

*Listeria monocytogenes*

Laboratorio Nazionale di Riferimento

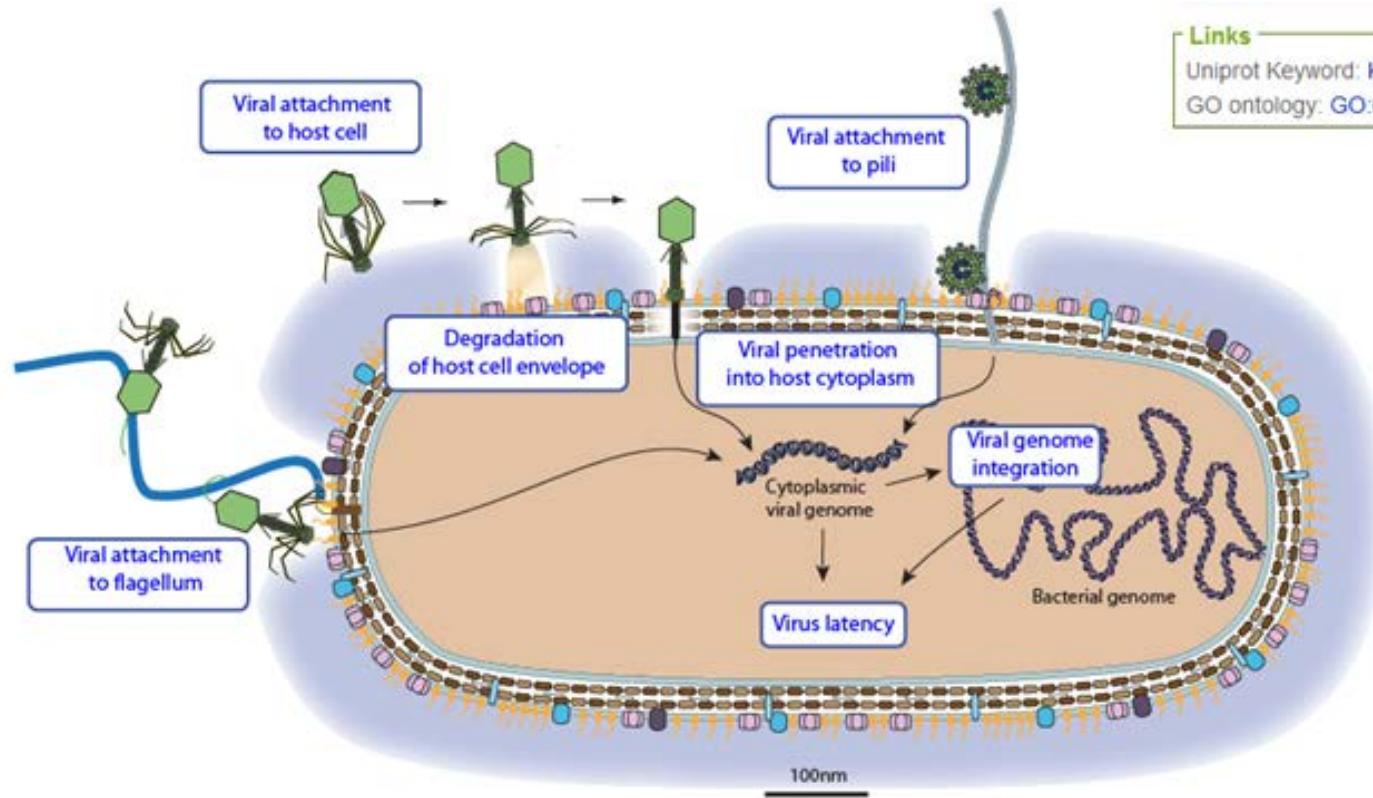
## Nuove tecniche di risanamento degli alimenti mediante l'utilizzo di batteriofagi

CIFIV, 25/11/2014

DVM, PHD  
Giuseppe Aprea



# Just to remember what is a «bacteriophage»



**Links**  
Uniprot Keyword: KW-1160  
GO ontology: GO:0046718

[http://viralzone.expasy.org/all\\_by\\_species/3996.html](http://viralzone.expasy.org/all_by_species/3996.html)



