Title: Optimization of the methods for monitoring bacteriophage infections and phage dynamics in artisanal milk cultures.

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Introduction.
Artisanal milk cultures are undefined cultures which are reproduced each day at the cheesemaking plant by heat treating raw milk and incubating it at 37-42°C, with or without backslopping (Parente et al., 2006). They are used for the production of several traditional Italian cheeses, including Mozzarella, and are usually dominated by *Streptococcus thermophilus*. The propagation of the cultures is carried out at the cheesemaking plant with limited or no asepsis and bacteriophage infections are known to play a role in their ecology (Bruttin et al., 1997). Because of their unpredictability, due to fluctuations in microbial composition and, as a consequence, of technological properties, they are often replaced by defined starter cultures. However, artisanal milk cultures are still required in the standard of identity of some Italian cheeses and, as many other artisanal cultures, they may be a rich source of new strains (Parente et al., 2006) and of scientific knowledge on the dynamics of microbial communities and on bacteriophage/host interactions.

In fact, *S. thermophilus* is the second most important phage species after *Lactococcus lactis*, because of its use as a starter in fermented milks, Italian, French and Swiss cheese varieties, and in Pizza cheese (low-moisture part-skim Mozzarella cheese) (Parente et al., 2004; Iyer et al., 2010). The biology of *S. thermophilus* has recently been reviewed (Quiberoni et al., 2006). Although *S. thermophilus* belong to a single polytene species of the class *Siphoviridae*, order *Caudovirales*, two packaging modes (*cos* and *pac*) have been identified and *cos* and *pac* phages have a slightly different organization of their genomes (Quiberoni et al., 2006). Conserved sequences have been identified in the genomes of both *cos* and *pac* phages and used to develop PCR assays (Del Rio et al., 2007, 2008). Recently, a PCR assay based on amplification of the conserved antireceptor gene has been used to characterize *S. thermophilus* bacteriophages (Binetti et al., 2005).

*S. thermophilus* phages usually have a very narrow host range, infecting 1 or few strains of *S. thermophilus*. This may be due to the main bacteriophage defense system present in *S. thermophilus*, the CRIPR/CAS system (Barrangou, 2012).

Traditional methods for bacteriophage detection and typing, based on inhibition assay, plaque assays and bacteriophage isolation and typing are cumbersome and labor intensive and are difficult to apply for long time monitoring of bacteriophage dynamics during reproduction of natural starters. On the other hand, the quantitative PCR assays which have recently been developed (Del Rio et al., 2008) allow only the quantitation of *cos* and *pac* bacteriophages, with little information on phage diversity. In addition, there is no systematic study on the relative merits of different phage DNA purification methods from milk, whey and cheese.

Proposed activities.
Detection of phage DNA by molecular methods has been carried out 1. by simply using milk as a template (Quiberoni et al., 2006; Del Rio et al., 2007, 2008), 2. by using multistep purification protocols (Binetti et al., 2008) or 3. by adapting commercial DNA extraction kits (Ly-Chatain et al., 2011). While the first alternative is simple, it may be less sensitive and hamper PCR amplification because of the presence of Ca, fat and proteins, although Ly-Chatain et al. (2011) have reported no inhibition of PCR reactions with up to 5 µl of milk in a 25 µL reaction volume. The second alternative is more labour intensive and may require the use of toxic chemicals. The third alternative, although more expensive, may be more amenable to large screenings and increase sensitivity. Naturally contaminated samples of milk,
milk culture, whey and cheese, and samples spiked with known amounts of \textit{S. thermophilus} bacteriophages will be used with all three methods. Methods 2 and 3 will be compared on the basis of yield and purity of DNA and samples obtained with all three methods will be used for PCR targeted at the variable VR2 region of the antireceptor gene of cos-type bacteriophages (Binetti et al., 2005), multiplex PCR for the detection of cos and pac-type phages (Quiberoni et al., 2006) and quantitative multiplex PCR (Del Rio et al., 2008). Both naturally contaminated samples and samples with artificial addition of 2 different phages will be used, with at least three replicates per sample, and the reproducibility and sensitivity (in terms of minimum amount of pfu detectable) will be tested.

The expected outputs of this miniprojects are: a. the training of a post-doc student of Università degli Studi della Basilicata in molecular techniques related to the detection of phages and real-time PCR; the optimization of simple or quantitative methods for the detection of \textit{S. thermophilus} bacteriophages in artisanal starter cultures.

**Proposed duration:** 45 days.

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**References.**


