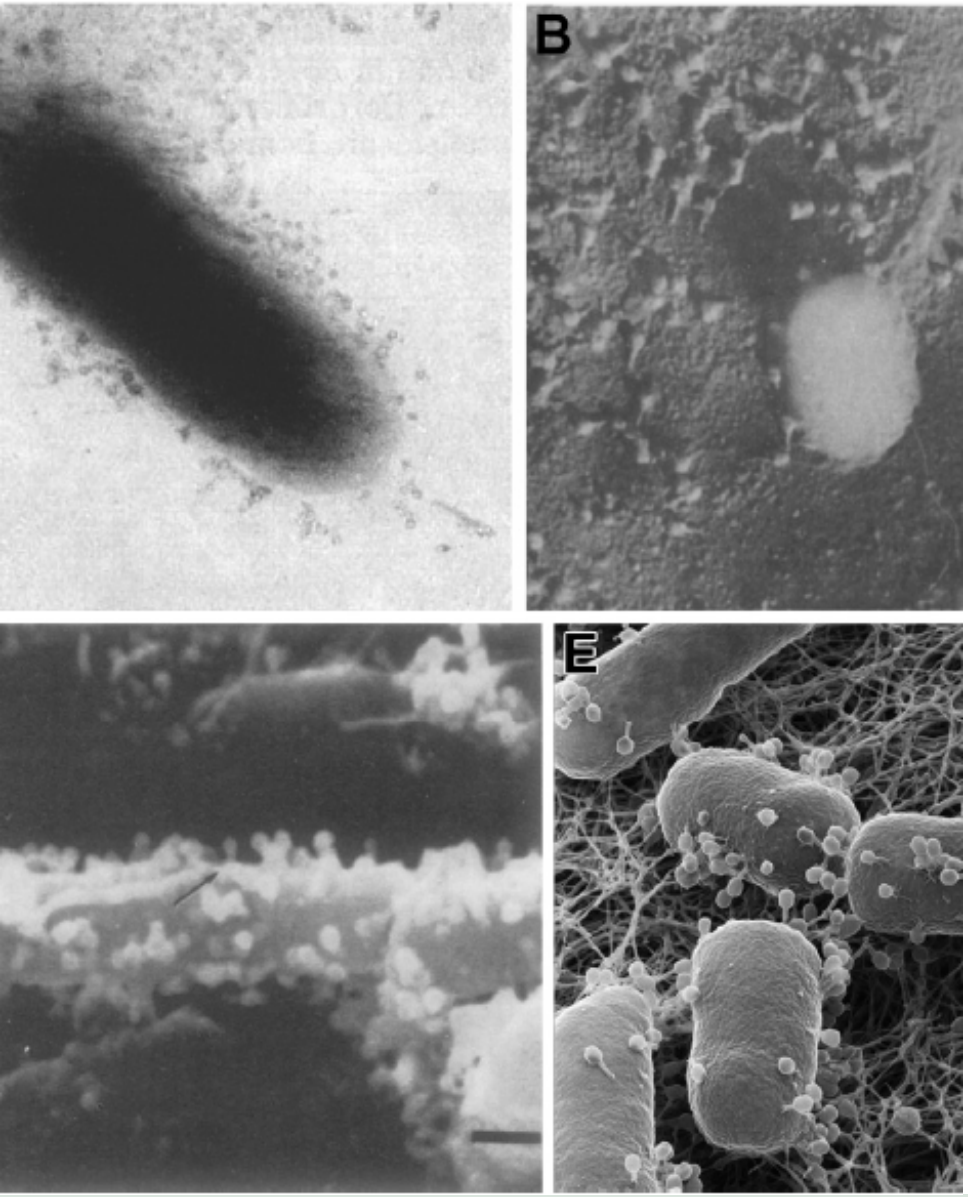
FAST DETECTION OF BACTERIOPHAGES AT DAIRIES

qPCR fast method for phage detection

14/10/2021

Billund, Hotel Legoland

Vittoria Piccini, Research Scientist



AGENDA

- Introduction
- *Lactococcus lactics* phages
- Phage host interactions
- Impact of phages in dairy enviroment
- Detection methods for phages
- qPCR fast detection method
- Case study
- Conclusion

Almeida, G. M., Leppänen, M., Maasilta, I. J., & Sundberg, L. R. (2018). Bacteriophage imaging: past, present and future. *Research in microbiology*, *169*(9), 488-494.