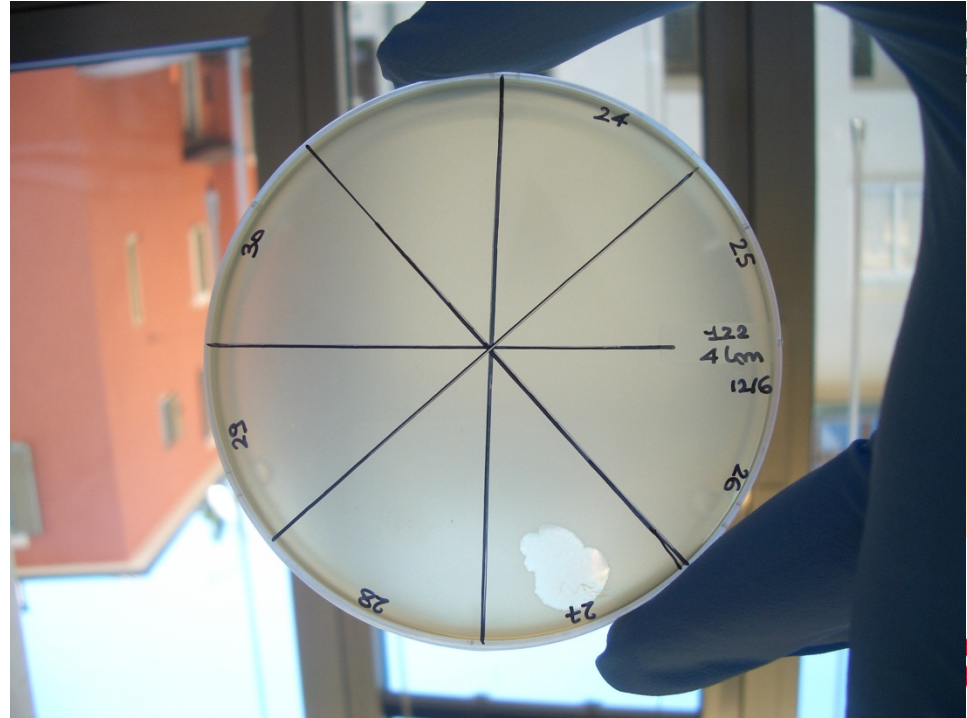


**Table 2.** CASE B: Sampling sites and microbiological results. *L. monocytogenes* was investigated in surfaces swabs (30 samples). Bacteriophages were investigated in drain water samples (samples n. 17 and 26) and in all surface swabs.

Cheese plant working area	Surfaces	Results
	1. Curdling tank	-
	2. Curdling tank	-
	3. Milk pipe to curdling tank	-
	4. Stainless steel curdling tool	-
	5. Stainless steel curdling tool	-
	6. Stainless steel curdling tool	-
	7. Sink	-
	8. Handrails	-
Cheese making area	9. Cutting machine (tape)	-
	10. Cutting machine (grid)	-
	11. Cutting machine (not contact surface)	11- <i>L. monocytogenes</i> serovar 1/2a
	12. Table	-
	13. Table	-
	14. Sink	-
	15. Wall	-
	16. Plastic stencil	-
	17. Drain + water	Phage 010120/1
	18. Whey pipe	-
'Ricotta' making area	19. Drain + water	-
'Ricotta' storage area	20. Stainless steel shelf	-
	21. Door handle	-
'Pecorino' salting area	22. Salting machine	-
	23. Wood shelf	-
	24. Wall	-
	25. Tape under vacuum machine	-
	26. Drain + water	-
Cutting/packaging area	27. Cutting machine (food contact surface)	Phage 10022/27 + 27- <i>L. monocytogenes</i> serovar 4b
	28. Cutting machine	-
Dressing room	29. Shoes	-
	30. Cabinet	-

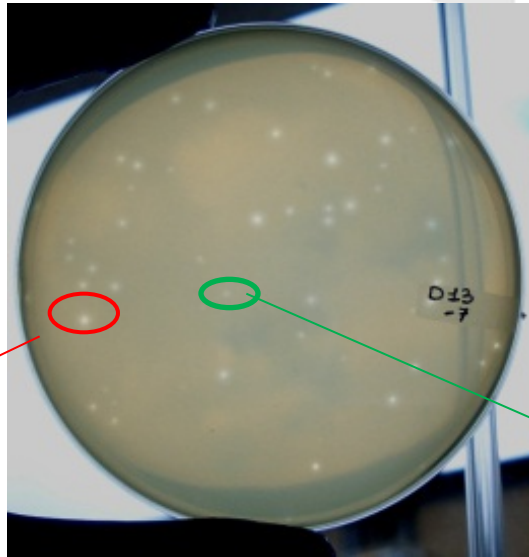
**Table 4.** *L. monocytogenes* strains used for the isolation of  $\phi$ 010120/1 and  $\phi$ 10022/27 and qualitative host range assessment of  $\phi$ IZSAM-1,  $\phi$ 010120/1 and  $\phi$ 10022/27.

	NCTC 1/2b (1LM)	ATCC 1/2c (13M)	NCTC 4c (3LM)	ATCC 4b (4LM)	NCTC 1/2a (11LM)	ATCC 4a (12LM)	11- <i>L. monocytogenes</i> serovar 1/2a	27- <i>L. monocytogenes</i> serovar 4b
$\phi$ IZSAM-1	+	++++	++	+	++++	++	-	-
$\phi$ 010120/1	-	-	++	+++	-	++	-	+
$\phi$ 10022/27	-	-	++	++++	-	++	-	-



Order  
Siphoviridae,  
nm, tail 170 nm,  
m.



