An Integrative Approach to Understanding the Ripening of Mould-Ripened Cheeses

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Overview …

I. The case of mould-ripened cheeses

1. The role of the eukaryotic organisms.
2. The Non-Starter Yeast and Moulds (NSYM).

II. A polyphasic and integrative approach to understanding the ripening

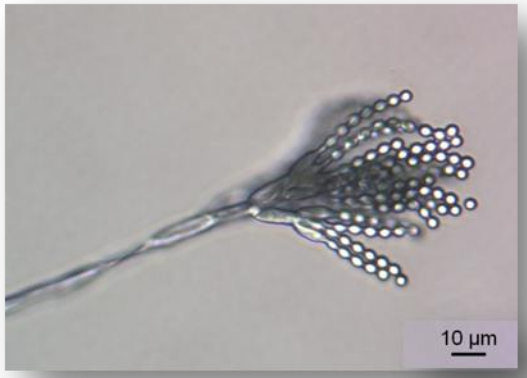
1. Molecular quantification.
2. The metatranscriptome.
3. The volatilome.
4. The contribution of the milk's native microflora.

I. The case of mould-ripened cheeses

Photo credit: http://vins-etonnants.blogspot.ca/2012_11_01_archive.html

The major fungal players

- ***Penicillium camemberti***
  - Filamentous mould conferring the white velvet characteristic to the cheese.
  - Considered a domesticated form of *P. commune*
  - Produce NH_3 causing alkalinization at the surface.
  - Participate in flavor formation through the production of methyl cetone, esters, aldehydes, lactones and volatile sulfur compounds



The major fungal players

- ***Geotrichum candidum***
  - Formally a yeast
  - Could show a dimorphic phenotype depending on the strain and the cheese conditions
  - Consume lactate and produce ammoniac, resulting in cheese alkalinization
  - Aids *P. camemberti* colonization
  - Flavor formation

Also inoculated

- ***Debaryomyces hansenii***
  - Lactose, lactate, citrate assimilation
  - Easily colonizes the cheese and competes with other fungi
- ***Kluyveromyces lactis / K. marxianus***
  - Lactose assimilation
  - Aroma production
- ***Yarrowia lipolitica***
  - Often found as secondary microflora
  - Tributyrin hydrolysis
  - Lactose, galactose, lactate assimilation
  - Aroma production

… but also Non-Starter Yeast and moulds

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ORIGINAL PAPER

Characterization of the fungal microflora in raw milk and specialty cheeses of the province of Quebec

Karine Lavoie · Marilyne Touchette · Daniel St-Gelais · Steve Labrie

Some milk contains interesting yeasts

Producer A

	June		July		August		September		October		Average
	Isolate	CFU/ml	Isolate	CFU/ml	Isolate	CFU/ml	Isolate	CFU/ml	Isolate	CFU/ml	CFU/ml
Yeasts											
Candida glaebosa	5	5.0E+02	6	3.0E+02	14	1.4E+03	2	9.1E+02	20	5.0E+02	8.4E+02
Cryptococcus curvatus	7	7.0E+02	2	1.0E+02	0	0.0E+00	0	0.0E+00	0	0.0E+00	1.3E+02
Issatchenkia orientalis	0	0.0E+00	1	5.0E+01	2	2.0E+02	0	0.0E+00	1	2.5E+01	7.9E+01
Rhodotorula mucilaginosa	1	1.0E+02	1	5.0E+01	0	0.0E+00	0	0.0E+00	0	0.0E+00	2.5E+01
Nectria mauriticaula	1	1.0E+02	0	0.0E+00	0	0.0E+00	0	0.0E+00	0	0.0E+00	1.7E+01
Unidentified yeasts	1	1.0E+02	1	5.0E+01	1	1.0E+02	6	2.7E+03	1	2.5E+01	5.2E+02
Total	**13**	**1.3E+03**	**10**	**5.0E+02**	**18**	**1.8E+03**	**9**	**4.1E+03**	**23**	**5.8E+02**	**1.7E+03**
Molds											
Lecythophora hoffmannii	2	2.0E+02	0	0.0E+00	0	0.0E+00	0	0.0E+00	1	2.5E+01	3.8E+01
Phoma macrostoma	0	0.0E+00	1	5.0E+01	0	0.0E+00	0	0.0E+00	0	0.0E+00	8.3E+00
Unidentified molds	0	0.0E+00	1	5.0E+01	0	0.0E+00	0	0.0E+00	1	2.5E+01	1.3E+01
Total	**3**	**3.0E+02**	**2**	**1.0E+02**	**0**	**0.0E+00**	**0**	**0.0E+00**	**2**	**5.0E+01**	**7.5E+01**
Total yeasts and molds	**16**	**1.6E+03**	**12**	**6.0E+02**	**18**	**1.8E+03**	**9**	**4.1E+03**	**25**	**6.3E+02**	**1.8E+03**

