Characterization of natural milk cultures for the production of high moisture Mozzarella cheese: variability in performance and species composition.

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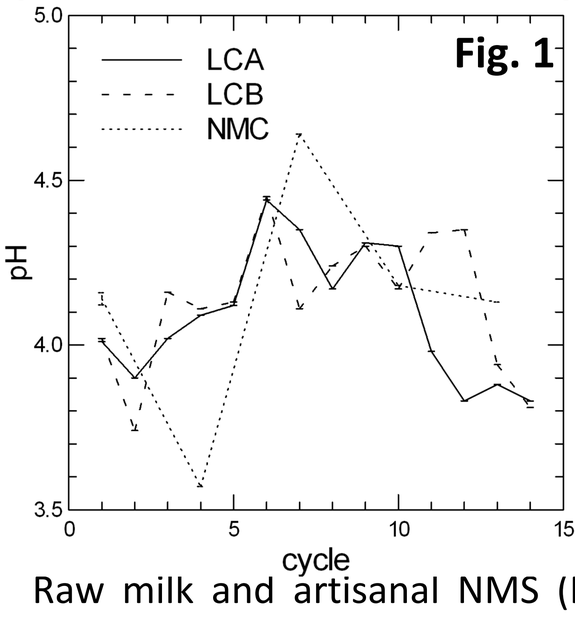
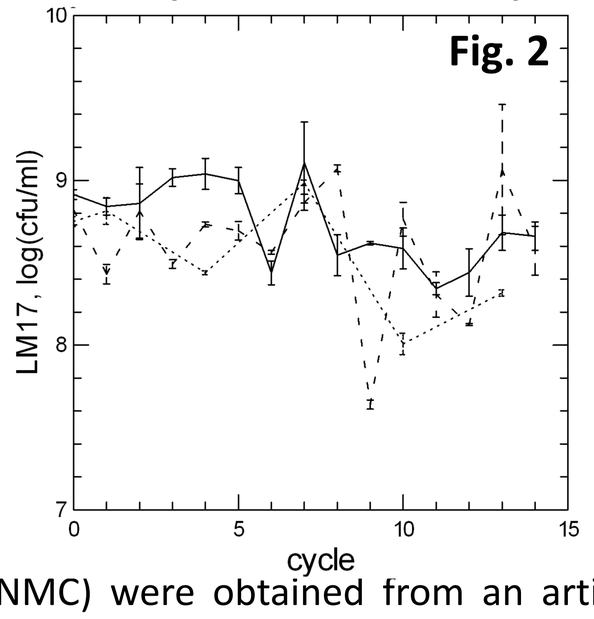
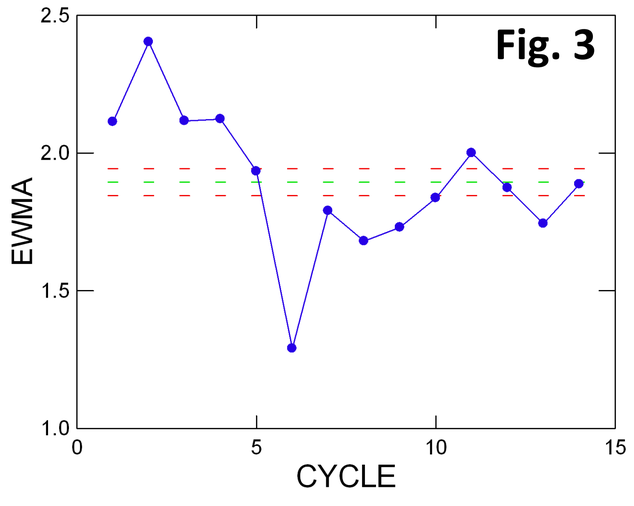
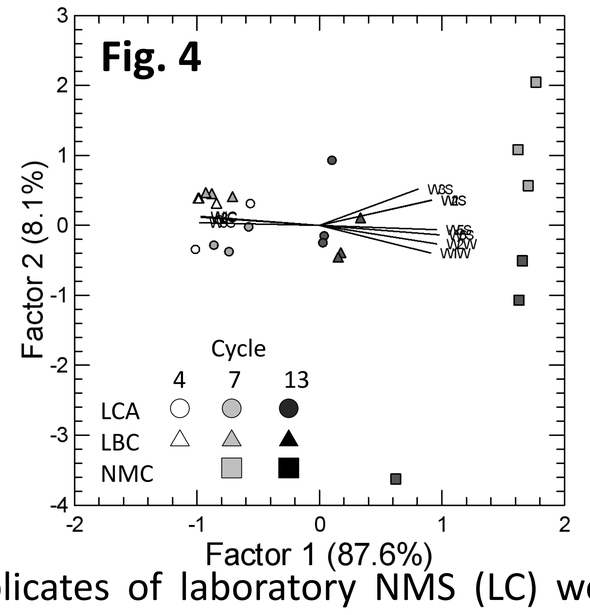
Natural milk starters (NMS) are undefined starter cultures used in Italy and elsewhere for several cheese types, including high moisture Mozzarella. NMS are produced by thermisation (63° C, 15 min) of raw milk followed by incubation at 42°C. The procedure is repeated daily with backslopping and the microbial community is usually dominated by *Streptococcus thermophilus*, enterococci and thermophilic lactobacilli (Parente, 2006). Little has been published recently on the microbiology and on the technological performances of these undefined starter cultures. Our objective was to use culture dependent and independent methods to evaluate the dominant bacterial community, to assess the variability in performances over repeated cycles of reproduction, and to assess the presence of *S. thermophilus* bacteriophages.