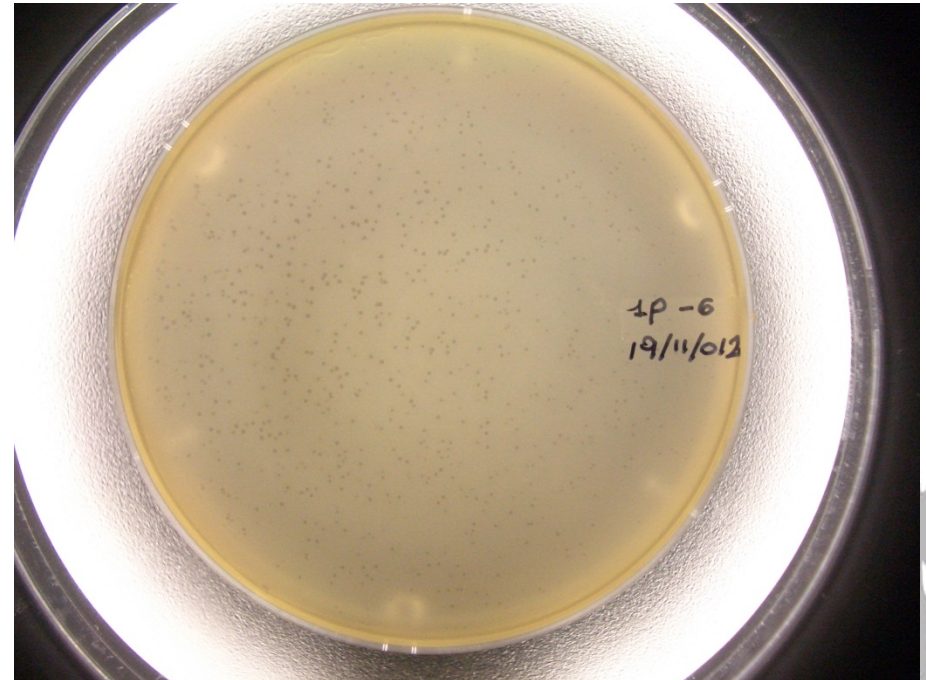
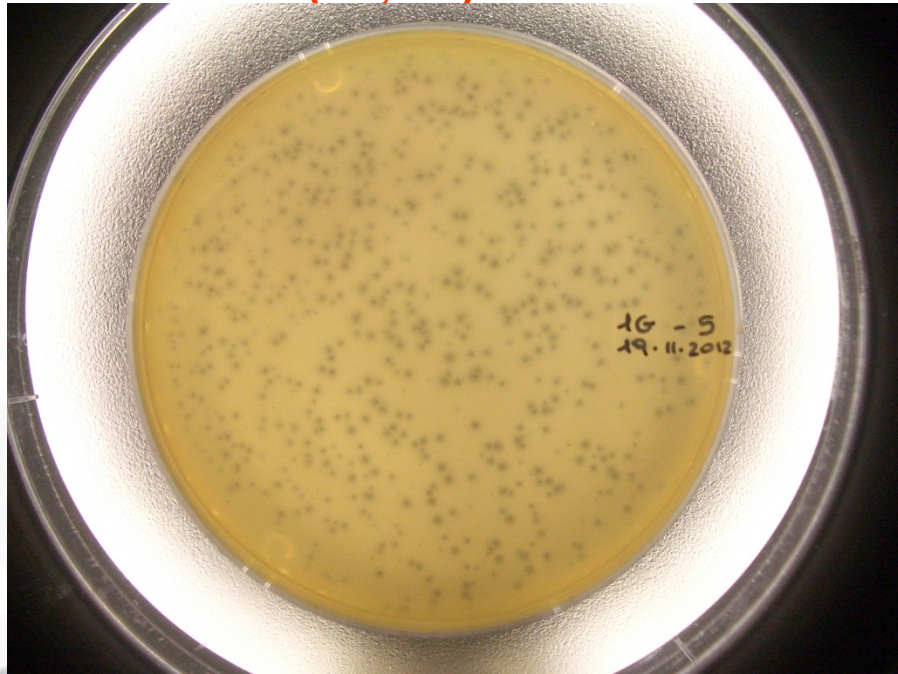


4 plaques x  
3 purification steps



**Φ 2 BIG plaques  
(1G; 2G)**

**Φ 2 SMALL plaques  
(1P; 2P)**

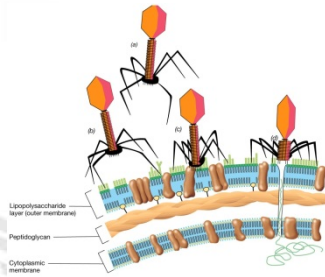
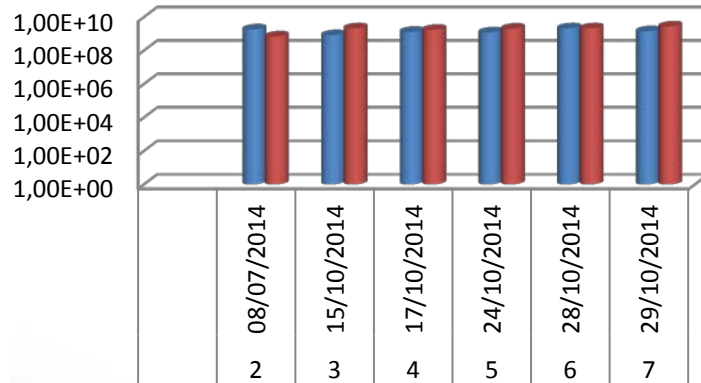