Isolates	Source	St	*ADE2*	*HIS3*	*LEU2*	*LYS2*D	*NMT*1	*TRP*1
LMA-519	Cheese 1* (Milk *a*) April 3	67	2	4	9	9	8	8
LMA-652	Cheese 1* (Milk *a*) June 12	67	2	4	9	9	8	8
LMA-805	Cheese 1* (Milk *a*) Aug. 12	67	2	4	9	9	8	8
LMA-545	Milk *a* April 2	67	2	4	9	9	8	8
LMA-250	Milk *e* February 11	67	2	4	9	9	8	8
LMA-422	Milk *a* March 5	01	22	2	1	14	2	25
LMA-783	Milk *a* July 14	07	22	2	5	14	2	25
LMA-546	Milk *a* April 2	02	22	2	1	14	13	26
LMA-503	Cheese 2** (Milk *a*) April 2	03	22	4	5	10	2	25
LMA-666	Milk *c* May 20	04	22	1	5	4	2	25
LMA-694	Milk *g* April 15	19	5	1	2	10	3	5
LMA-696	Milk *h* April 15	05	6	1	18	10	11	1
LMA-726	Milk *f* April 15	06	2	1	12	13	3	10

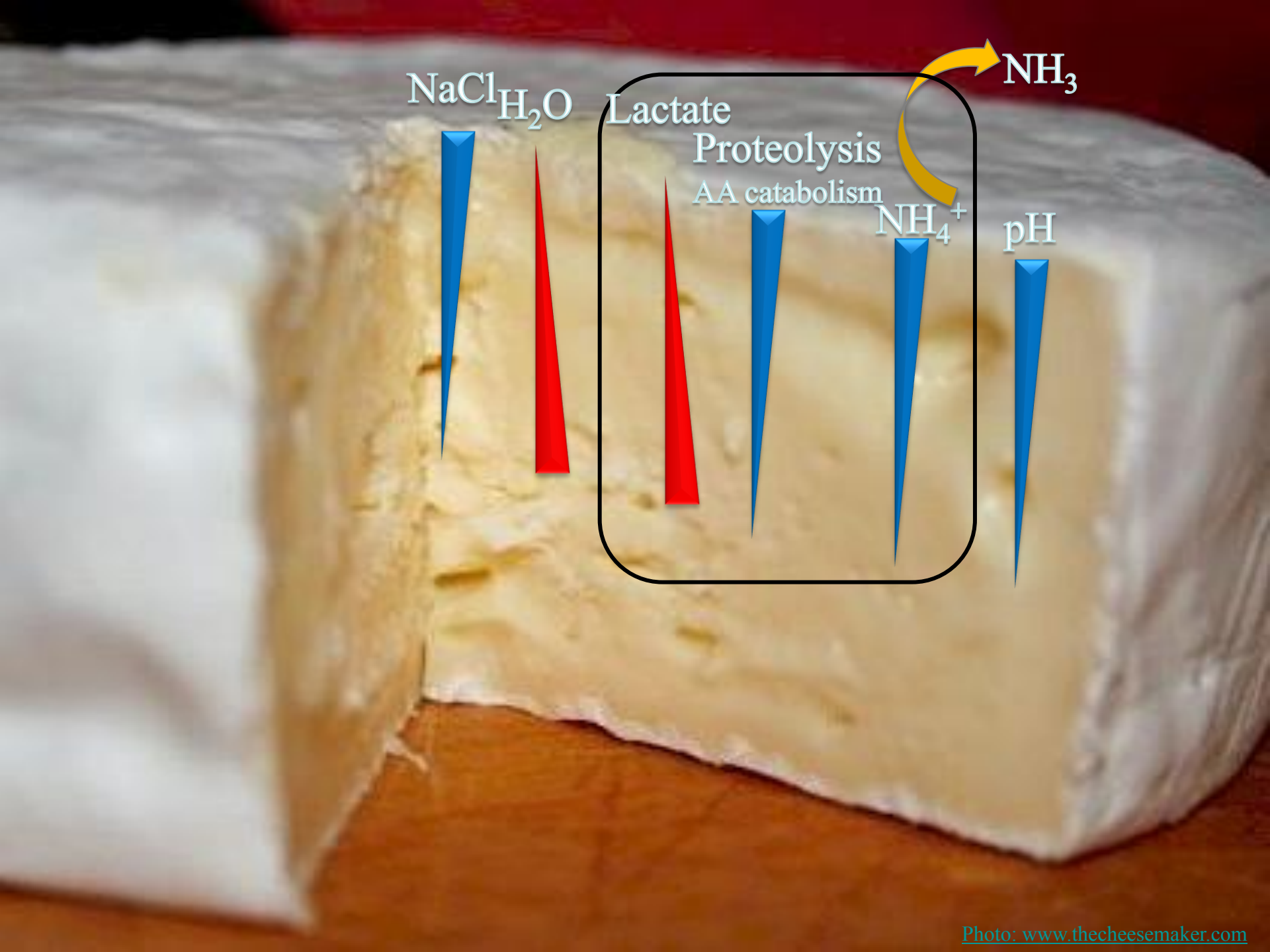


Photo: www.thecheesemaker.com

II. A polyphasic and integrative approach to understanding the ripening

Photo credit: http://vins-etonnants.blogspot.ca/2012_11_01_archive.html

Ripening culture quantification

Activity of the fungal microflora

Identification of the Y&M secondary microflora

Effect on the cheese aroma

Molecular quantification vs. traditional microbiology

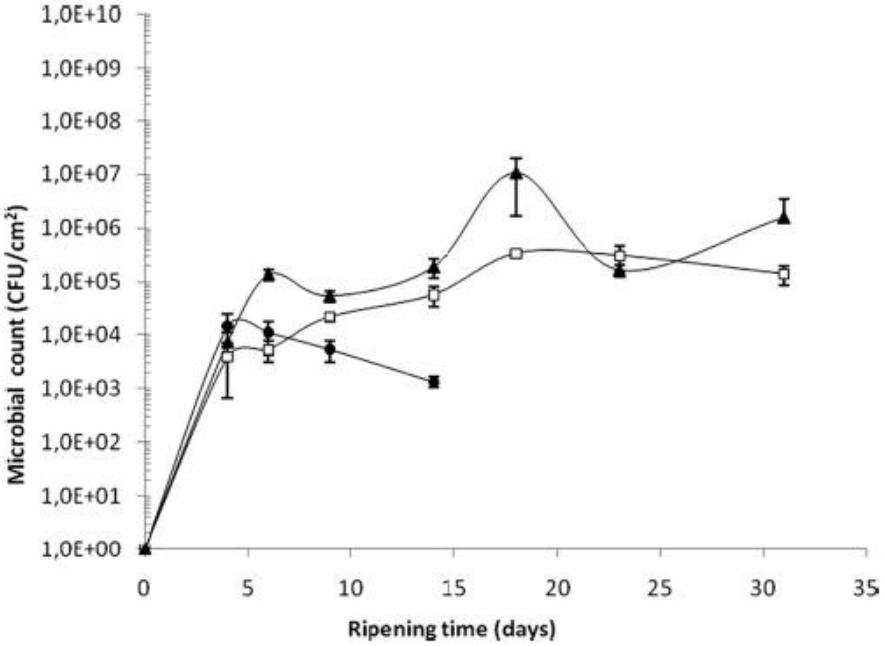


FIG 2 Microbial count on acidified PDA medium (pH 3.5) of the *K. lactis* LMA-437 (●), *G. candidum* LMA-436 (□), and *P. camemberti* (▲) ripening culture.

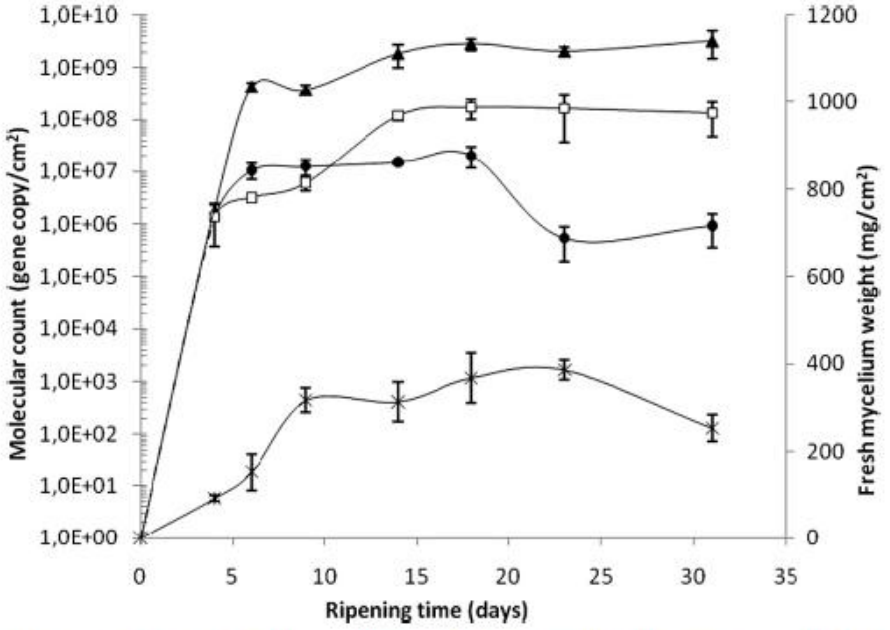
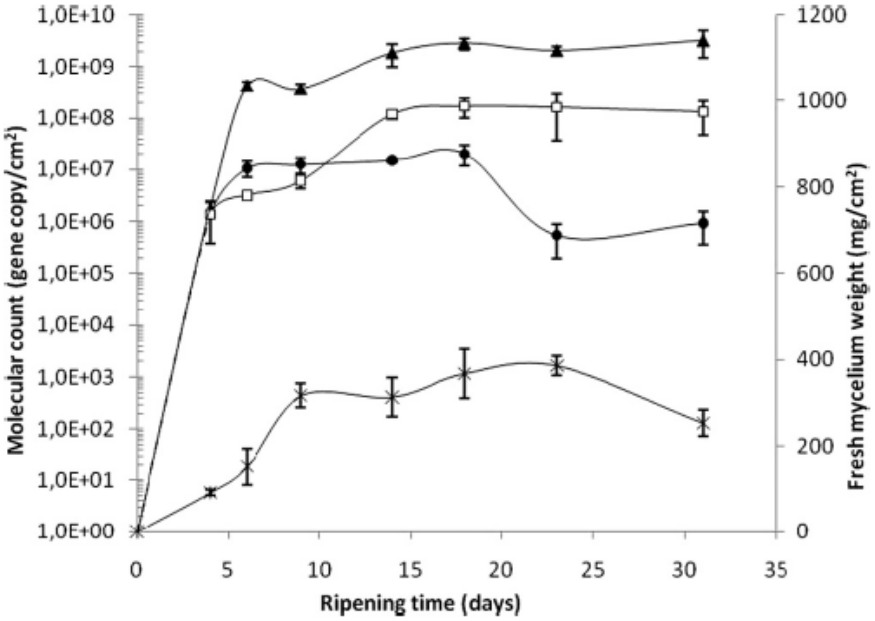


