Raw milk for direct human consumption inoculated with the probiotic *Lactobacillus rhamnosus GG*

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INTRODUCTION

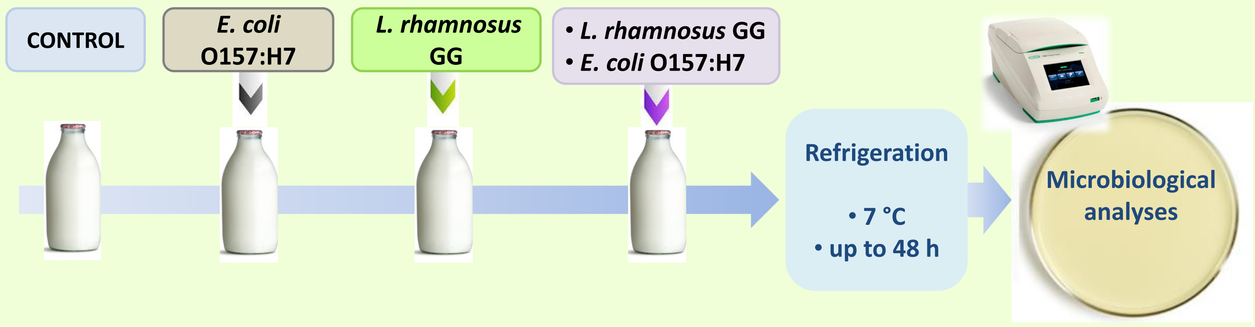
Although highly debated, **raw milk** for direct human consumption is spread in some countries, being sold through **vending machines**. The current Italian legislation (Italian Republic, 2007) requires the refrigeration of vending machine raw milk at a temperature comprised between **0 °C** and **4 °C** also during the storage at home. Nevertheless, not all consumers **boil** raw milk before consumption and often administer raw milk even to **children** (Giacometti et al., 2012). Moreover many of them do not use an insulated bag to carry the raw milk at home and, in addition, **household refrigerators** usually have temperatures **above 4 °C** (Godwin et al., 2007).

AIM

We investigated the possibility to use the **probiotic *Lactobacillus rhamnosus* GG** strain, to control the microbiological **quality** and **safety** of **raw milk** for the **direct** human **consumption**. This probiotic was chosen since it is commonly used for the production of probiotic foods and supplements due to its recognized beneficial effects on consumer health (Goldin and Gorbach, 2008), and because it produces antimicrobial peptides which inhibit both Gram-negative and Gram-positive bacteria (Lu et al., 2009).

MATERIALS AND METHODS

- ***Lactobacillus rhamnosus* GG ATCC 53103** and ***Escherichia coli* O157:H7 Ad565** were added, alone or together (final concentrations of ca. 7.5 log and ca. 4 log cfu/ml respectively), in **raw milk** bought from a **vending machine** located in the **Apulia Region**. Not spiked raw milk was used as a control.
- **Microbial population changes** after sampling (**T0**), and after 6 (**T6**), 24 (**T24**) and 48 (**T48**) hours in conditions mimicking storage at home (**7 °C**), were investigated by plating raw milk aliquots on appropriate substrates. **pH** was measured at the same times.
- **PCR-based identification** was performed for presumptive *Salmonella* spp., *L. monocytogenes*, *L. rhamnosus* GG, *E. coli* O157:H7 and *S. aureus* (Di Lena et al., 2015; Fusco et al., 2011; Fusco et al., 2012, Josefsen et al., 2007; Quero et al., 2014).
- Three trials were performed in three different days (on three different raw milk samples).



RESULTS AND DISCUSSION

Raw milk microbiota

Raw milk from vending machine revealed an etherogeneus microbiota (**Fig. 1** and **Fig. 5**). In particular, the **aerobic mesophilic microbiota** was over 5 log cfu/ml which was the maximum amount allowed by the European legislation (Reg. EC n. 853/2004). High loads of total aerobic mesophilic microorganisms have been found also in raw milk from vending machine in other Italian countries (Giacometti et al., 2013).

Pathogens such as ***L. monocytogenes***, ***Salmonella*** spp., and ***E. coli* O157:H7** have not been found in the raw milk and the amount of ***S. aureus*** was compliant with the current Italian legislation (Italian Republic, 2007).

Spoilage *Pseudomonas* spp. was present in high concentration that remained constant during the first 6 hours of refrigeration, probably due to the bacteriostatic and/or bactericidal action of the endogenous raw milk enzymes and/or metabolites of the microbiota, and then increased, consistently with their psychrotophic nature (**Fig. 5**).