**4 pure lysates for efficacy tests**

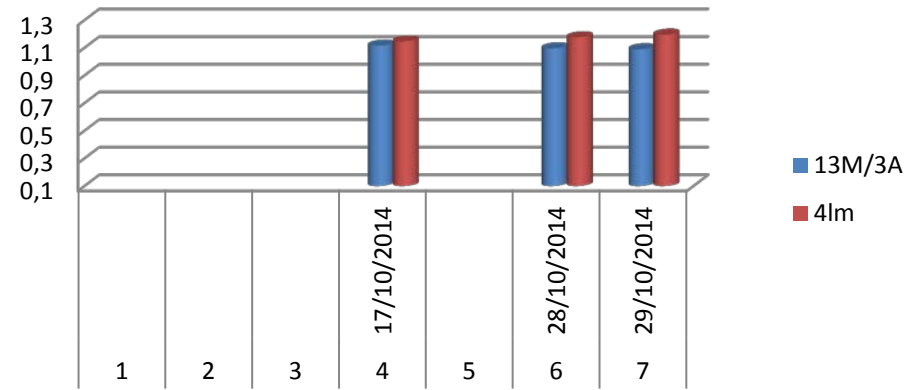


## Host cultures

**B)**  
Titre *L. monocytogenes* o.n.



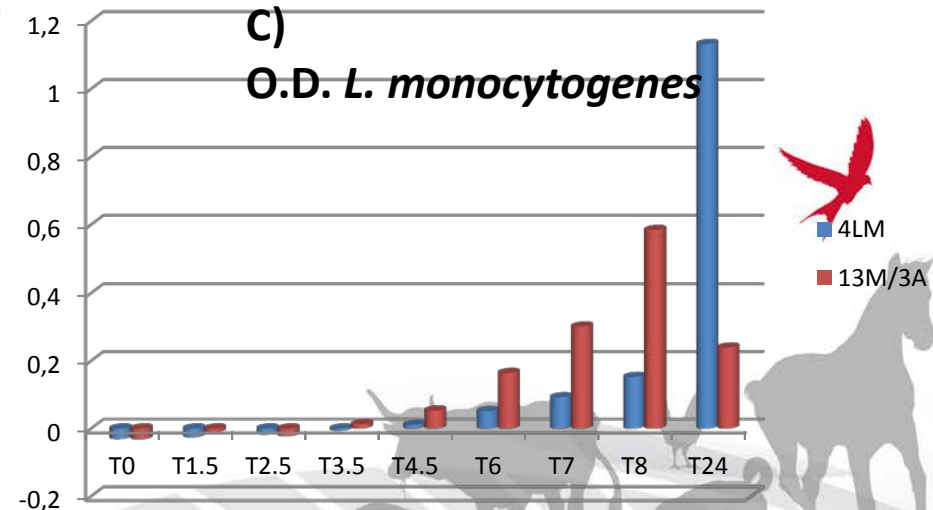
**A)**  
O.D. *L. monocytogenes* o.n.



■ 13M/3A

■ 4Im

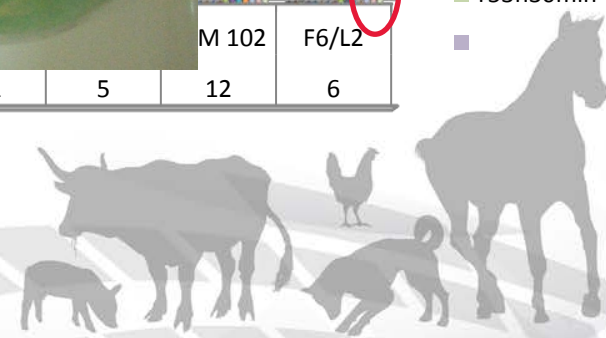
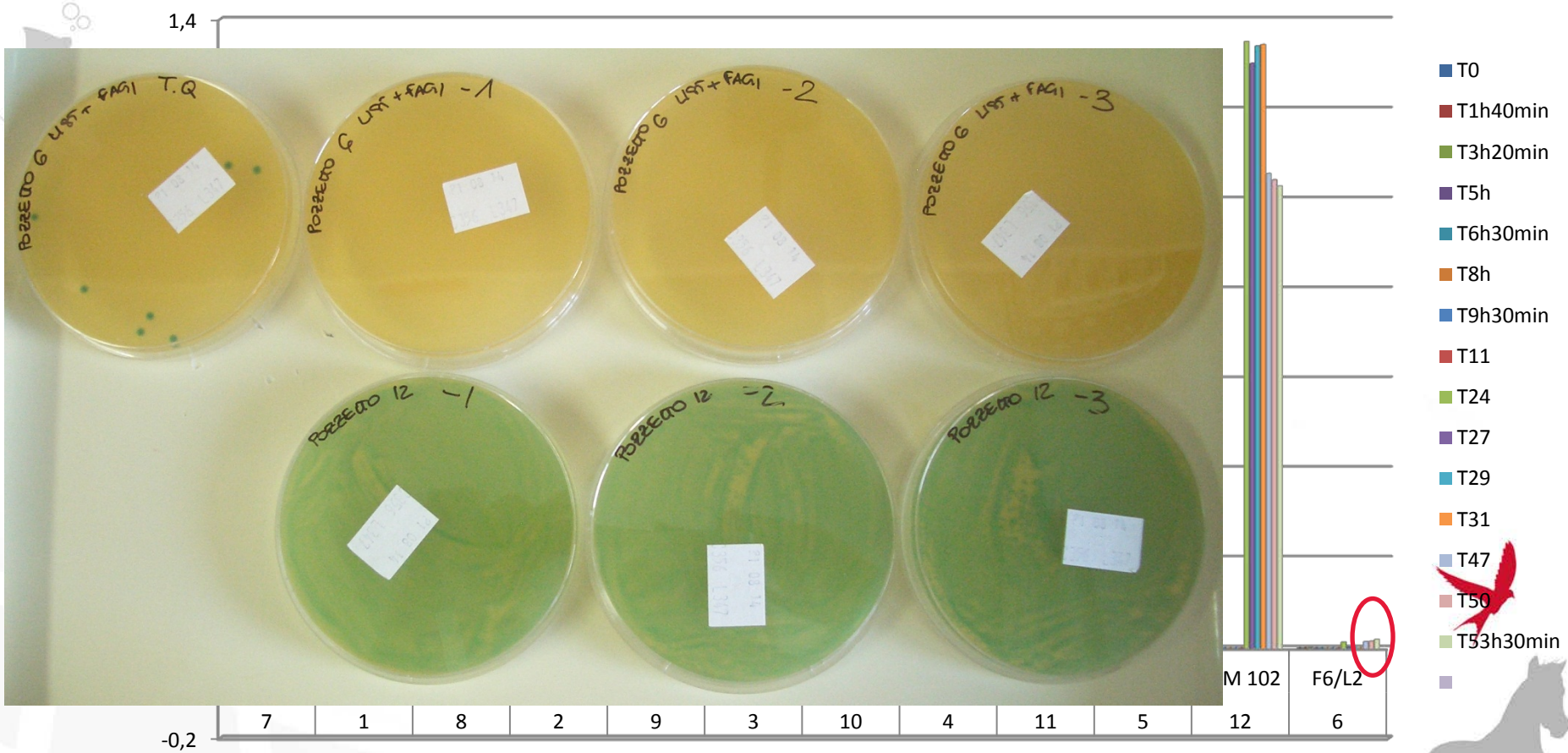
**C)**  
O.D. *L. monocytogenes*