pH, microbial counts, head space composition and acid production of natural milk cultures are variable over reproduction cycles



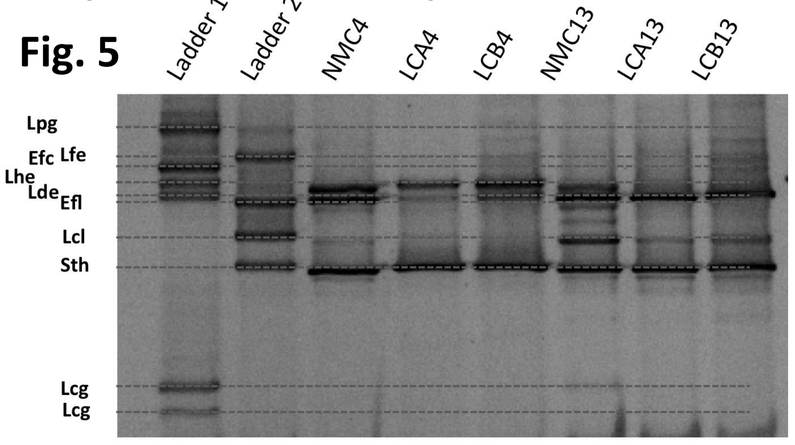


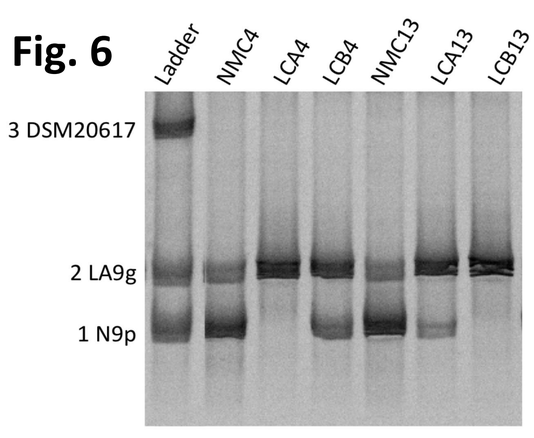




Raw milk and artisanal NMS (NMC) were obtained from an artisanal cheese making plant. Two replicates of laboratory NMS (LC) were prepared. From the 2nd cycle raw milk was inoculated (0.5% v/v) with the previous day batch and incubated for 16 h, and the procedure was repeated 13 times. pH (**Fig. 1**), microbial counts (**Fig. 2**), acid production ability (**Fig. 3**) were evaluated. Counts of enterococci (KAA), aciduric LAB (Rogosa) and coliforms (VRBA) were always below 10^3, 10^5 and 10^2 cfu/ml, respectively. Counts of thermophilic streptococci (**Fig. 4**) were variable (10^8-10^9 cfu/ml). An example of an Exponentially Weighted Moving Average chart for acid production ability in milk (5% inoculum, 4 h, 42°C) is shown in **Fig. 3** for culture LCA: the process is obviously not under statistical control but, although acid production ability was variable, it was always sufficient for the production of Mozzarella cheese (pH decrease of curd to 5.3), and tended to stabilize over time. No correlation was found between culture pH, counts on LM17 and acid production ability. Volatiles profiles in the headspace of selected samples was evaluated using a 10 MOS sensors electronic nose. The results are presented as a Principal Component Analysis in **Fig. 4**. There were little differences between the two laboratory cultures LCA and LCB, although an evolution in time was evident, while the artisanal NMC was clearly different from the two LCs. Most of the differences were due to sensors which responded mainly to aromatic and non polar organic compounds (W3C, W1C. W5C), with broad range sensitivity (W1S, W3S) or sensitive to alcohols (W2S).

Streptococcus thermophilus is the dominating species, with several biotypes present. Phages contaminate the cultures.