FIG 3 Comparison of the evolution of the mycelium biomass (×) and the surface microbiota quantification of a soft-cheese model curd using a TaqMan real-time qPCR method. The ripening culture contained *K. lactis* LMA-437 (●), *G. candidum* LMA-436 (□), and *P. camemberti* (▲).

Lessard et al. 2012. Appl. Environ. Microbiol. 78(6):1813

Changing the ecosystem



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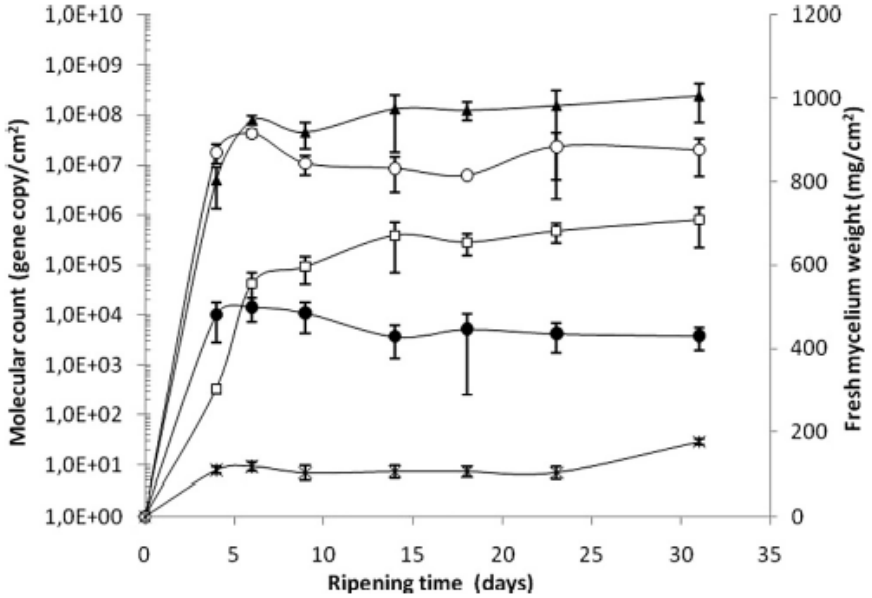

FIG 5 Comparison of the evolution of the mycelium biomass (×) and the surface microbiota quantification of a soft-cheese model curd using a TaqMan real-time qPCR method. The ripening culture contained *K. lactis* LMA-437 (●), *D. hansenii* LMA-1019 (○), *G. candidum* LMA-436 (□), and *P. camemberti* (▲).