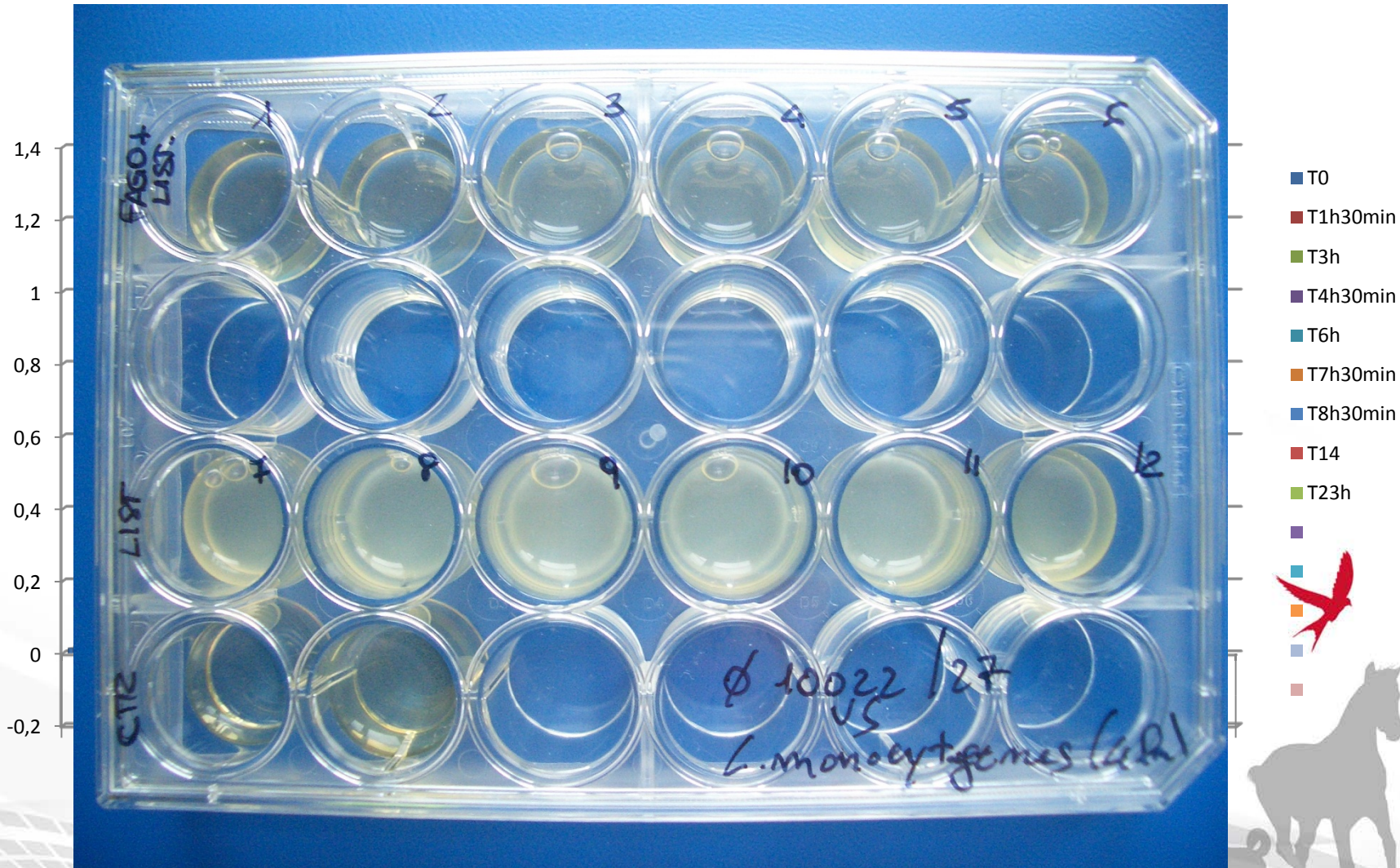
■ 4LM

■ 13M/3A

# O.D. Phage P100 vs *L. monocytogenes* 13M/3A



# O.D. Phage 10022/27 POOL vs *L. monocytogenes* 4LM





PO 22E 110 14

-5