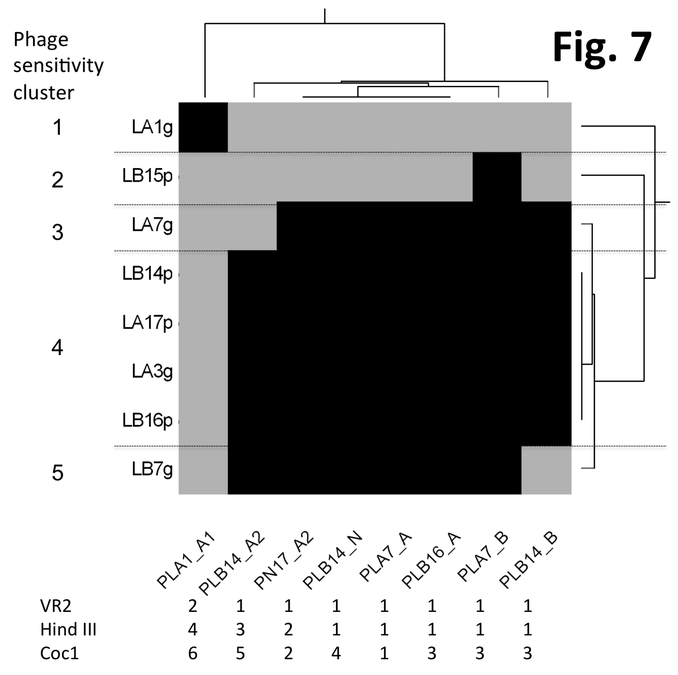




Culture independent methods were used to identify dominant species and biotypes in laboratory and artisanal milk cultures at cycles 4 and 13. Bacterial DNA was extracted using MoBio PowerFood kit and used in PCR-DGGE of the v3 region of 16S rDNA (**Fig. 5**, Ercolini et al. 2004) and *lacS* PCR-DGGE (**Fig. 6**, Ercolini et al. 2005). *S. thermophilus (*Sth*)* was always present, while other species (*Lactococcus lactis*, Lcl; *Lactobacillus helveticus,* Lhe; *L. delbrueckii*, Lde; *Enterococcus faecalis*, Efl) were found only in some cultures: only some species were recoverable on culture media. A higher diversity was found in the artisanal culture probably because of the lack of control of contamination and incubation conditions. From 1 to 2 *lacSZ* biotypes were detected (**Fig. 6**) with type 1 dominating NMC and type 2 dominating LC. *cos*-type bacteriophages were detected in all cultures by multiplex PCR (Quiberoni et al., 2006; not shown). These results were confirmed by a culture dependent approach (**Table 1**) based on random isolation from cycle 13, RAPD-PCR of isolates, identification by ITS-PCR or 16S rDNA sequencing and typing of *S. thermophilus* by partial sequencing of *lacSZ* (Ercolini et al., 2005) or *serB* (El Sharoud et al., 2012). From 3 to 6 *S. thermophilus* biotypes were found. All *S. thermophilus* were lysogenic, and were positive in the amplification of both *cos* and *pac* bacteriophages. However, isolation of lytic phages was difficult and required an enrichment step. Eight unique phages were identified by a combination of host range, VR2 sequencing (Binetti et al., 2005), RAPD-PCR and restriction analysis (**Fig. 7**).

Table 1. Distribution (%) of isolates laboratory (LCA and LCB) and artisanal (NMC) natural milk cultures at cycle 13 in Coc1 RAPD-PCR clusters. *lacSZ* and *serB* groups are also shown.

RAPD-PCR Cluster	LCA (%)	LCB (%)	NMC (%)	Species	*lacSZ* group	*serB* group
1	0	70	0	*S. thermophilus*	2	1
2	0	25	0	*S. thermophilus*	2	1
3	21	0	0	*S. thermophilus*	2	1
4	5	0	4	*S. thermophilus*	1/2	3/2
5	26	0	0	*S. thermophilus*	2	1/4
6	0	0	4	*S. thermophilus*	1	4
7	0	0	15	*S. thermophilus*	1	4
8	11	0	22	*S. thermophilus*	1/2	2/1
9	11	5	0	*S. thermophilus*	2	2
10	21	0	0	*S. thermophilus*	2	5/1
11	5	0	0	*S. thermophilus*	2	1
12	0	0	4	*S. thermophilus*	2	1
13	0	0	15	*E. casseliflavus*	na	na
14	0	0	4	*Lact. garviae*	na	na
15	0	0	15	*Enterococcus spp.*	na	na
16	0	0	8	*E. faecalis*	na	na
17	0	0	11	*E. faecium*	na	na
isolates	19	20	27			



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