Bacteriophages

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Dairy Innovation Australia

Webinar for NCDEA, April 2014

To begin at the beginning….

What is a bacteriophage?

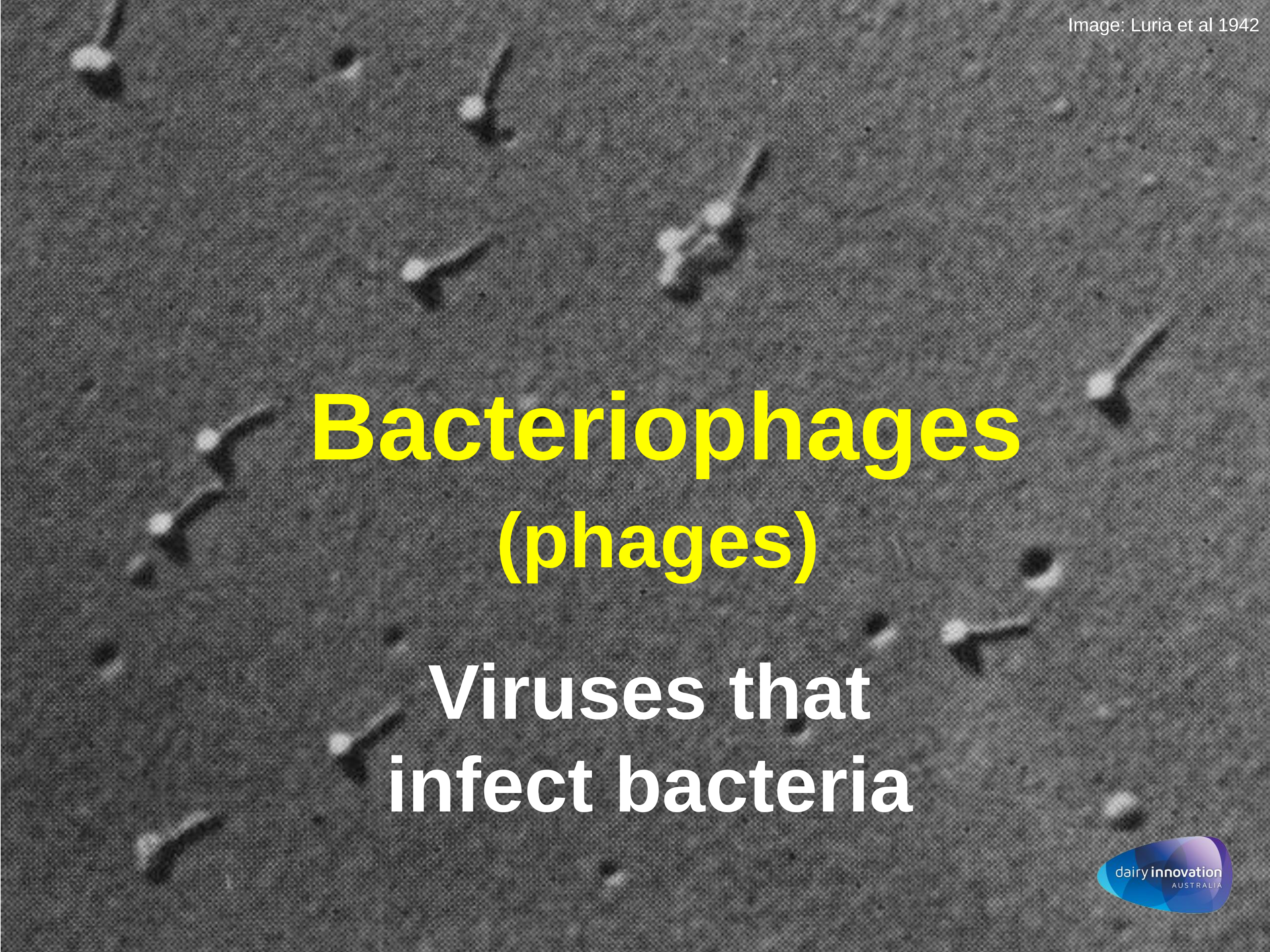
Image: Luria et al 1942
Bacteriophages
(phages)
Viruses that
infect bacteria
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Image: Luria et al 1942

“The Twort-d’Herelle Phenomenon”

Frederick Twort, 1915. An investigation on the nature of ultra-microscopic viruses. Lancet 2:1241-1243.

Félix d'Herelle, 1917. Sur un microbe invisible antagonistic des bacilles dysenterique. C. R. Acad. Sci. Paris 165:373-375.