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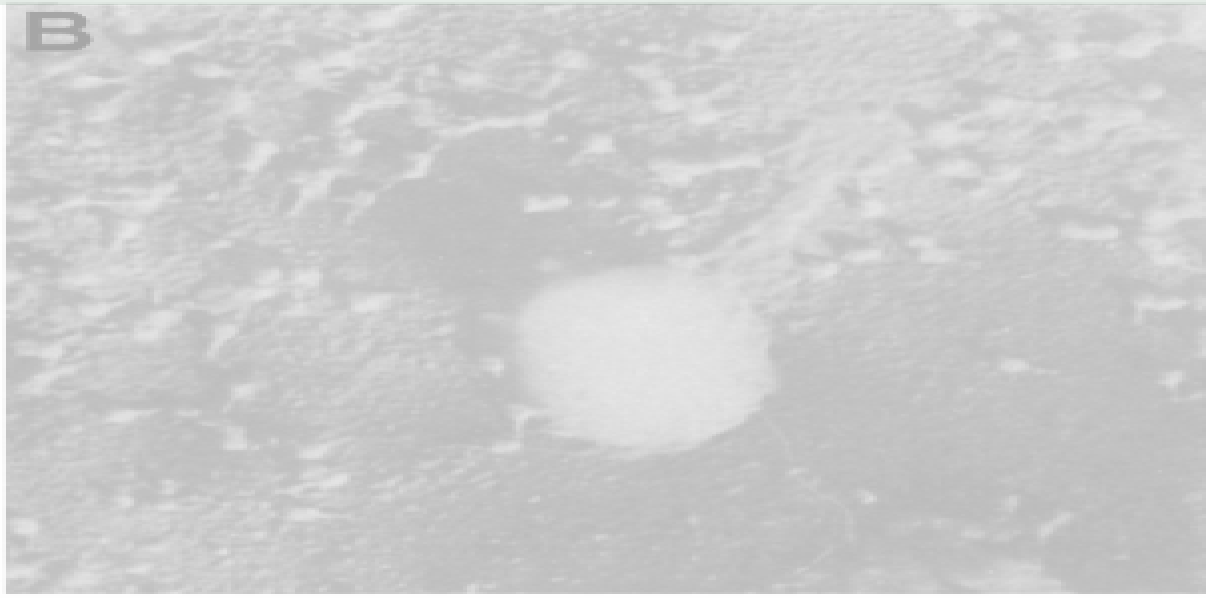
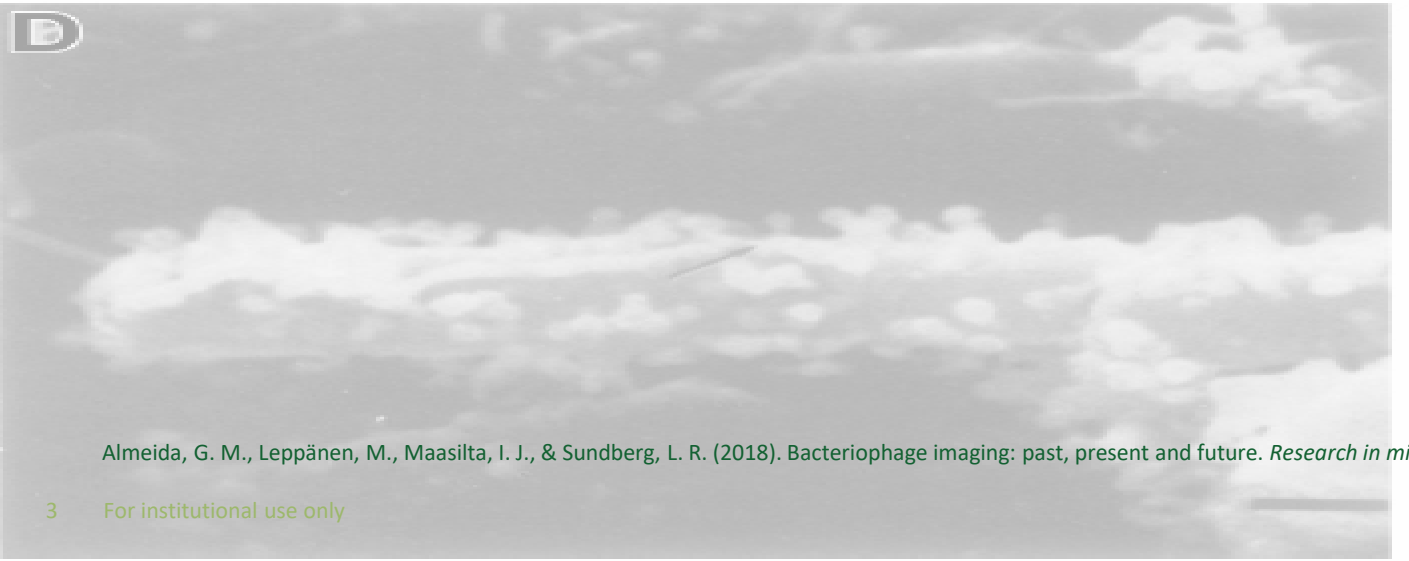
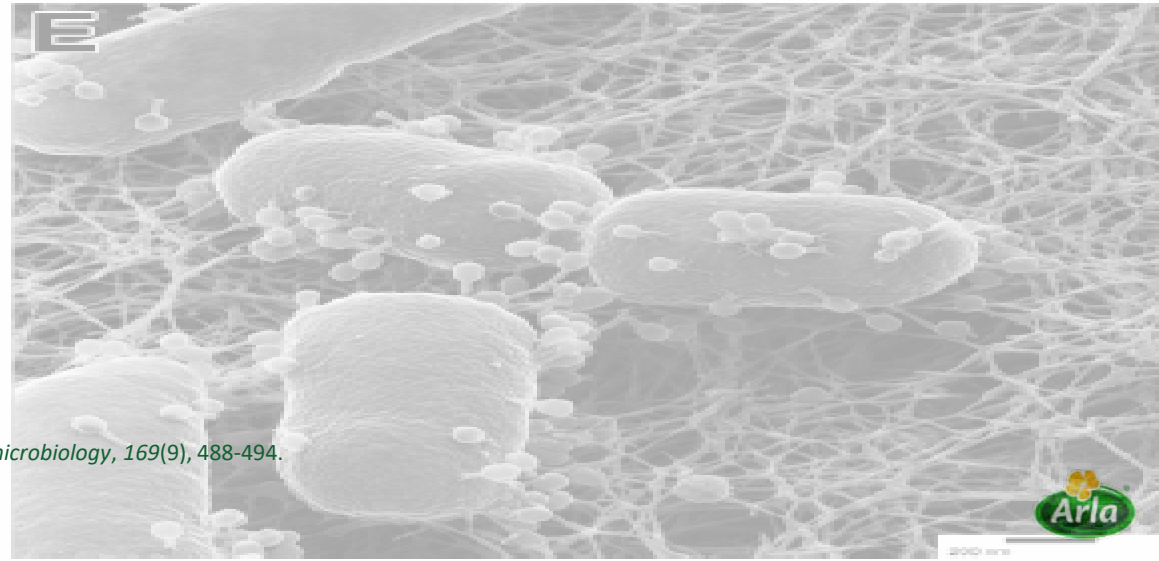
Why are bacteriophages an issue in the dairy enviroment?

