Vitality of Lactococcus lactis throughout cheese ripening.

Vitality of *Lactococcus lactis* throughout cheese ripening

Marianna Ruggirello*, Paola Dolci, Luca Cocolin
Department of Agriculture, Forest and Food Sciences, University of Turin, Italy
***email: marianna.ruggirello@unito.it**

Aim of the work

Quantitative PCR (qPCR) and retrotranscription-qPCR (RT-qPCR) assays were optimized to detect, quantify and determine the vitality of *Lactococcus lactis* in ripened cheeses, where it is known that no starter lactic acid bacteria prevail on lactococcal microbiota. The new protocols were used to screen the presence and vitality of *L. lactis* in commercial cheeses characterized by different ripening times.

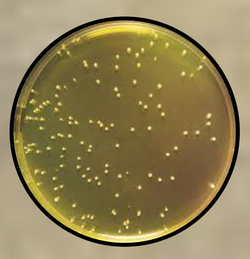
Traditional culture-dependent analysis were also carried out in order to evaluate the presence of metabolically active *L. lactis* cells in a viable but not culturable (VNC) state. In previous researches, a few authors detected the presence of this microorganism throughout cheese ripening and hypothesized its contribution to the characteristics of the final product. In the present work we are considering these preliminary evidences.

Products

PRODUCTS	COD.
ASIAGO PDO 40 GG	ADF40
FONTINA PDO 180 GG	FD180
PECORINO FIORETTO 140 GG	PF4-5
RASCHERA PDO CISALPINO	RDC
TOMA PIEMONTESE PDO PEZZANA	TPDP
PECORINO SARDO DOLCE PDO	PSDD
TOMA DI LANZO	TL
PEC. TOSCANO PDO STAG. 90/180 GG	PTD3-6
FONTINA AOSTA PDO 90 GG CPFA	FAD90
CASTELMAGNO PDO	CDT
BRA PDO	BTD
ASIAGO D'ALLEVO PDO 90/120 GG	AAD90-120

Culture – dependent approach

4. Microbiological analysis



The cheese samples resulted positive for the presence of *L. lactis* populations by RT-qPCR, were analysed by traditional plating on lactococci selective medium M17 agar. Twelve colonies for each sample were submitted to DNA extraction and *L. lactis* species-specific PCR (His-PCR) was performed.

5. HIS - PCR

Figure 2. His-PCR profile of L. *lactis* species (+). Line 1: marker; line 2: positive control; line 3: negative control.

His-PCR was carried out with primers HIS I and HIS II, amplifying the operon for histidine biosynthesis in *L. lactis*. The resulting band of 933 bp specific for *L. lactis* is showed in Fig. 2.

The results obtained by RT-qPCR and microbiological analysis were compared and were not always in agreement. In fact, the presence of *L. lactis* detected by RT-qPCR was not always confirmed by traditional plating and His-PCR. It could be hypothesized that M17 medium is not suitable to recovery *L. lactis* cells in stress conditions.

COD.	Plate counts Log CFU/g	His-PCR
ADF40	6,94	2
FD180	6,38	0
PF4-5	7,79	7
RDC	6,40	0
TPDP	8,38	0
PSDD	7,76	0
TL	8,15	1
PTD3-6	6,51	0
FAD90	6,86	0
CDT	5,40	1

Culture – independent approach

1. Optimitation of qPCR and RT-PCR protocols

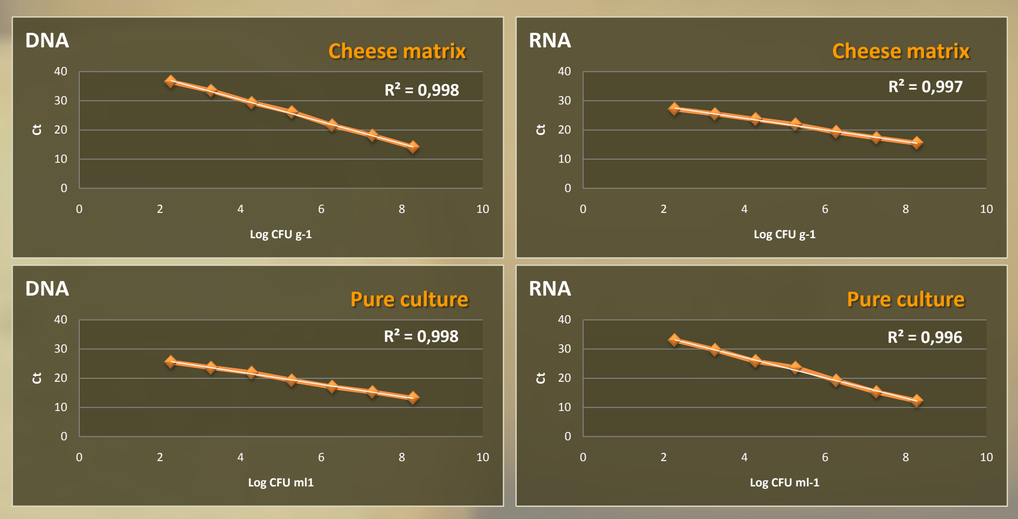
Amplification conditions	Amplification Cycle	
SsoAdvanced™ SYBR@ Green Supermix 1X (Biorad)	98°C 2'	
Tuf2 f 0,4 µM Tuf2 r 0,05 µM	95°C 5'' 68.7°C 30''	40 cycles

