WHEY PHAGE ECOLOGY IN ARTISANAL TRADITIONAL GRANA-LIKE CHEESE: SUSTAINABILITY IN DAIRY PRODUCTION

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Cheeses and phage: what's wrong?

Natural whey starters (NWS) are cultures of lactic acid bacteria (LAB) obtained from the incubation of cheese whey collected at the end of the day before cheese-making process . NWS are characterized by the presence of thermophilic LABs, and used for the manufacture of Italian, long ripened Grana-like cheeses such as *Trentingrana*. Several factors could affect the NWS activity such as bacteriophage attack, leading to an impairment in milk clotting, whey drainage, product texturing, and microbial growth with lost in cheese production yield.

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Phage and dairies: the problem

LABs bacteriophage are a risk issue for the dairy industry because their complete removal is not realistically possible.
Different sources of phage contamination are present in dairy factories :

- milk (10^1-10^4 pfu/ml), thermic treatment resistance (by casein)
- NWS maintenance and propagation
- dairy environment (operator, whey aerosol, work surfaces)

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Phage and dairies: our study

AIMs

- to study the phage population biodiversity in *Trentingrana* cheese production
- to find a solution in phage infection-attack maintaining the sustainability in cheese production

WHAT WE DID

- whey sampling in six Trentingrana dairies (A – F; see figure) in the Trento province (Italy) during the four different seasons in 2018 for a total of: 230 NWS samples collected, more than 1600 thermophilic LABs and 163 phages isolated from NWS samples

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Conclusions

Phages and NWSs have a long ecological story, driven both by human activities and by the natural evolution. Our first results showed how whey phages consist of a complex community underling 1) the potential presence of more virulent-active viruses in this ecosystem, 2) the potential ability to attack more than one LAB biotype-species. Further molecular analysis on the remaining isolates will close this overview, allowing to select the main viruses characteristic of *Trentingrana* production. Phage genome sequencing associated with selection of LAB strains phage-resistant, will characterize further steps.

References

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[3] Mahony J. et al. 2016, *Phage-Host Interactions of Cheese-Making Lactic Acid Bacteria*, Annual Review of Food Science and Technology
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Phage and LAB host: a *kill the winner* strategy

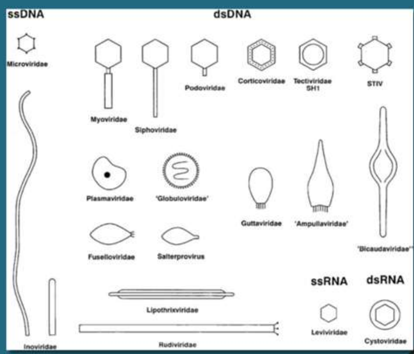
Similarly to other ecosystems, bacteriophages are suggested to play an ecological role within NWS, acting as a biological pressure, leading to the spontaneous selection of phage-resistant strains, being able to counteract the loss of the sensitive ones, thus preserving the overall technological performances of the cultures [1, 2, 3].
The most studied phages are for *Lactococcus lactis* and *Streptococcus thermophiles*; nowadays phage genome are available for *Lactobacillus* sp. (*Siphoviridae* e *Myoviridae*) or *L. plantarum*, *L. grassei*, L. *casei/paracasei*, *L. rhamnosus*, *L. delbrueckii* and *L. helveticus*

Morphology:

- Capside
- Nucleic acid (ssDNA, dsDNA, ssRNA, dsRNA)

Classified by:

- electronic microscopy
- serotyping
- structural proteins
- molecular genotyping



adapted from Hans-W- Ackermann 2009 [4]

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First results: LABs characterization

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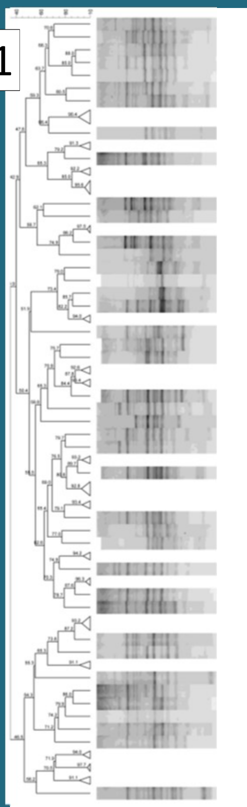

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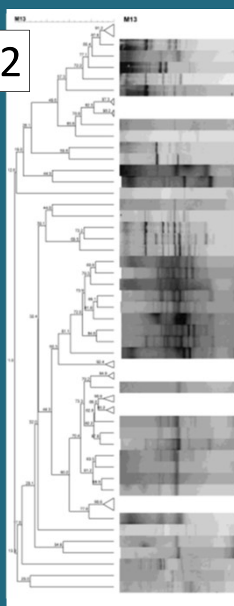

Fig. 1) UPGMA dendrogram obteined clustering the M13 RAPD fingerprinting patterns of 81 whey isolated LAB strains.

Fig. 2) UPGMA dendrogram derived from the M13 RAPD fingerprinting patterns of 56 isolated phages.

White triangles are representing strains or phage with same biotypes in Fig. 1 and 2 respectively

The genomic LAB characterization by RAPD-PCR clustered about 650 strains in 397 biotypes showing and high biodiversity both inter- and intra- dairy factory and season. Similarly phage diversity was widely spread across and within the same dairy, showing a rich whey phages population.

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