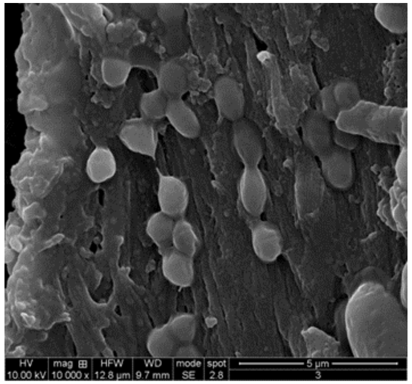
1. Calabrian chestnut



2. Sicilian chestnut

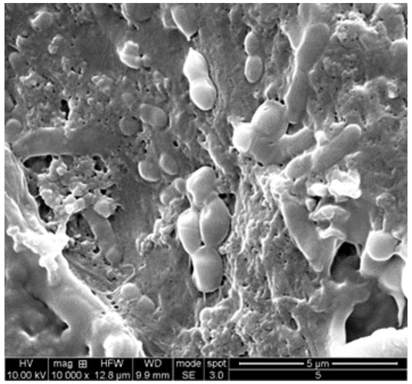


3. Cedar

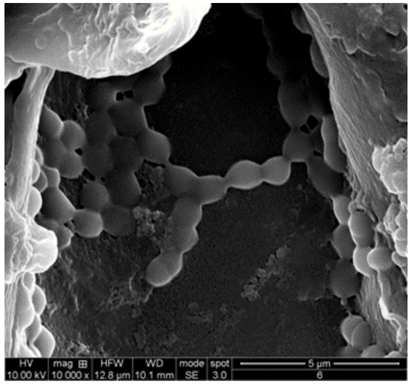


4. Cherry

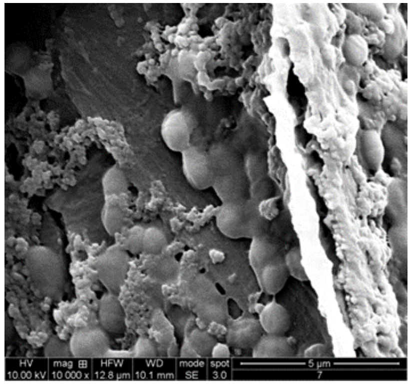
5. Ash



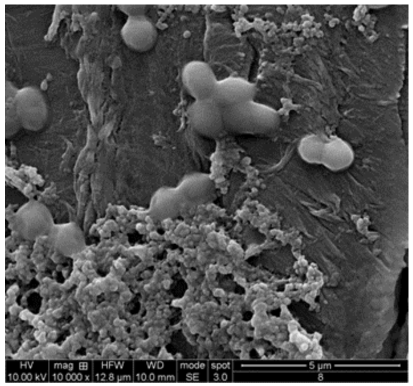
6. Walnut



7. Black Pine



8. Poplar



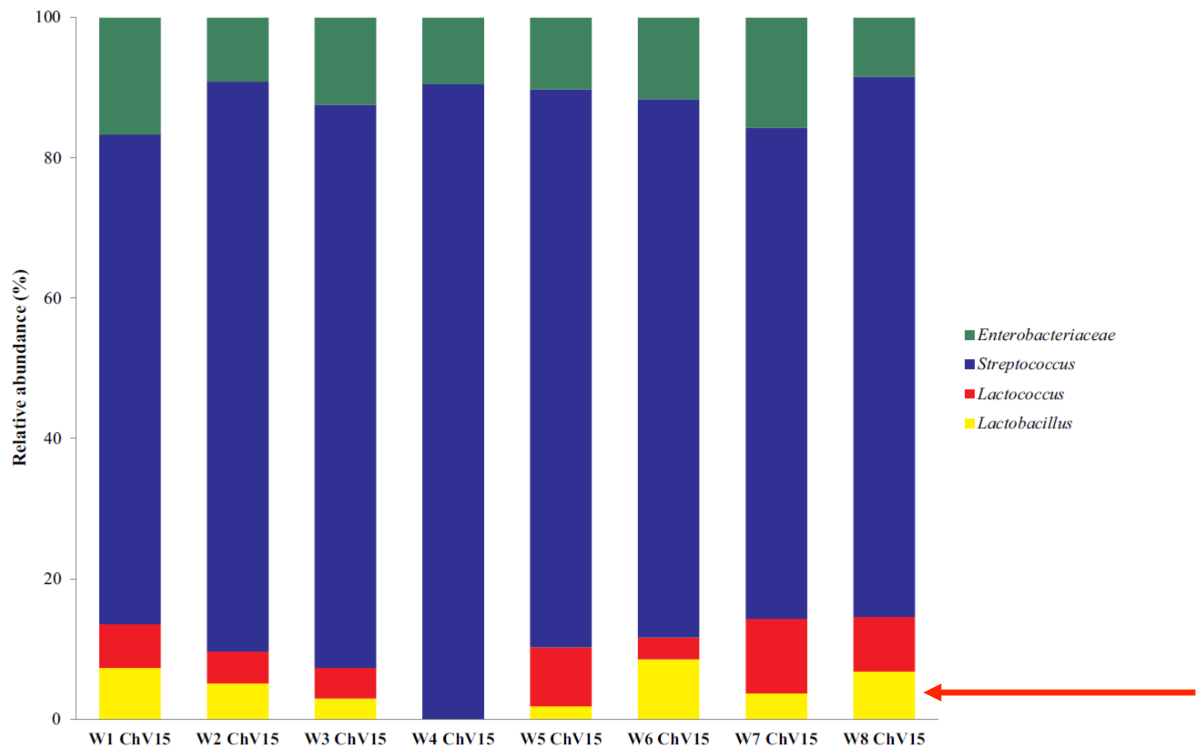
Influence of different woods on cheese

Culture-dependent analysis

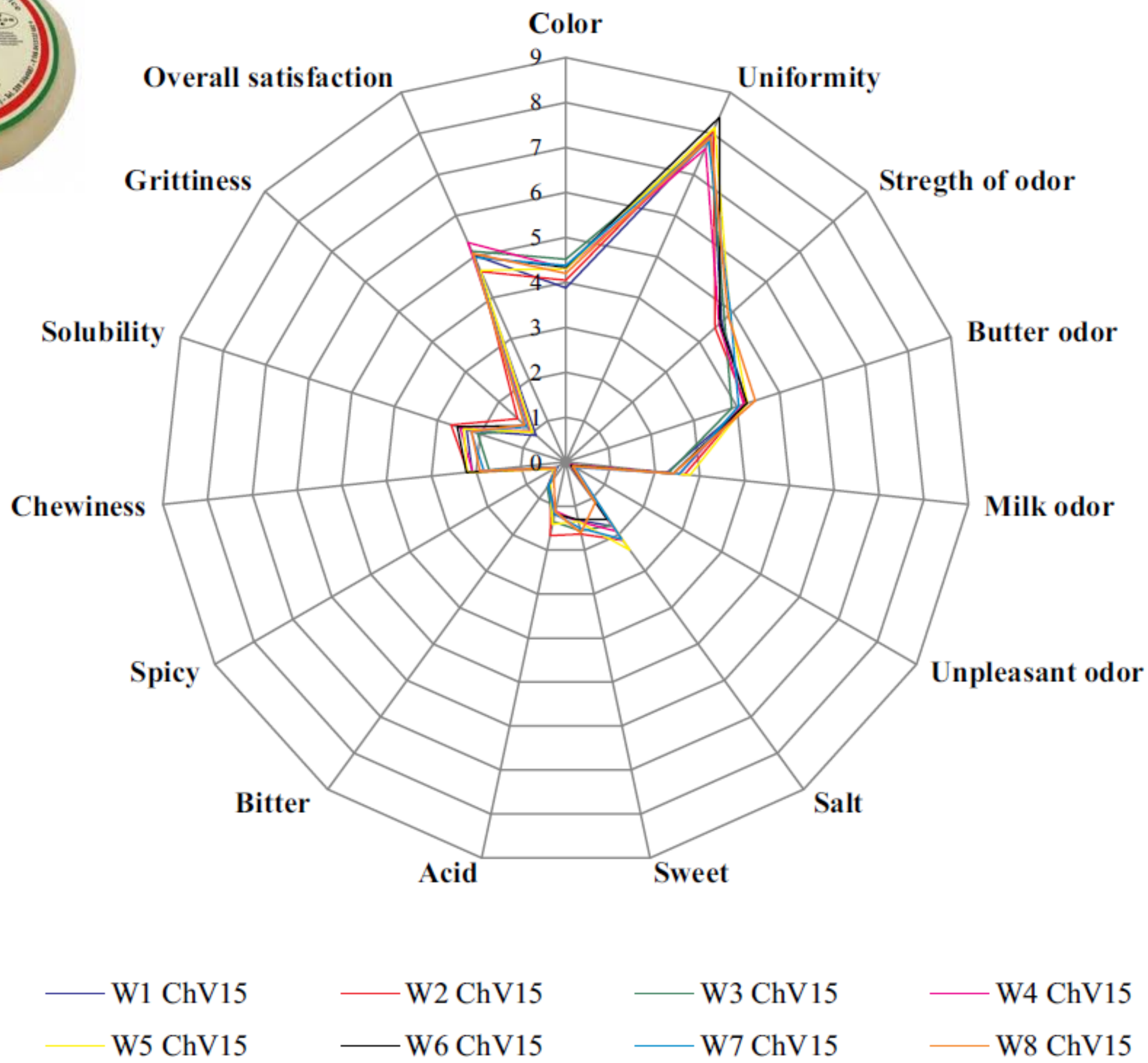
Table 3
Distribution of LAB species within VdB cheeses.

Species	W1 ChV_{15}	W2 ChV_{15}	W3 ChV_{15}	W4 ChV_{15}	W5 ChV_{15}	W6 ChV_{15}	W7 ChV_{15}	W8 ChV_{15}
Lactococcus lactis	■	■	■	■	■	■	■	■
Lactobacillus paracasaei	■	■	■	■	■	■	■	■
Lactobacillus rhamnosus		■	■	■	■	■	■	■
Lactobacillus fermentum			■	■	■	■		
Pediococcus pentosaceus	■	■						

Culture-independent analysis



Influence of different woods on cheese



Gaglio et al. 2019. Int. J. Food Microbiol 291: 91-103

Conclusions

- Wooden vat biofilms host both SLAB and NSLAB
- Enterococci denied of risk factors are often present at dominanting levels over undesired strains
- The development of *ad hoc* biofilms with selected strains on «virgin» wooden vats determined the microbial stabilization of the final cheeses
- **Wooden vats are safe systems and determine a self-containment of the cheese making spoilage risks**

↓

Use of wooden equipments in other Countries

Agricultural Microbiology group – Dip. SAAF, University of Palermo

GRACIAS POR SU ATENCIÓN