Isolation and characterization of new filamentous phages active on Gram-positive bacteria in Swiss-type cheese

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Isolation and characterization of new filamentous phages active on Gram-positive bacteria in Swiss-type cheese

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INTRODUCTION

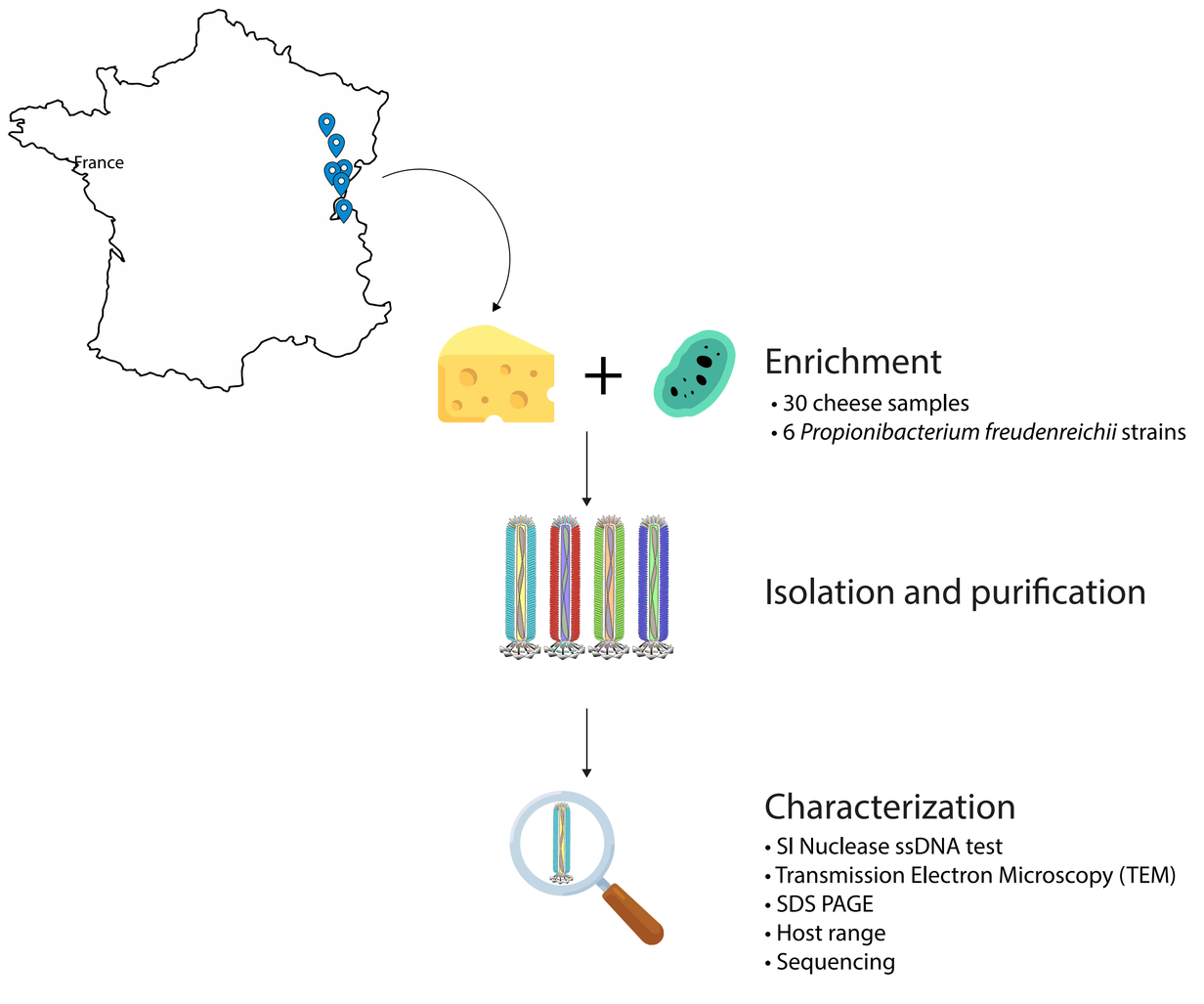
In 1998, our team isolated the **first filamentous phages active and culturable on Gram-positive Bacteria** [1]. The phage infected *Propionibacterium freudenreichii* and was isolated from Swiss-type cheese. It is a single-stranded DNA phage, that was named B5. Its genome contains 5.8 kb, codes for about 10 open reading frames and the organization is very similar to the organization of the genomes of filamentous phages active on Gram - bacteria. It was also showed that the structural properties of its proteins indicate that it is related to filamentous phage family and probably the same life cycle.

Since then, nothing has been done to follow up on this work and no related phage was found ; little is known about B5-like phages diversity.