0257 0330  
04 12 14

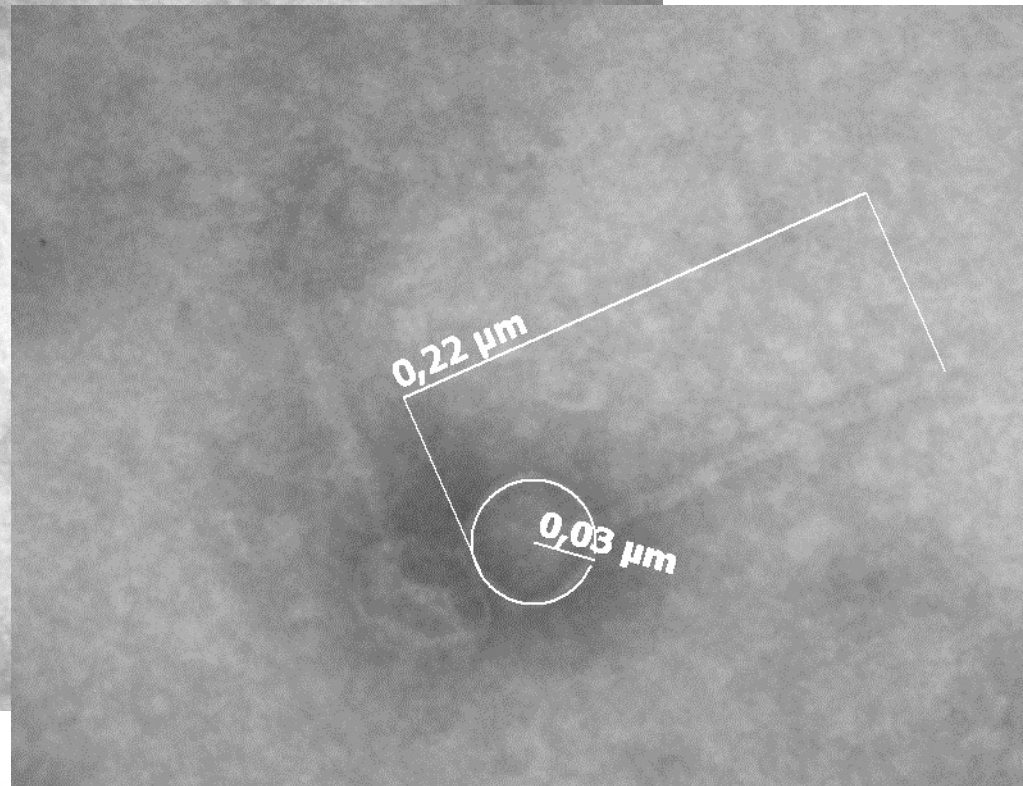
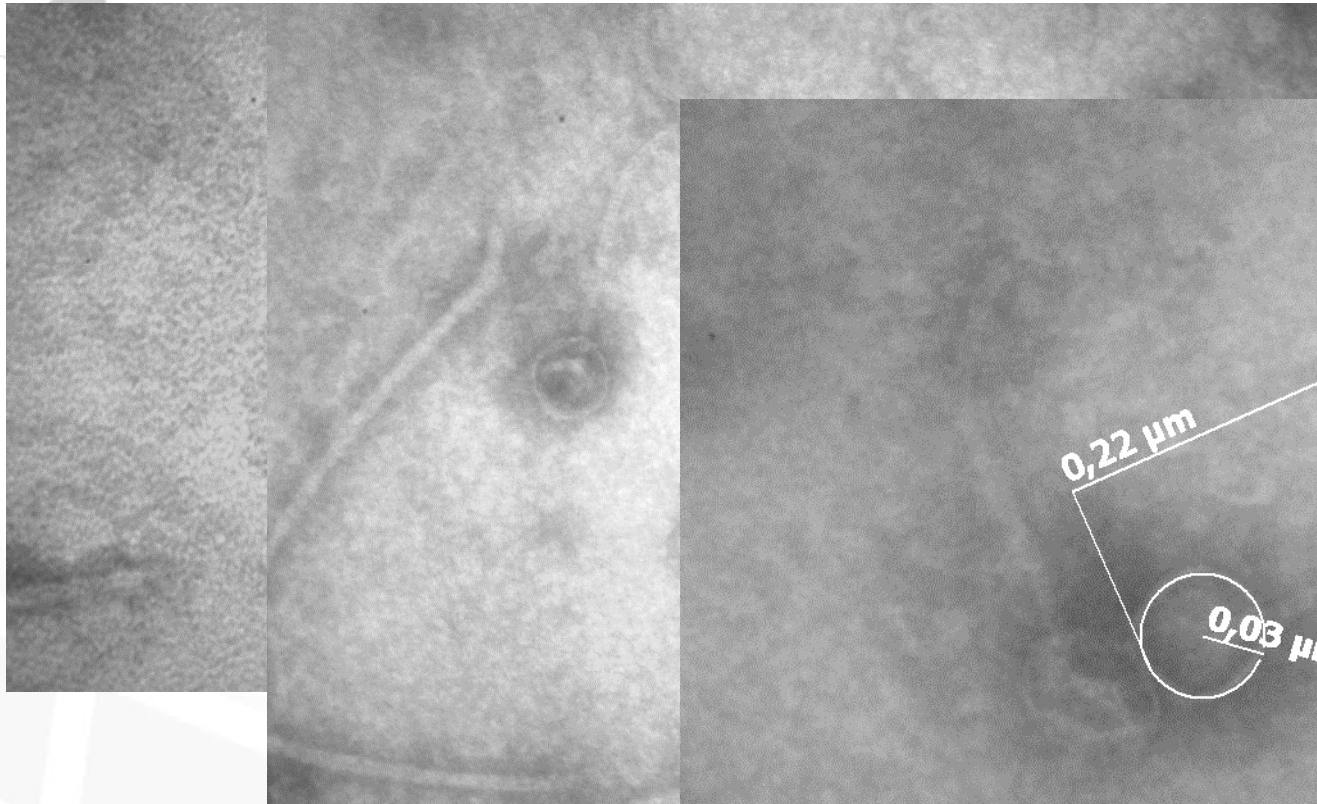
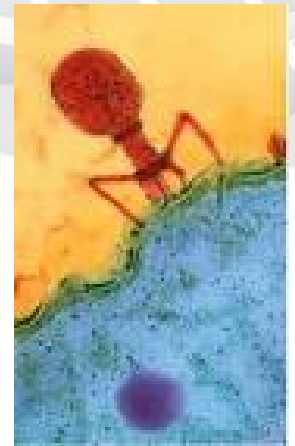
1,000  
1,000  
1,000  
1,000  
1,000