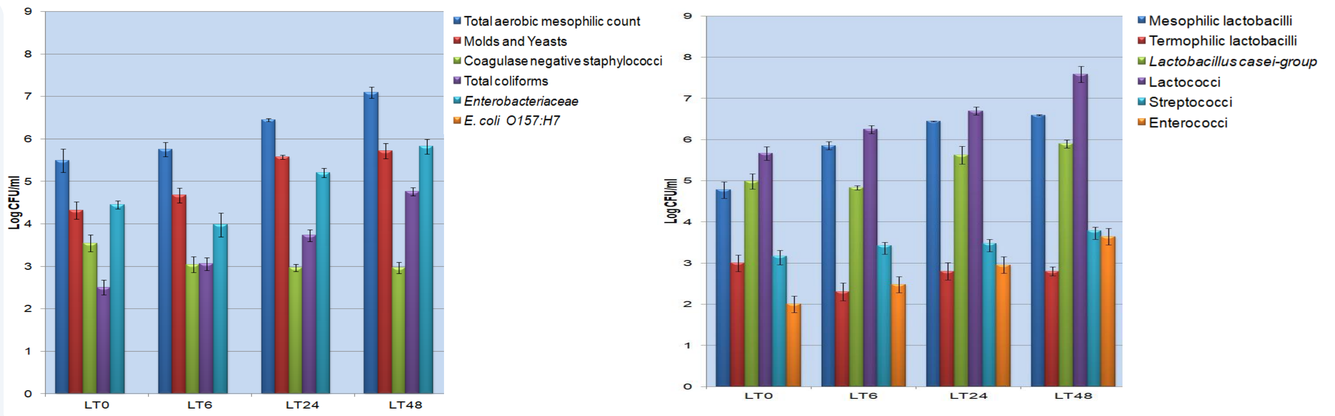


Figure 1. Microbial populations of raw milk after purchase from the vending machine (T0) and after 6 (T6), 24 (T24) and 48 (T48) h of incubation at 7 °C. Error bars indicate standard deviations

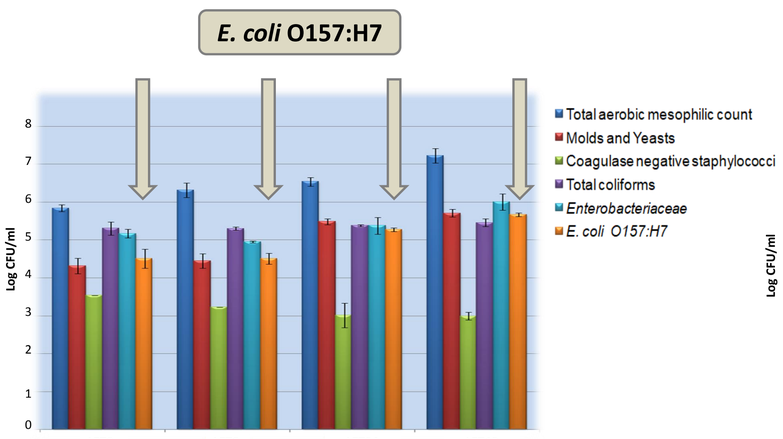


Figure 2. *E. coli* O157:H7 Ad565 concentrations in raw milk from vending machine immediately after the inoculum (T0) and after 6 (T6), 24 (T24) and 48 (T48) h of incubation at 7 °C. In the figure some of the microbial populations concurrently enumered are also reported. Error bars indicate standard deviations

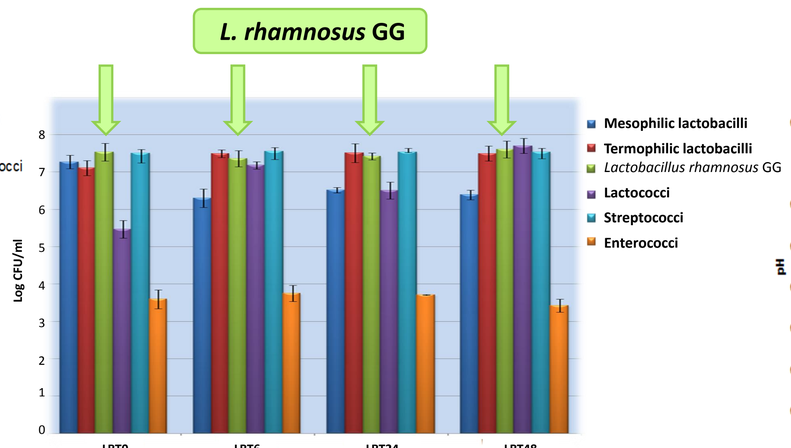


Figure 3. *Lactobacillus rhamnosus* GG concentrations in raw milk from vending machine immediately after the inoculum (T0) and after 6 (T6), 24 (T24) and 48 (T48) h of incubation at 7 °C. In the figure some of the microbial populations concurrently enumered are also reported. Error bars indicate standard deviations