- Product waste
- Cheese defects
- Food safety related concerns







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Bacteriophages in history

1896 – Hankin reported bacteriological activity of Ganges filtered water on *Vibrio cholera*

1915 – Twort first observed ”transmissible glassy transformation” of bacteria

1916 – D’Herelle discovered the basic facts about phages in filtrates of dysentery fluids

1934 – Whitehead and Cox isolated the first bacteriophages from lactic acid bacteria

Sharp, R. (2001). Bacteriophages: biology and history. *Journal of Chemical Technology & Biotechnology*, *76*(7), 667-672.

Summers, W. C. (2012). The strange history of phage therapy. *Bacteriophage*, *2*(2), 130-133.

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Lactococcus lactis phages and their presence in dairies

Host	Bacteriophage family	Bacteriophage species	Taxonomy references
L. lactis	*Siphoviridae*	936 P335 c2 1358 Q54 P087 1706 949	Deveau et al., 2006
	Podoviridae	P034 KSY1	

Deveau, H., Labrie, S. J., Chopin, M. C., & Moineau, S. (2006). Biodiversity and classification of lactococcal phages. *Applied and environmental microbiology*, *72*(6), 4338-4346.

Mahony, J., & Van Sinderen, D. (2014). Current taxonomy of phages infecting lactic acid bacteria. *Frontiers in microbiology*, *5*, 7.

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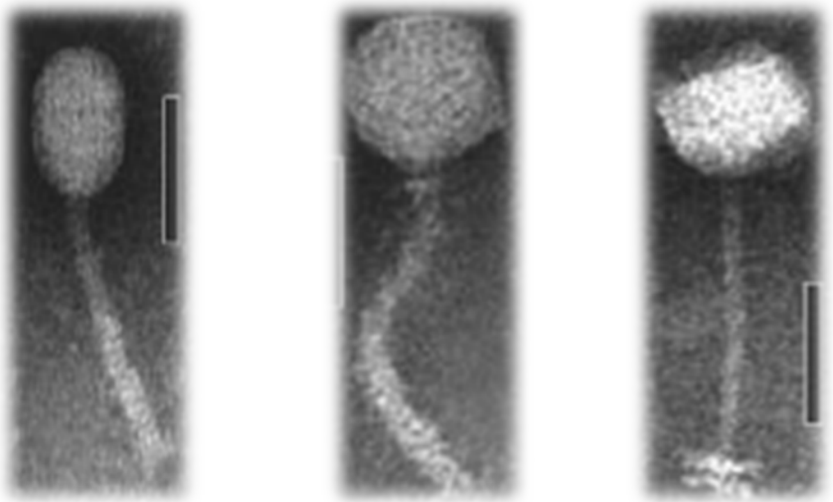
Lactococcus lactis phages and their presence in dairies

- Among the 10 currently recognized lactococcal phage groups 936, c2 and P335 are the most frequent in dairy enviroments
- 936-group phages showed to be the most problematic group for fermentations
- P335 group showed significant genetic diversity and absence of a core genome
- According to Madera et al., 936 around 24%, P335 around 4%, c2 around 72% of phages in milk and after pasteurization 936 type becomes more dominant



c2 936 P335

*bars 50 nm

Deveau, H., Labrie, S. J., Chopin, M. C., & Moineau, S. (2006). Biodiversity and classification of lactococcal phages. *Applied and environmental microbiology*, *72*(6), 4338-4346.

Madera, C., Monjardín, C., & Suárez, J. E. (2004). Milk contamination and resistance to processing conditions determine the fate of Lactococcus lactis bacteriophages in dairies. *Applied and environmental microbiology*, *70*(12), 7365-7371.

Oliveira, J., Mahony, J., Hanemaaijer, L., Kouwen, T. R., & van Sinderen, D. (2018). Biodiversity of bacteriophages infecting Lactococcus lactis starter cultures. *Journal of dairy science*, *101*(1), 96-105.