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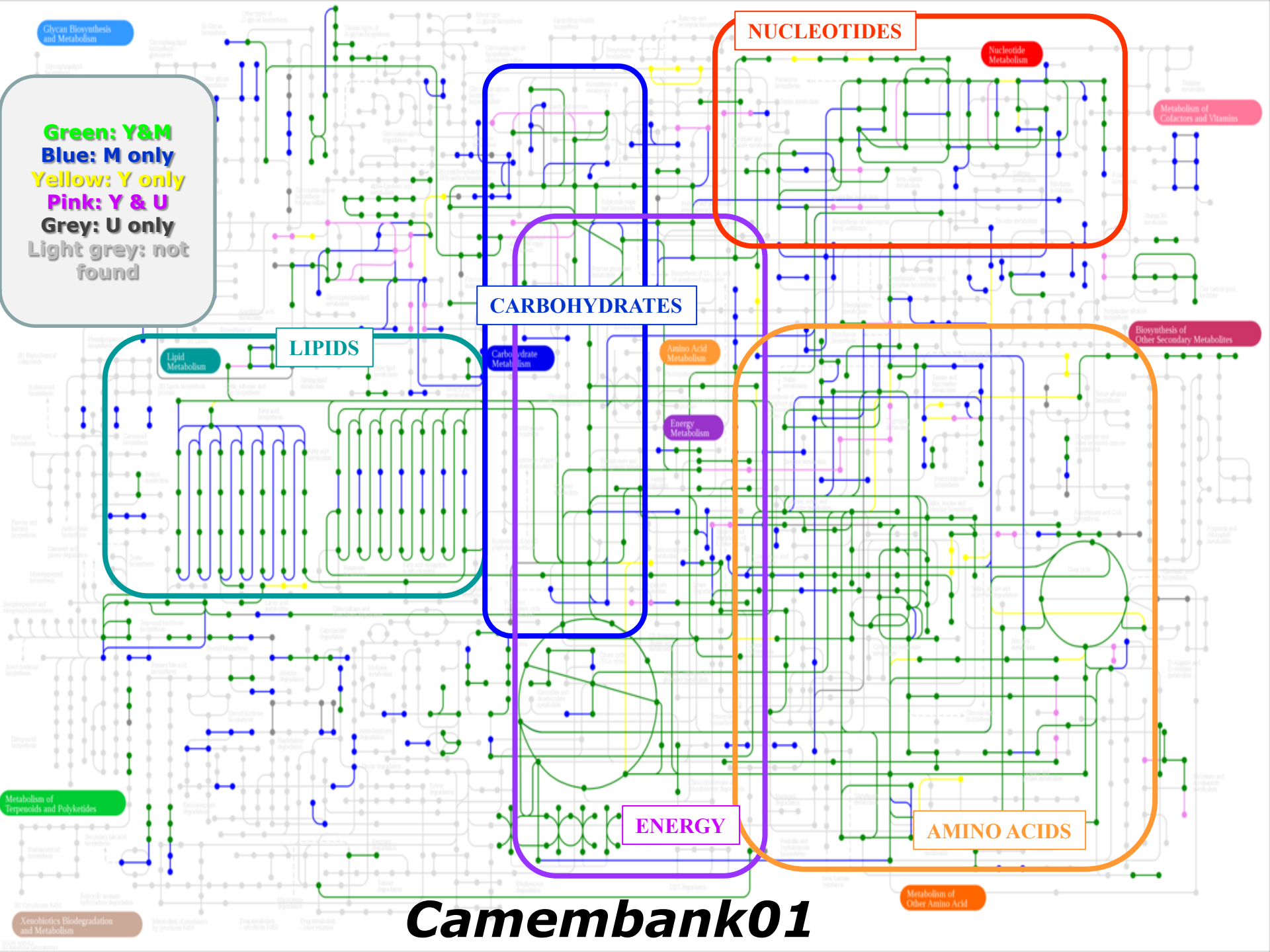
Studying the metatranscriptome of a simple industrial mould-ripened cheese

- Industrial cheese inoculated using only *Penicillium camemberti* and *Geotrichum candidum*
- Identification of the genes expressed using a metatranscriptomic approach
- Gene expression monitored at seven sampling points during the ripening


Green: Y&M
Blue: M only
Yellow: Y only
Pink: Y & U
Grey: U only
Light grey: not found
NUCLEOTIDES
CARBOHYDRATES
LIPIDS
ENERGY
AMINO ACIDS
Glycan Biosynthesis and Metabolism
Nucleotide Metabolism
Metabolism of Cofactors and Vitamins
Lipid Metabolism
Carbohydrate Metabolism
Amino Acid Metabolism
Energy Metabolism
Biosynthesis of Other Secondary Metabolites
Metabolism of Terpenoids and Polyketides
Metabolism of Other Amino Acid
Xenobiotics Biodegradation and Metabolism
Camembank01