Fig. 1. qPCR and RT-qPCR protocols

The primers Tuf2 were unbalanced (Fig. 1) and different annealing temperatures were tested to optimize the PCR conditions. The protocols were specific for *L. lactis* and did not amplify the other dairy lactic acid bacteria species considered.

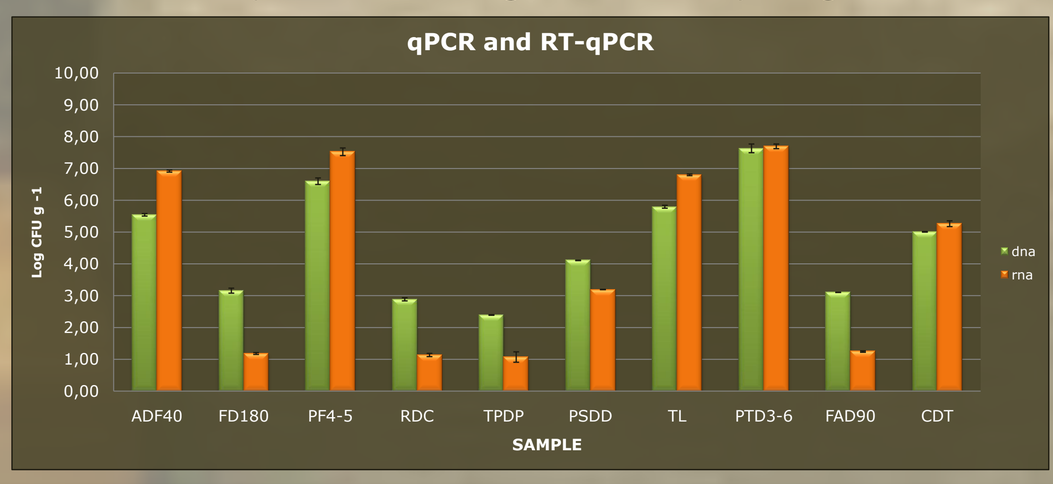
2. Standard Curves

Calibration curves were constructed from serially diluted cells of *L. lactis* as pure culture and inoculated in fresh grated cheese used as biological model. The signals produced in terms of Ct by the decimal serial dilutions were plotted against the Log_{10} CFU and standard curves were defined for both DNA and RNA analysis with a quantification limit of 10^2 CFU/g (in cheese).



3. Application of the qPCR protocols in commercial cheeses

All the cheese samples (10 g) were homogenized with 40 mL of Ringer solution and an aliquot (1 mL) was subjected to both DNA and RNA extraction and qPCR and RT-qPCR. In ten of the cheeses analysed the load values were varying from 10^1 to 10^7 CFU/g. Specifically in four cheeses were higher than 10^6 CFU/g. In two of the cheeses (Bra PDO and Asiago d' Allevo PDO) no signal was detected.



Conclusions

On the basis of our results, it can be concluded that the optimized qPCR protocols are suitable for the specific quantification of *L. lactis* species in cheese matrix, showing a good quantification limit of 10^2 CFU/g at both DNA and RNA level.

The culture-indipendent analysis highlighted the presence of the microorganism even at late ripening in contrast with traditional plating unables to detect microbial cells in VNC state.

A cheese-making pilot production will be assessed to follow the presence and activity of *L. lactis* from the inoculum in milk, as starter culture, until the end of the cheese ripening.