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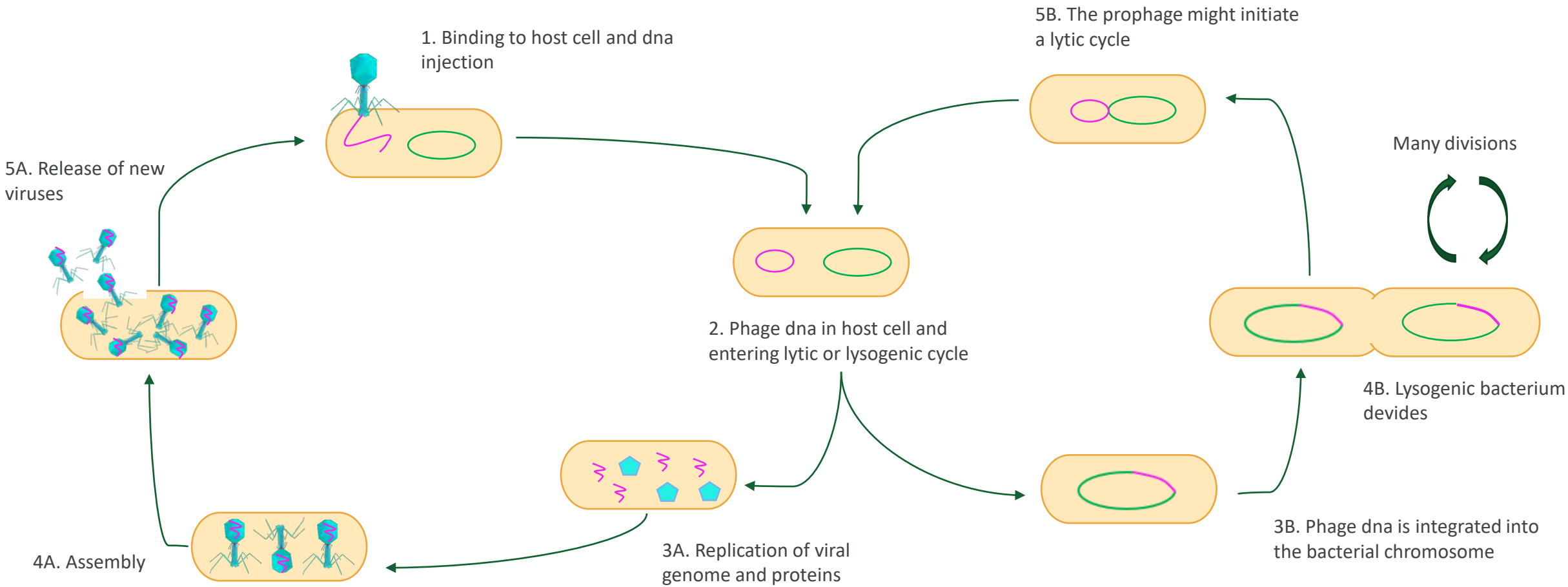
Phage-host interactions



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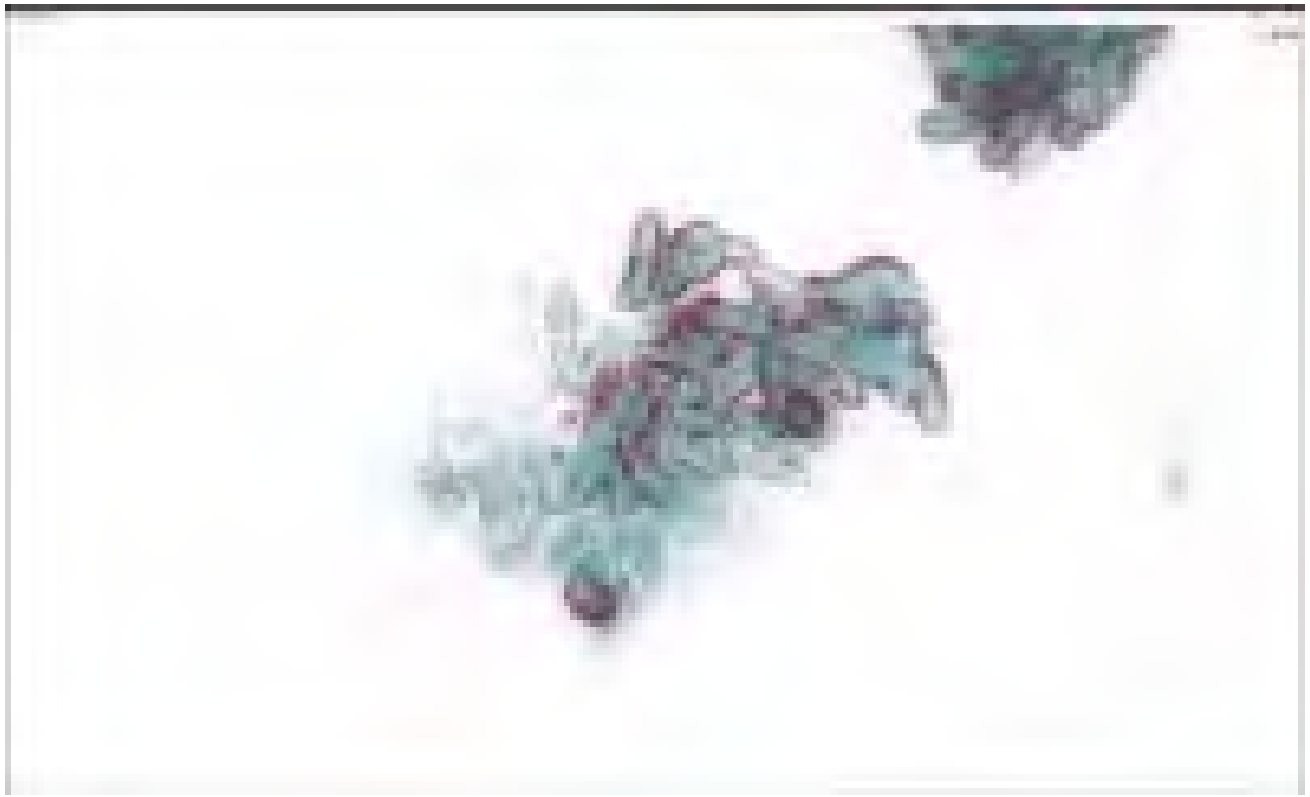
Phage-host interactions