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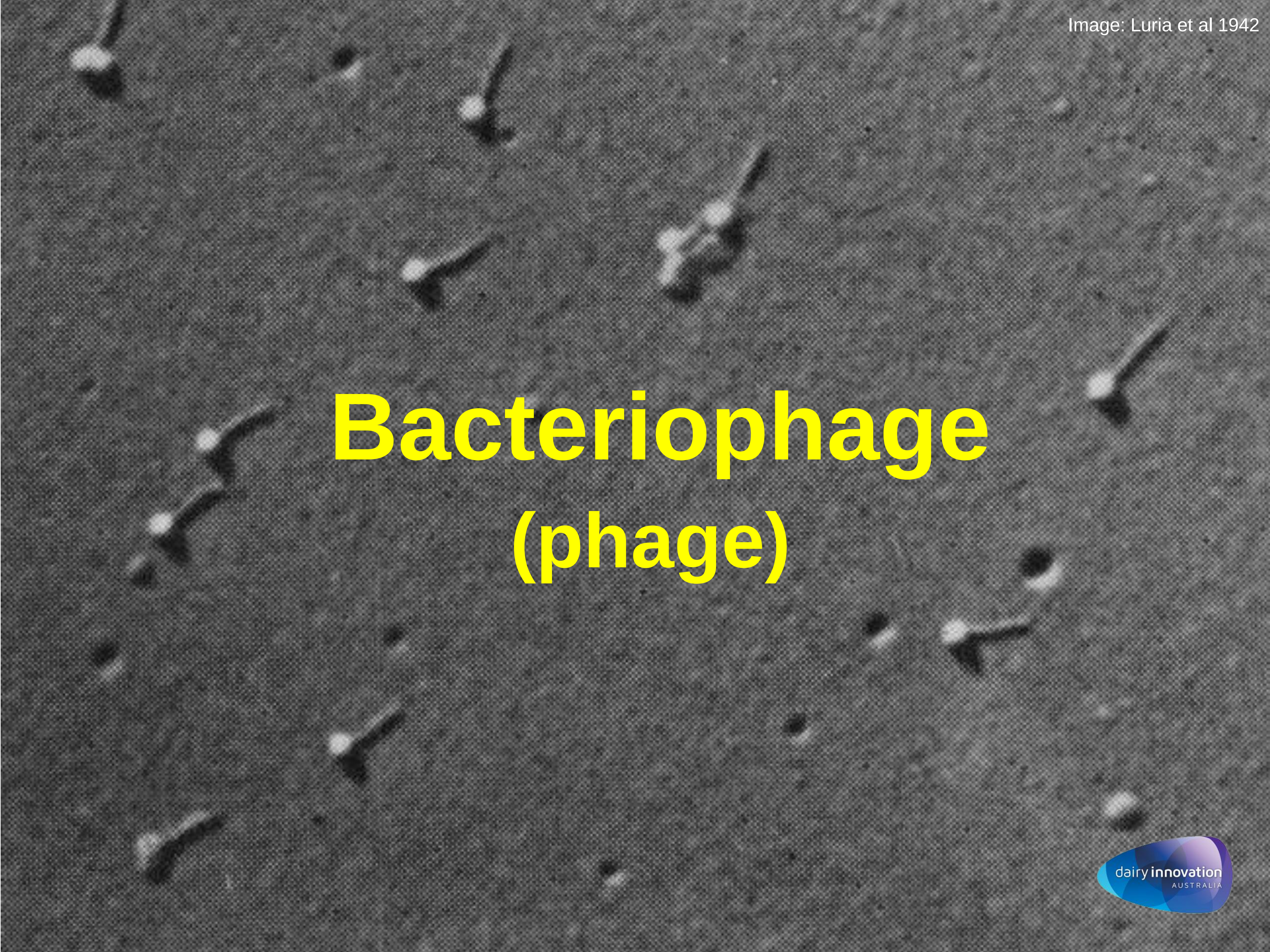

Image: Luria et al 1942

Bacteriophage

(phage)

The basic facts …..