Proteolysis

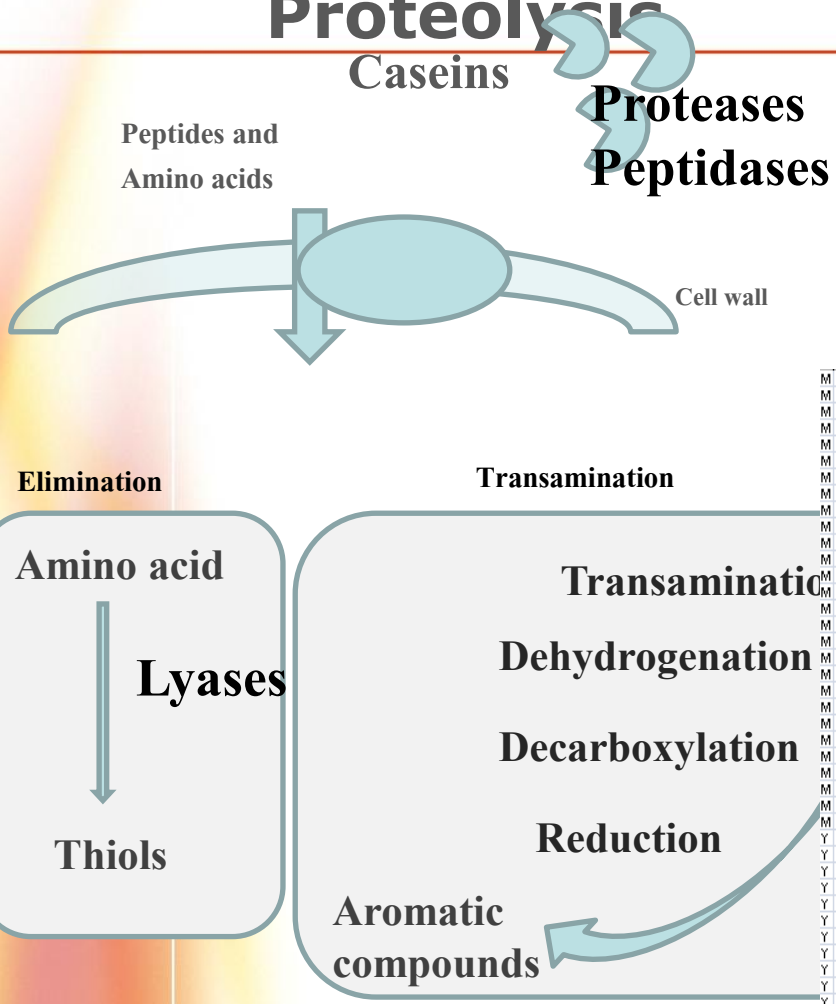
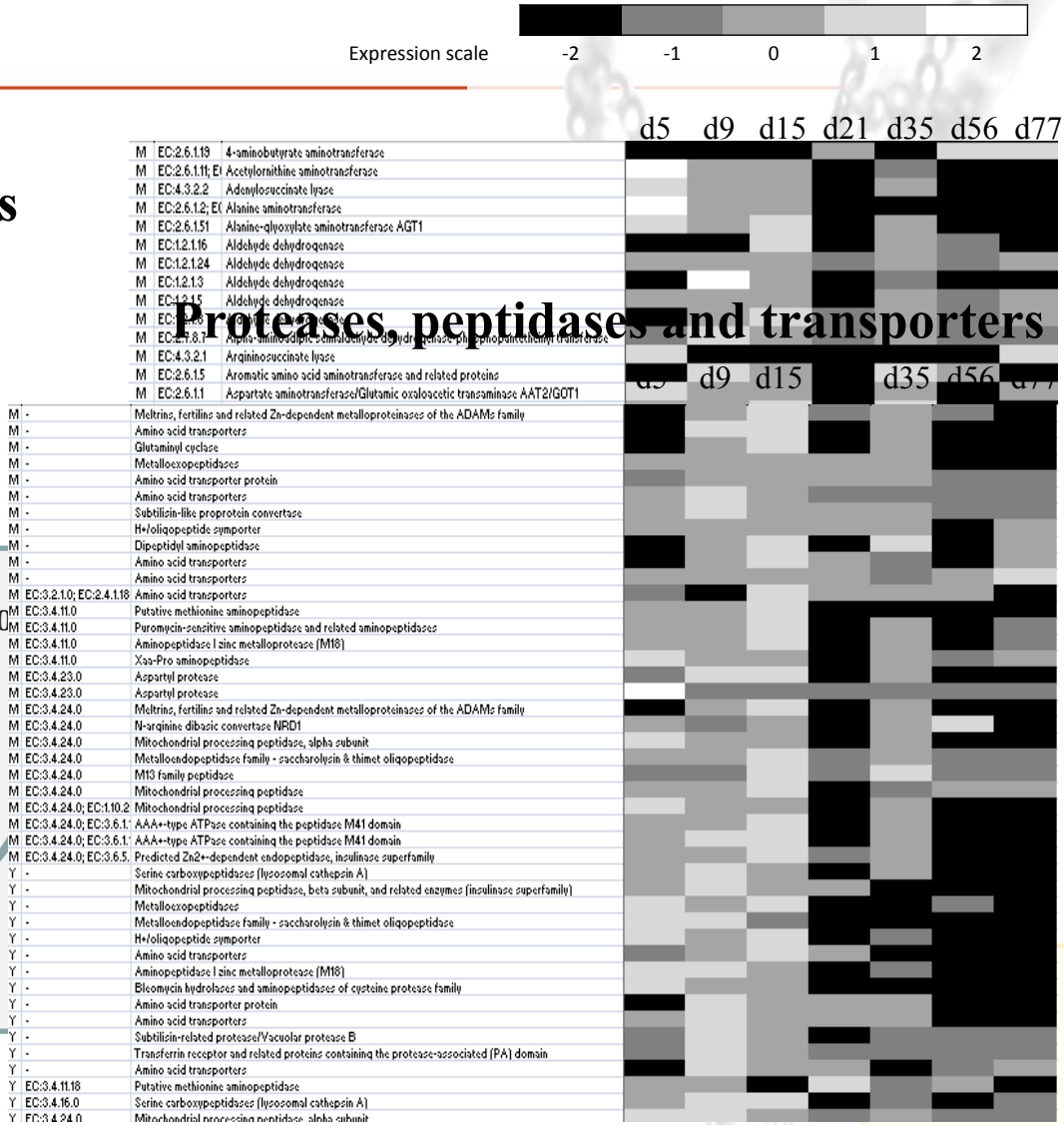