https://www.youtube.com/watch?v=HfqCxrMQHpQ

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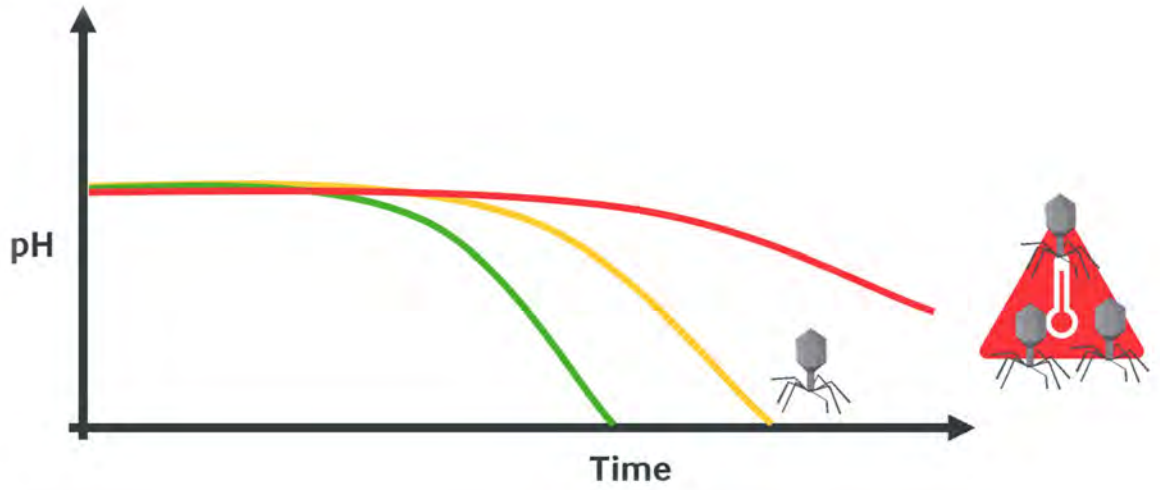
Estimated economical impact of bacteriophages at dairies

- 2000 tons per year of cheese produced → 50.000.000 DKK gaining
- If there is a phage attack, there will be a fluctuation in cheese moisture (2%) and consequently out of spec
- Loss of around 1.000.000 DKK per year

- Food safety related risk and consequent costs



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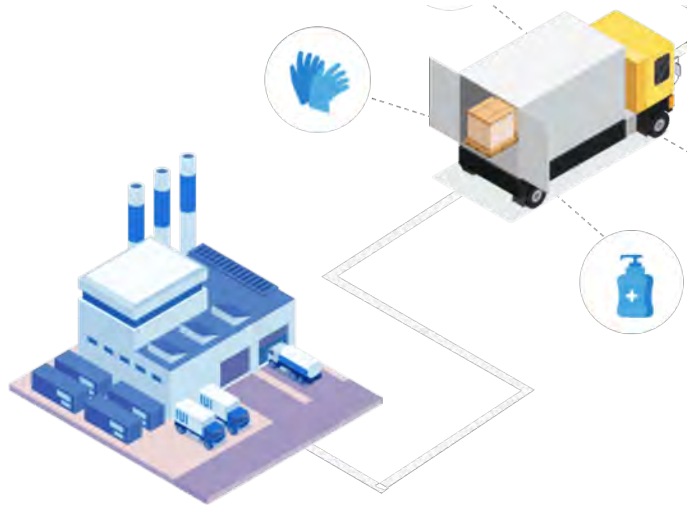
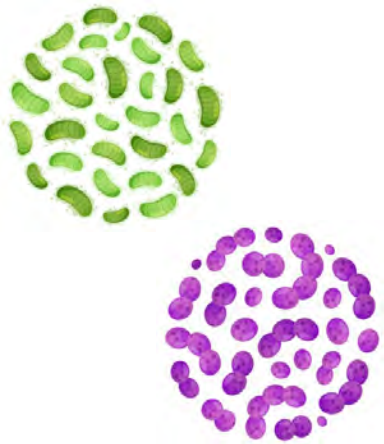
Fight phages at dairies