For this study, Swiss-type cheese sample were enriched in liquid YEL culture medium. After removal of bacterial cells using centrifugation and filtration, the enrichment was tested for phage plaques using the double agar layer assay. The newly isolated phages were then identified and the first steps of the characterization were performed.

The aim of this project is to isolate new filamentous phages from Swiss-type cheese to study their diversity and discuss, by comparison with phage B5, a possible evolution in recent years.

Method



RESULTS & DISCUSSION

Nine phages isolates from different cheeses were gathered after the purification step. They were isolated and propagated on the bacterial strain 434. Their DNA was digested by the SI nuclease, which showed they are all ssDNA phages. TEM images confirmed they were filamentous phages, and allowed to measure the virions, which measure 620 nm by 12 nm (Fig 1 and Fig 2). SDS page showed identical profiles for each phage. DNA sequencing revealed that 2 phages were isolated twice, in different cheese : 7 phages were kept for the rest of the analysis (L, f, c, d, D', e, and h).

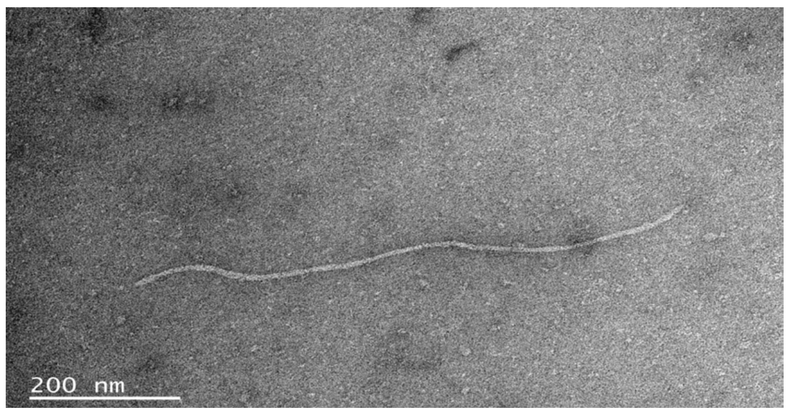


Fig 1. Electron micrograph of filamentous phage c. Image courtesy of Agnes BUREL*.

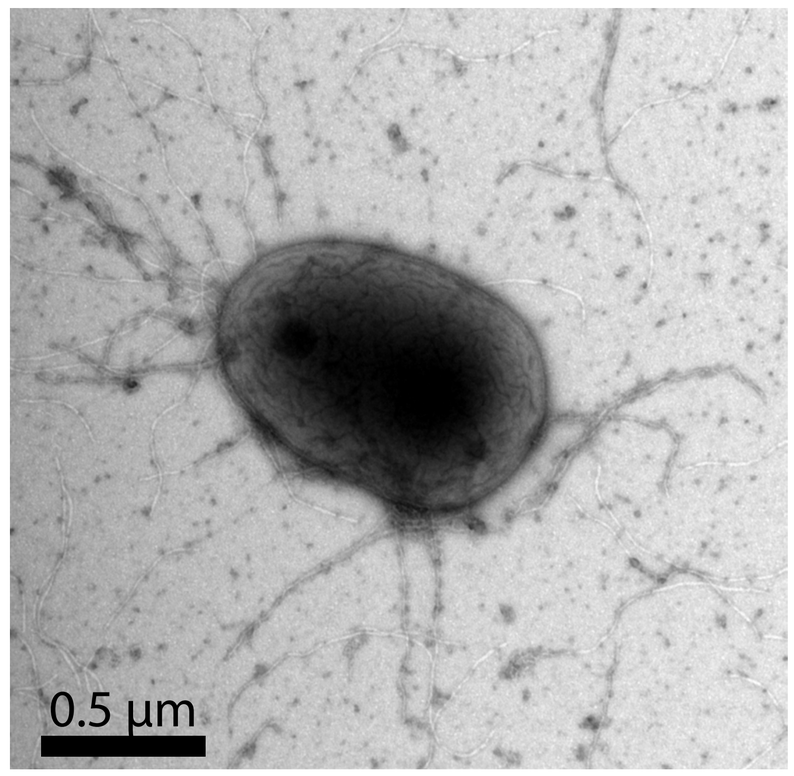


Fig 2. Electron micrograph of adsorption step between filamentous phage c and bacterial host strain 434. Image courtesy of Agnes BUREL*.

Interestingly, all the newly isolated phages share the genome size and structure of the original B5 phage. Sequence alignment also revealed a high sequence identity with B5 phage (Fig 4). The lowest identity percentage of each phage couple compared in Fig 3 is 93.75, which indicates that all these viruses share a close common ancestor, and were able to drift away from it. At what step of the cheese manufacturing do they come from ? (Fig 3) They could be part of the ferments used to process the milk, which cheese makers are known to exchange and mix, or be part of the livestock's diet. As such information is hard to access, this question will likely stay unanswered.