Figure adapted from Mariley, 2004

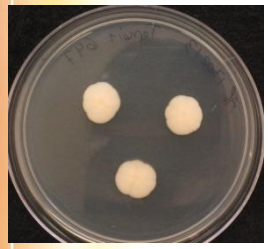


Study of the organic volatile compounds produced by the native yeast of Quebec province terroir milk

- Selection of 5 Non-Starter Yeast species from raw milk and cheese :
  - *Saccharomyces servazii*
  - *Pichia fermentans*
  - *Pichia jadinii*
  - *Issatchenkia orientalis*
  - *Rhodotorula mucilaginosa*
- Ripening cultures:
  - *Geotrichum candidum*
  - *Penicillium camemberti*

G. candidum

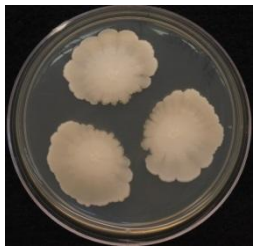
P. camemberti



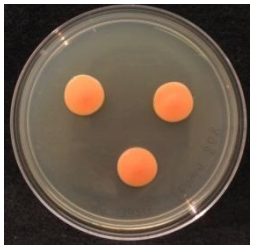
S. servazii

P. fermentans

P. jadinii



I. orientalis

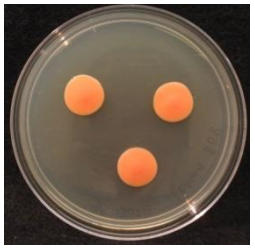


R. mucilaginosa

Sample preparation

- Camembert-type slurries
- Inoculation of slurries using yeast alone or in combination with standard ripening culture.
- Ripening conditions:
  - 10°C and 90% humidity, 10 days.
  - 4°C and 90% humidity, up to day 35.

+



R. mucilaginosa

+

G. candidum

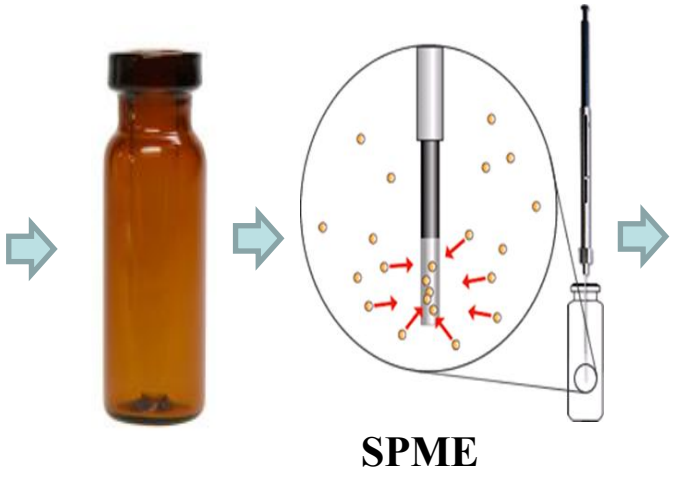
P. camemberti

=

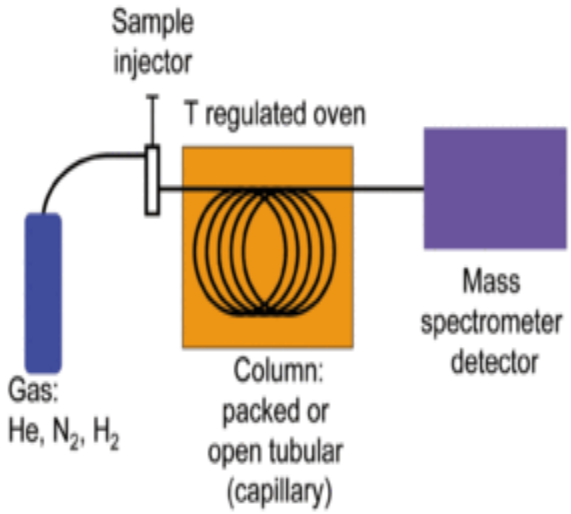
Volatile compound extraction using SPME-GC-MS