- Performing rotation of starter cultures
- Sanitation of dairy facilities
- Application of efficient heat-treatments

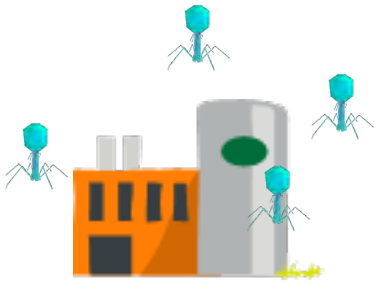


FAST DETECTION OF BACTERIOPHAGES AT DAIRIES

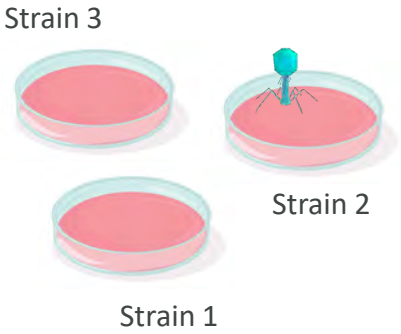
Fight phages at dairies - Performing rotation of starter cultures

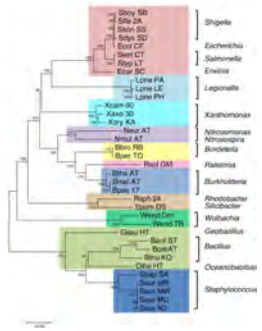


Culture development

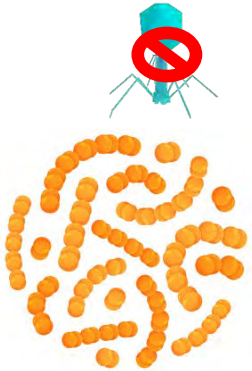


Investigation of phage ”outbreak” at site





Phage characterization



Renewal with cultures resistant to phages

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Phage tests currently available

Overlay assay

- Insight which strain is attacked by phage
- Insight which rotations/strains are not affected by the phage
- Free service provided by supplier

Long lead time for test results does not prevent phage problems

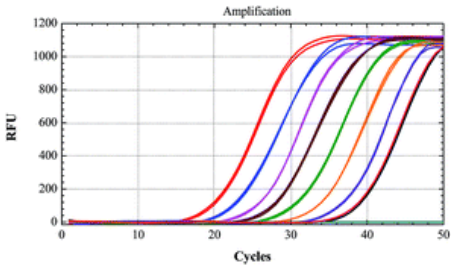
Acidification assay

- Insight which rotation is attacked by phage

Medium lead time for test results gives limited opportunity to prevent phage problems

- No information obtained on which specific strain is attacked

qPCR test



- Allows switch to new rotations before slow down of vats occur
- Phage titer is determined which gives an early warning
- Facilitates hygiene testing
- Quick indication if phages are the cause for the slow down of acidification

No information obtained on which specific strain is attacked

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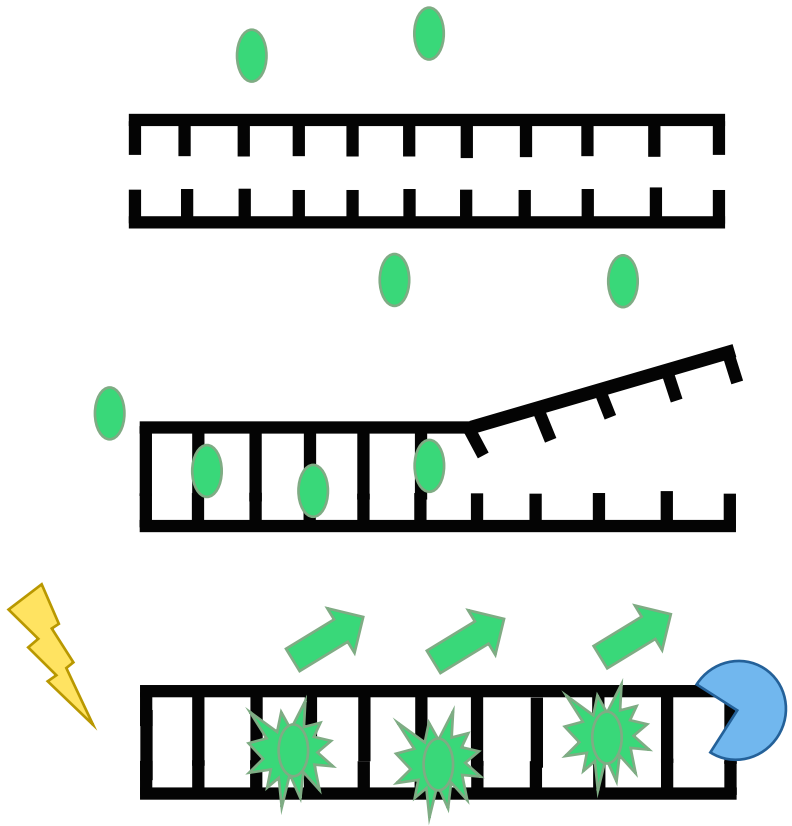
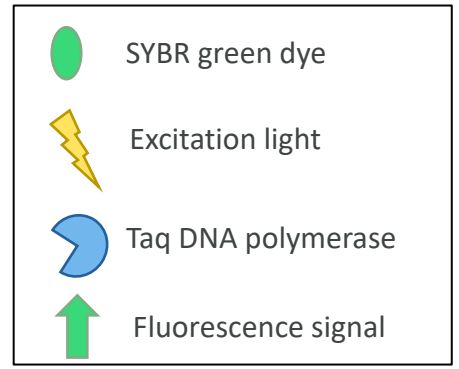
Principle of qPCR detecting phage DNA