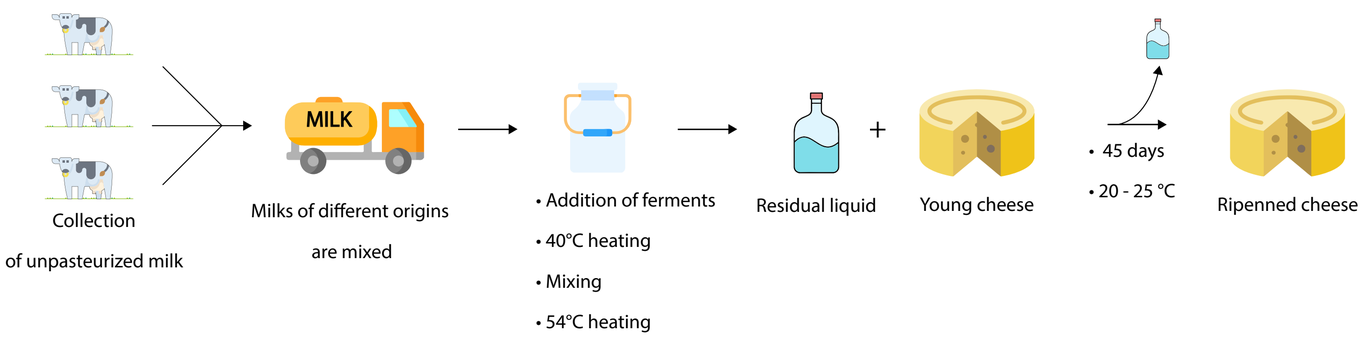


Fig 3. Fabrication process of Swiss-type cheese

Efficiency of Plating assay yielded EOP values ranging from 1 to 10^{-8} (Fig 4). 434 is the most sensitive bacterial strain, ITG 20 and ITG 21 were sensible but exhibited the highest level of resistance to all the phages. No correlation was found between EOP values and sequence identity.

	B5	L	f	c	d	D'	e	h
B5	100	97	95,03	96,62	96,77	96,2	93,75	95,12
L	97	100	95,74	96,38	96,53	96,2	95	95,3
f	95,74	95,03	100	95,11	95,62	95,42	94,69	95,04
c	96,38	96,62	95,11	100	100	96,41	94,59	94,95
d	96,53	96,77	95,62	100	100	96,65	94,88	95,25
D'	96,2	96,2	95,42	96,41	96,65	100	95,23	95,51
e	95	93,75	94,69	94,59	94,88	95,23	100	99,19
h	95,3	95,12	95,04	94,95	95,25	95,51	99,19	100

Fig 4. Identity percentage matrix of the phage genomes from this study. The genome of phage B5 was also used in the comparison.

	B5	L	f	c	d	D'	e	h
ITG 20	1,E-06	3,E-06	1,E-08	2,E-07	2,E-07	5,E-07	2,E-06	5,E-03
ITG 21	1,E-07	3,E-07	1,E-08	4,E-08	2,E-08	5,E-08	2,E-08	1,E-02
2500	2,E-01	1,E-05	3,E-07	8,E-04	1,E-01	2,E-01	5,E-06	2,E-03
2502	5,E-01	3,E-04	1,E-07	1,E-03	2,E-01	5,E-01	3,E-07	8,E-03
434	1,E+00	1,E+00	1,E+00	1,E+00	1,E+00	1,E+00	1,E+00	1,E+00

Fig 5. Efficiency of Plating of the phages of this study. Bacterial strain 434 was used as a reference. Low sensibility phage/host couples (EOP < 10^{-4}) are in red, medium sensibility phage/host couples (10^{-1} < EOP ≤ 10^{-4}) are in orange. High sensibility phage/host couples (EOP ≥ 10^{-1}) are in green.

$$EOP = \frac{\text{Number of plaques produced on a strain}}{\text{Number of plaques produced on the reference strain}}$$

CONCLUSION

Searching for new filamentous phages active on gram-positive bacteria, two main results were found :
– There is a diversity of B5-like phages in Swiss type cheese.
– The newly isolated phages are all closely related. No new exotic filamentous phage was found.

Very little is known yet about this family of phages : the next steps of our project should include genome annotation and infection cycle study.

Obtaining more detailed information from cheesemakers on the origins of their ferments would also be helpful to understand the environment in which those phages evolve and maybe find the original phage that evolved in the B5 family of phages.

Acknowledgement & Bibliography

* The TEM images were taken by **Agnes BUREL**, Plateforme microscopie électronique MRic/TEM - BIOSIT Biologie Santé Innnovation UMS CNRS 3480 INSERM 018 Université Rennes 1

[1] M.-C. Chopin, A. Rouault, E. Dusko, M. Gautier. Filamentous Phage Active on the Gram-Positive Bacterium Propionibacterium freudenreichiit, Journal of Bacteriology, 184 (7).