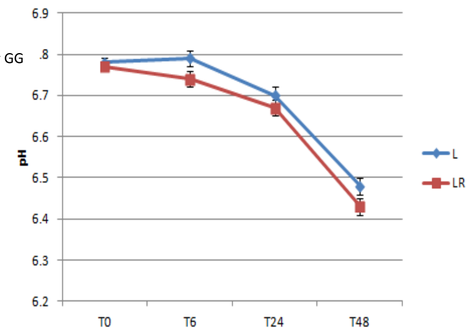


Figure 4. pH of raw milk with (LR) or without (L) the probiotic *L. rhamnosus* GG after purchase from the vending machine (T0) and after 6 (T6), 24 (T24) and 48 (T48) h of incubation at 7 °C. Error bars indicate standard deviations

***E. coli* O157:H7 Ad565** was able to multiply in raw milk increasing ca. 1 log cfu/ml (**Fig. 2**). Its presence did not affect the raw milk microbiota. The probiotic ***L. rhamnosus* GG survived** during refrigeration remaining at levels similar to those **normally encountered in probiotic products** (Di Lena et al., 2015) **(Fig. 3**), and acidified only slightly the raw milk (**Fig. 4**).

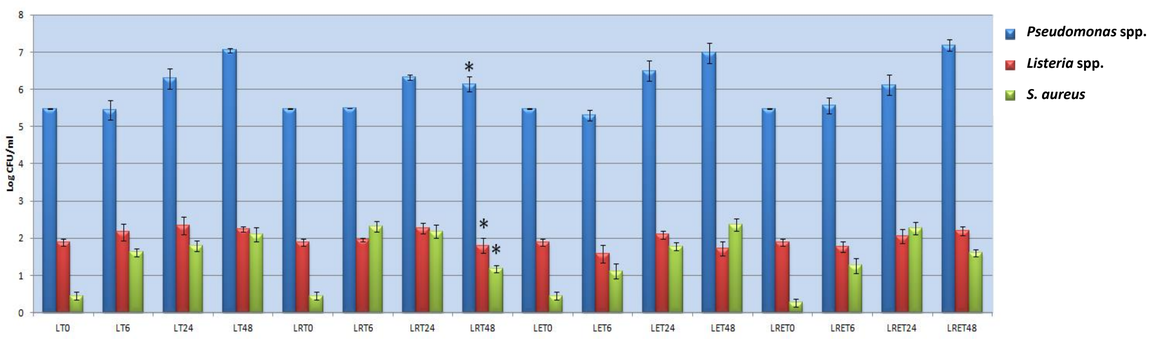


Figure 5. *Pseudomonas* spp., *Listeria* spp. and *S. aureus* populations of raw milk (L), raw milk added with the probiotic *L. rhamnosus* GG (LR), raw milk added with *E. coli* O157:H7 Ad565 (LE) and raw milk added with the probiotic *L. rhamnosus* GG and *E. coli* O157:H7 Ad565 (LRE) after purchase from the vending machine (T0) and after 6 (T6), 24 (T24), and 48 (T48) h of incubation at 7 °C. Error bars indicate standard deviations. Asterisks indicate statistically significant differences ($p<0.05$)

The presence of ***L. rhamnosus* GG** in raw milk exerted a certain antagonistic activity against **three** groups of microorganisms **after 48 h of refrigeration**:
the amount of **pseudomonads** in raw milk with the probiotic was ca. 0.92 log cfu/ml lower than in raw milk without the probiotic ($P<0.05$) (**Fig. 5**).
Similarly, for the populations of ***Listeria*** spp. and of ***S. aureus*** lower amounts, of ca. 0.5 and ca. 0.9 log cfu/mL, respectively ($P<0.05$), were reached in raw milk with the probiotic (**Fig. 5**).
Such a positive effect was **not found** in the probiotic raw milk artificially **contaminated** with ***E. coli* O157:H7 Ad565** maybe because the presence of this pathogen in the heterogeneous microbial ecosystem of the raw milk negatively affected the performances of the probiotic *L. rhamnosus* GG (**Fig. 5**).

CONCLUSION: the use of **protective** and **probiotic adjuncts** may be a **valuable strategy** to control **spoilage** and **pathogenic bacteria** in **raw milk** with an adequate microbiological quality (at least compliant with the current legislation), but probiotic strains **more effective** than *L. rhamnosus* GG should be used.

REFERENCES: Di Lena et al., 2015. *Int Dairy J*. 47, 27-36. Fusco et al., 2011. *J Food Microbiol*. 144, 528-537. Fusco et al., 2012. *J Food Microbiol*. 159, 1-8. Giacometti et al., 2012. *Ital J Anim Sci*. 11, e24. Godwin et al., 2007. *Food Prot Trends*. 27, 16-21. Goldin and Gorbach, 2008. *Clin Infect Dis*. 46, S96-S100. Italian Republic, 2007. Intesa n 5/CSR del 25 gennaio 2007. Josefsen et al., 2007. *Appl Environ Microbiol*. 73, 3040-3048. Lu et al., 2009. *J Pediatr Gastroenterol Nutr*. 49, 23-30. Quero et al., 2014. *Food Microbiol*. 38, 179-191.