Denaturation

Annealing

Extension





Muhammed, M. K., Krych, L., Nielsen, D. S., & Vogensen, F. K. (2017). A high-throughput qPCR system for simultaneous quantitative detection of dairy Lactococcus lactis and Leuconostoc bacteriophages. *PLoS One*, *12*(3), e0174223.

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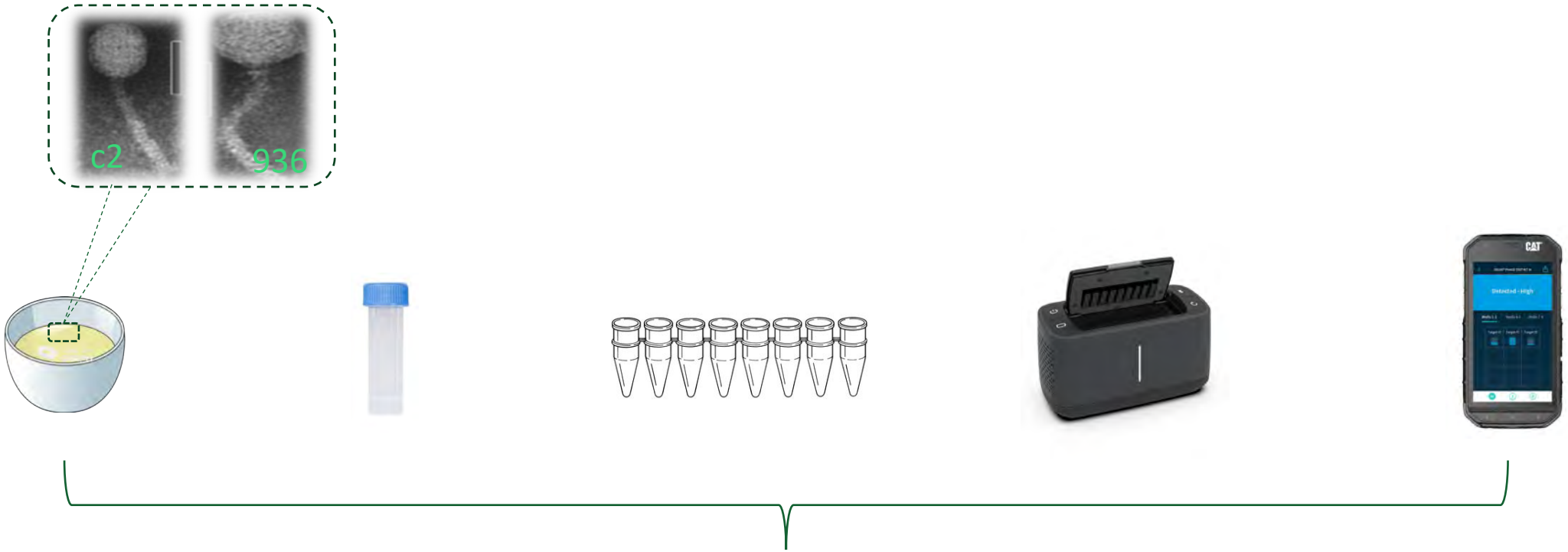
Phage test kit – Workflow



< 1 hour

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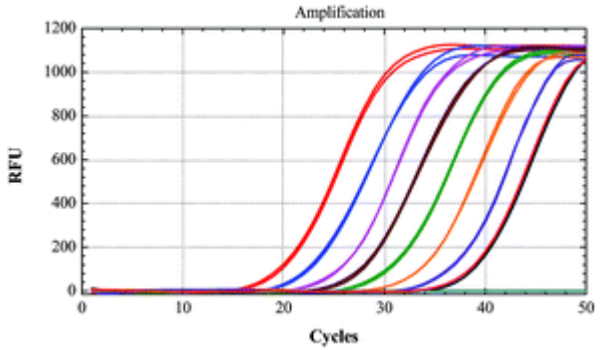
Phage test kit – Interpretation of results

TARGET	RESULT	STARTING QUANTITY (SQ)
	Detected - High	30,001 - 300,000
	Detected - High/Medium	3,001 - 30,000
	Detected - Medium	301 - 3,000
	Detected - Low/Medium	31 - 300
	Detected - Low	1-30
	Not Detected	0



FAST DETECTION OF BACTERIOPHAGES AT DAIRIES

Fighting bacteriophages at dairies is a never-ending challenge…

- Factory and equipment design
- Process design (heat treatments)
- Sanitation

- Use of phage-resistant starter culture
- Culture rotation programs
- Sanitation

- Use microbiologically safe water
- Avoid stagnation of water in drains and production area

- Adequate whey handling
- Correct disposal
- Correct thermal treatments

- Monitoring phage pressure

Marcó, M. B., Moineau, S., & Quiberoni, A. (2012). Bacteriophages and dairy fermentations. *Bacteriophage*, *2*(3), 149-158.