- **SPME-GC-MS** : *Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry*

- Day 14
- Day 21
- Day 28



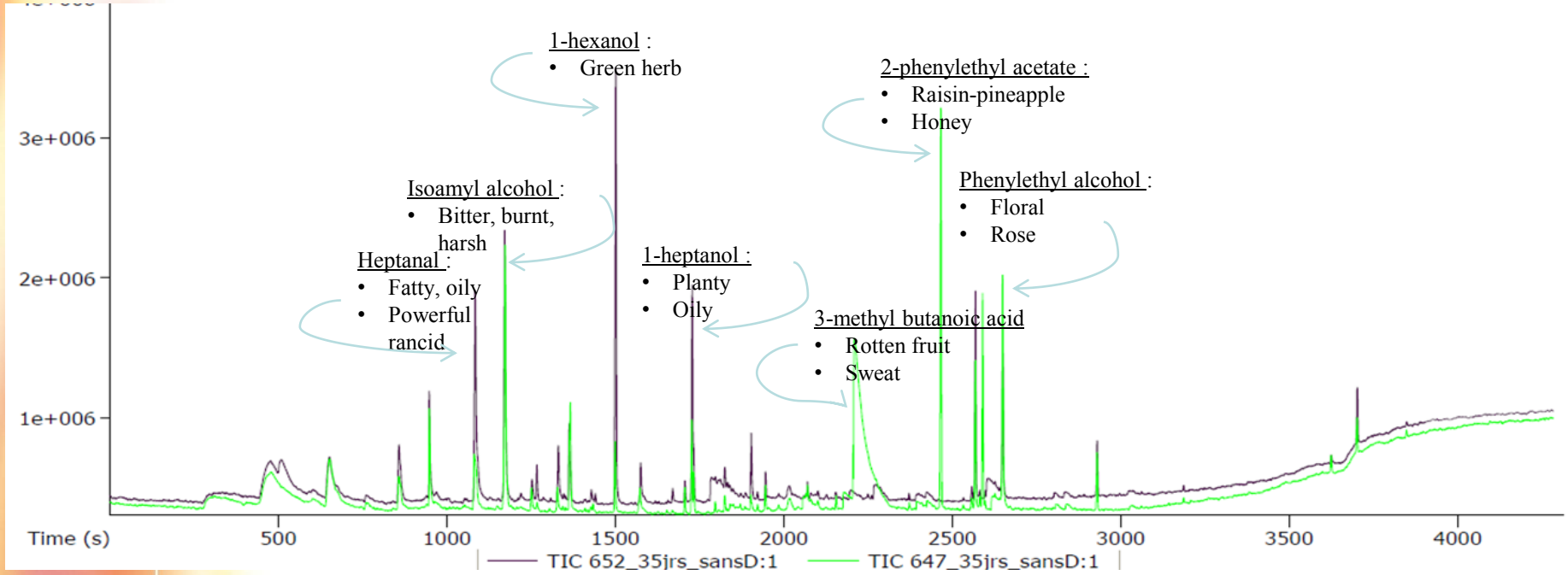
SPME



GC-MS

Comparison of COV products of two yeasts

- *I. orientalis* (day 35) : _____
- *S. servazzii* (day 35) : _____



Conclusions

- **Non-Starter Yeast and Molds (NSYM)**
  - Potentially a source of new aroma and flavors
- **What to expect?**
  - New genetic methods unravel the complexity of the mould-ripened cheese ecosystem
  - A more precise picture of the ripening activities will become available

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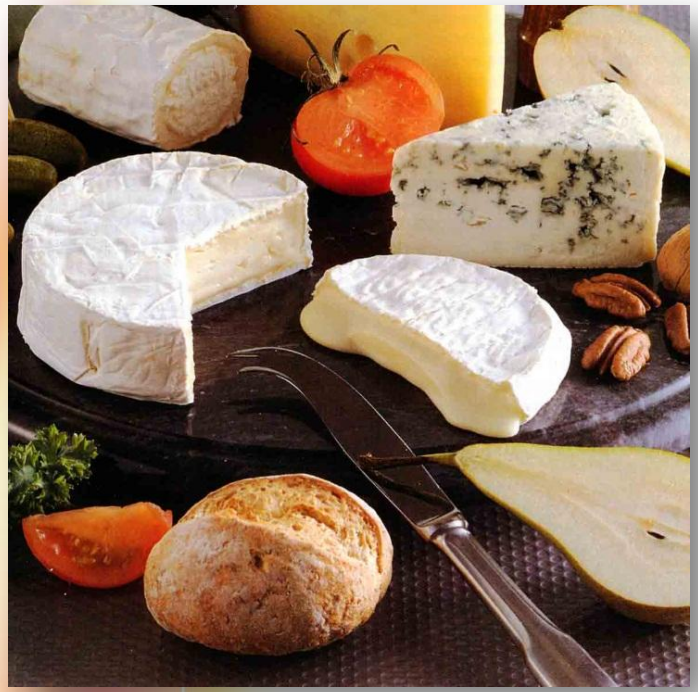

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Merci / Thank you!

Image source: http://gianacliscaldwell.wordpress.com/tag/making-white-mold-ripened-cheese/