1 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 11 | 13 | 14 | 15 | 16 | :

6 | 1 | 2 | 3 | 4 | 5 | 12 | 7 | 8 | 9 | 10 | 11 | 18 | 13 | 14 | 15 | 16 | 17

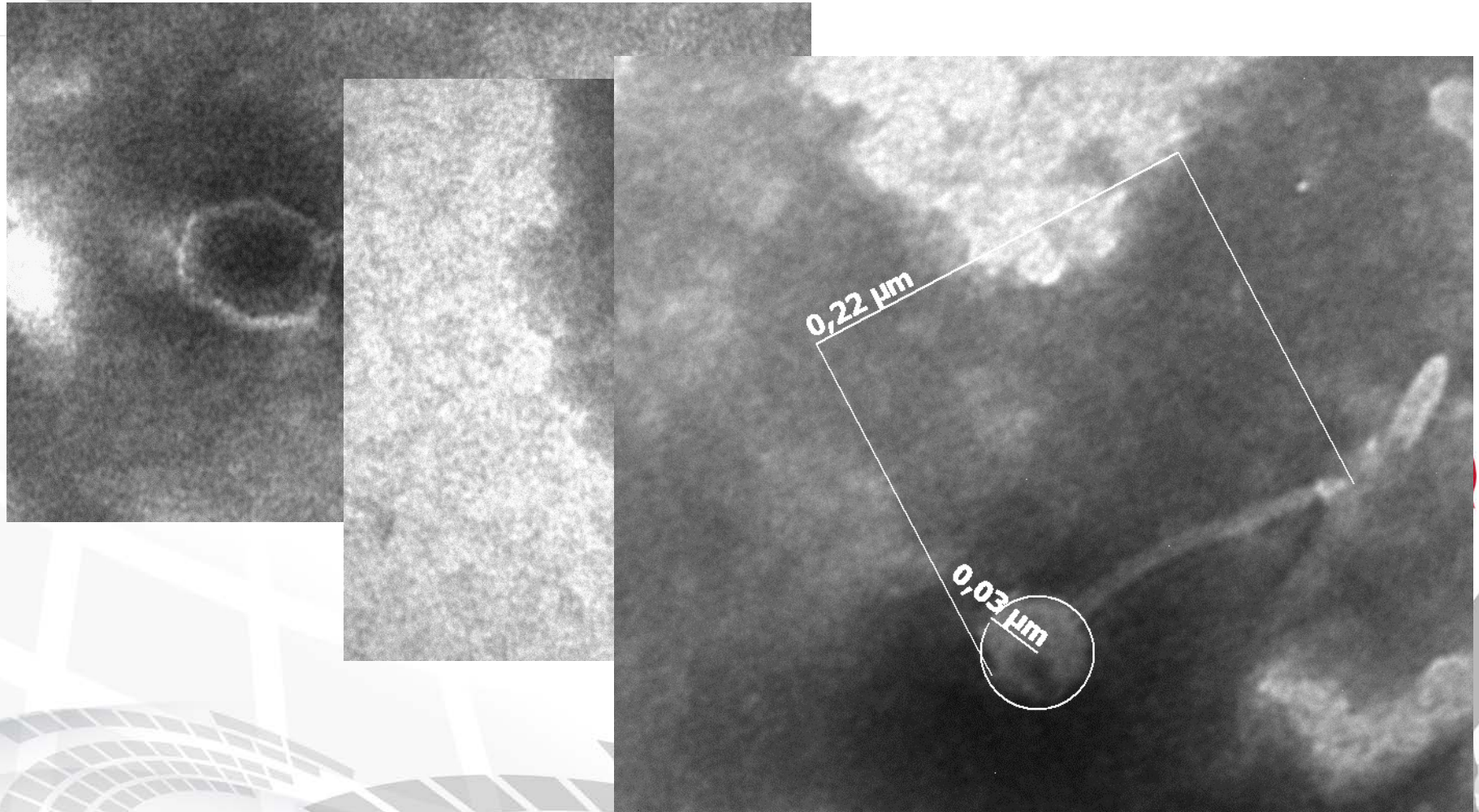
# TEM PHAGE 10022/27 PURE LYSATES

## 1G



# TEM PHAGE 10022/27 PURE LYSATES

1P



# Ordine **Caudovirales**, Famiglia **Siphoviridae**

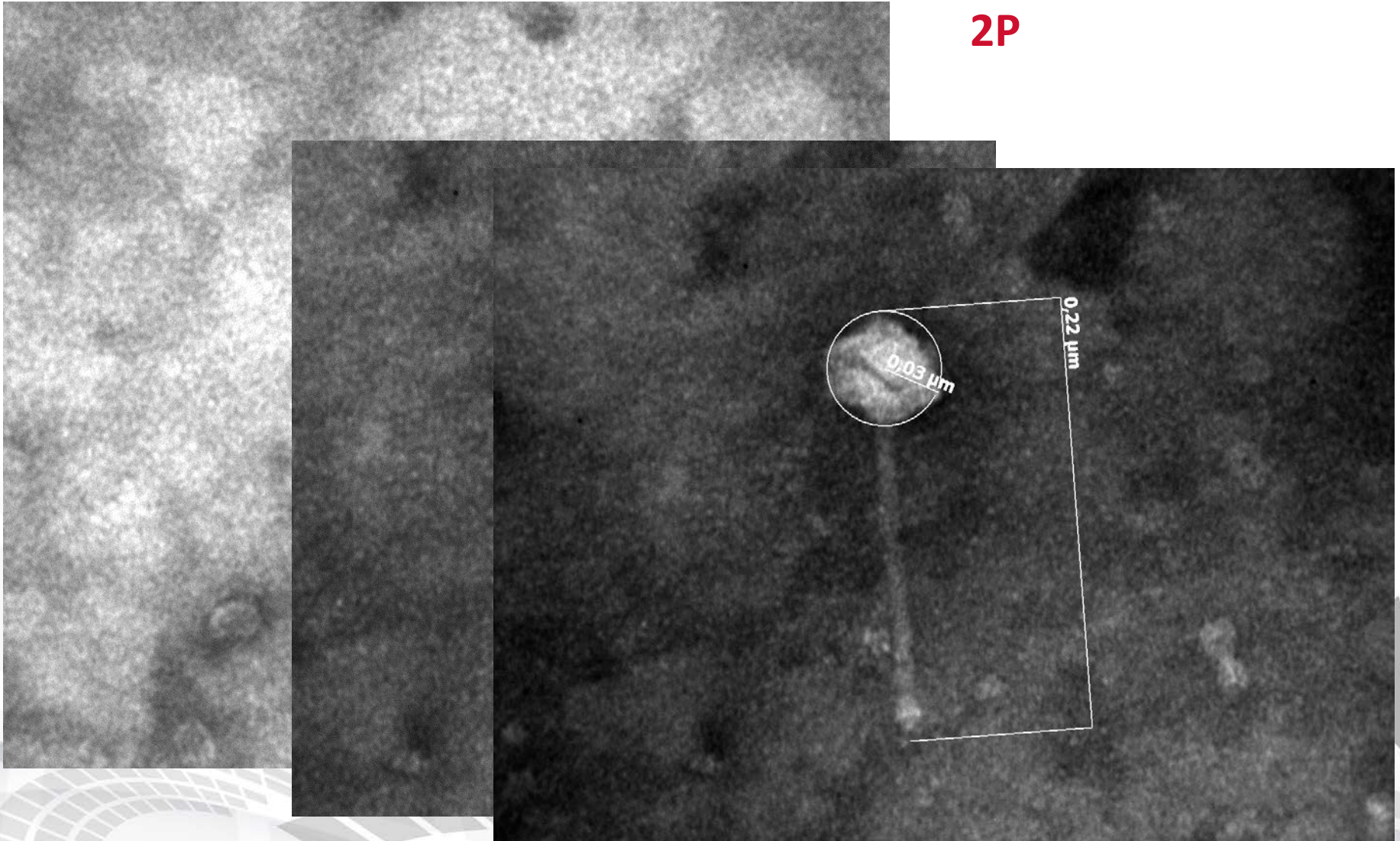


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## TEM

## PHAGE 10022/27 PURE LYSATES

### 2P





## Future activities:

- Evaluation of the nature of «resistent hosts»
- Evaluation of phage efficacy at different MOIs and combining the pure lysates
- Restriction enzymes profiles + sequencing
- Efficacy on food/surfaces





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## Special thanks to:

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