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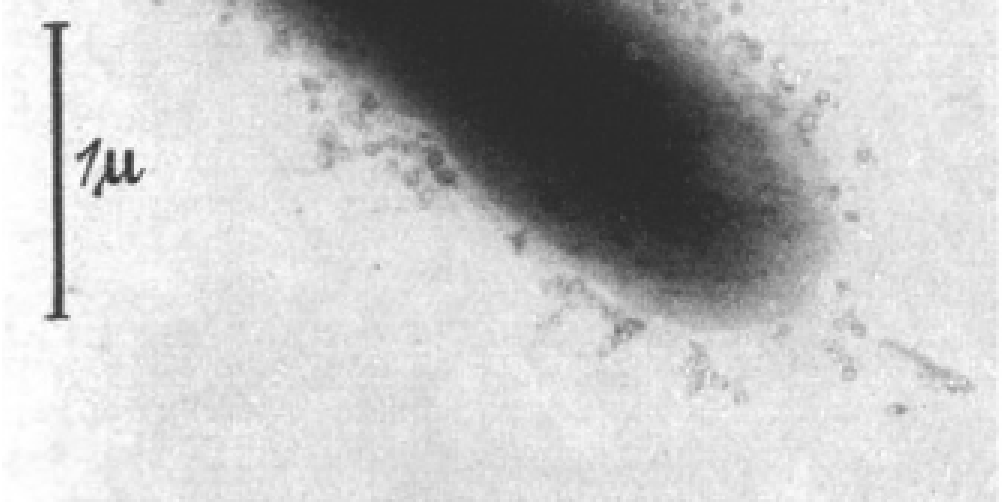
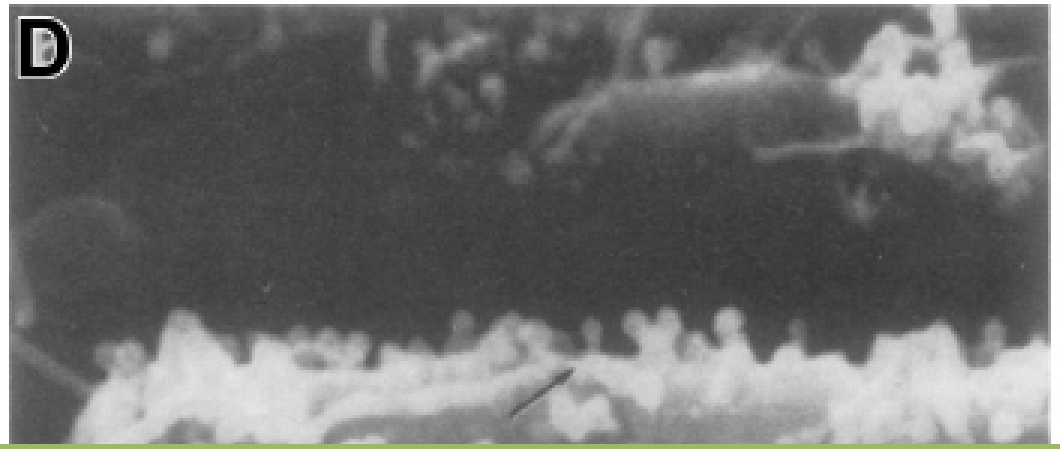
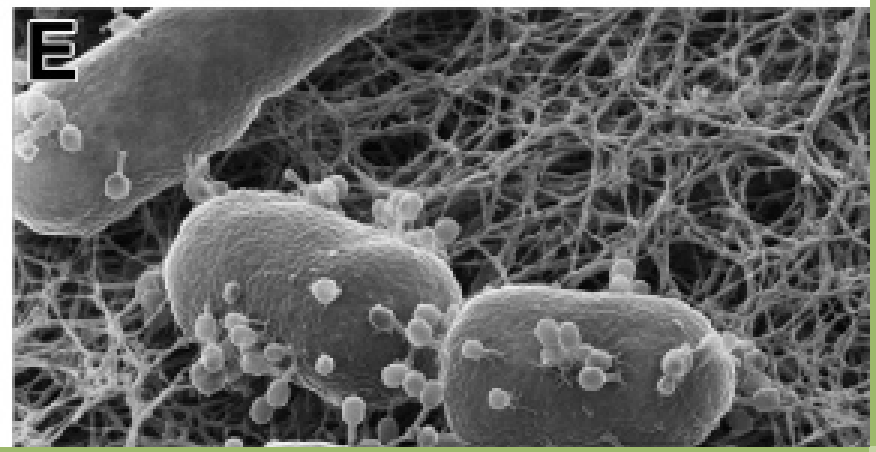
Collaborations and acknowledgment

- Eric van den Berg – Application Expert Phages – DSM
- The Danish Society of Dairy Technology







THANKS FOR YOUR ATTENTION ☺

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List of references

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