Fact 1:
Phages are very small

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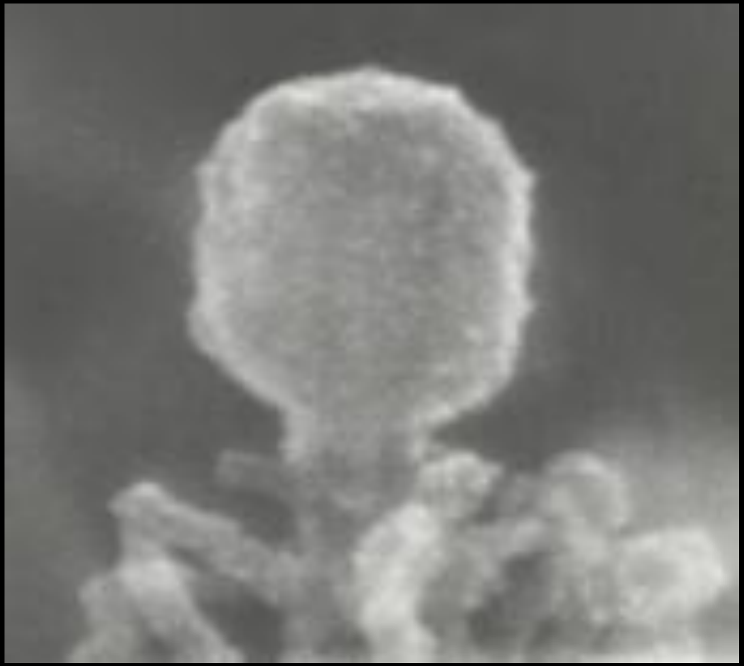
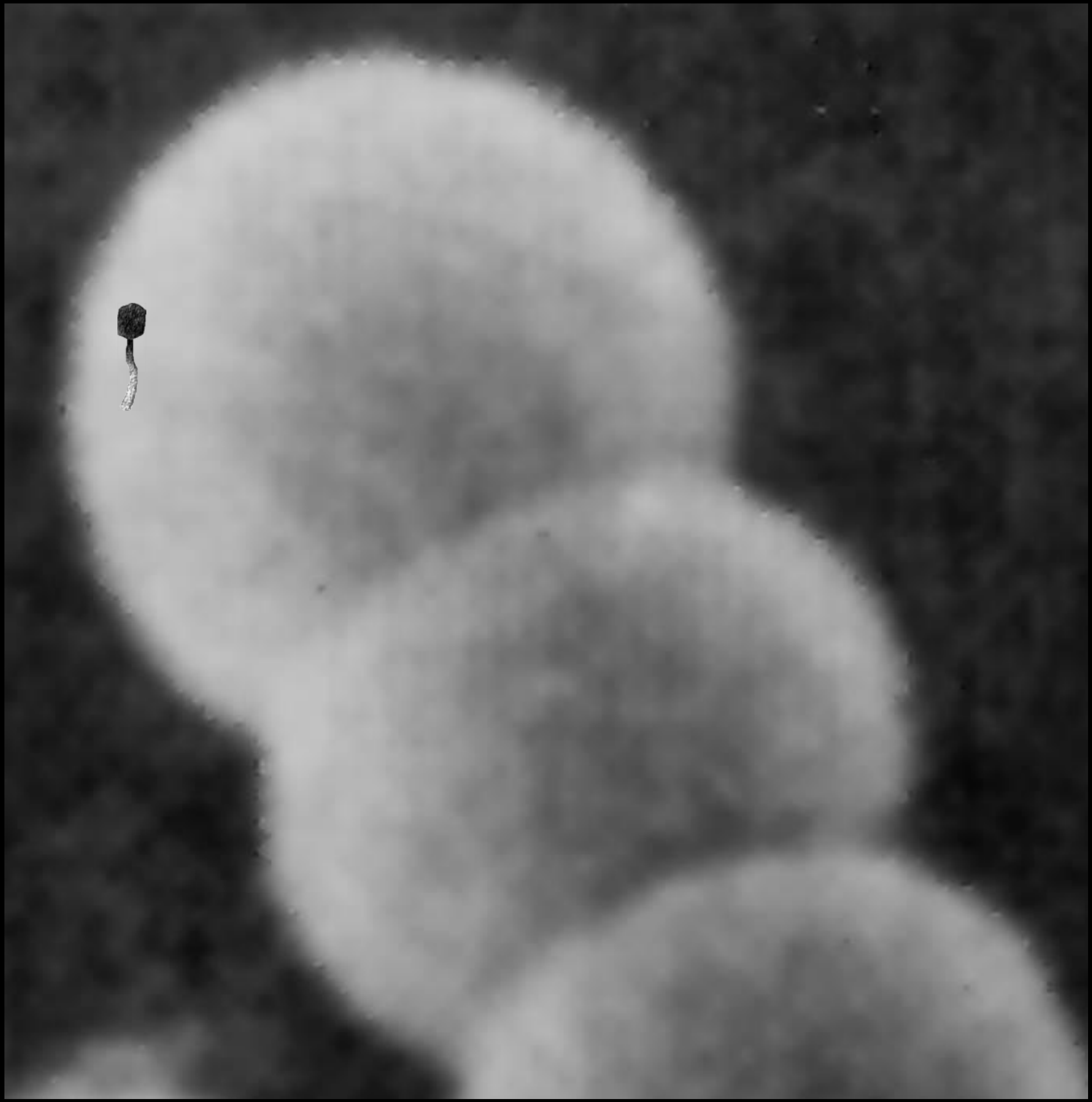
Phages are very small

Phages are very small



Tanaka 1984 – Coliphage T2, magnified approximately 1 million times

Phages are very small



Lactococcus: Bottazzi and Bianchi, 1984

Fact 2:

Phages are relatively simple, structurally stable biological entities

A typical phage

Head

Tail

Structural material: protein

Genome: packed inside the head

Tail: hollow tube

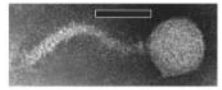
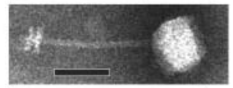
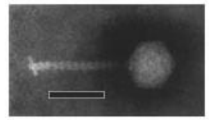
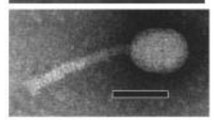
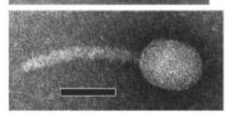
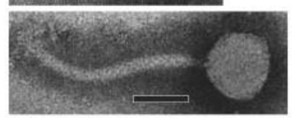
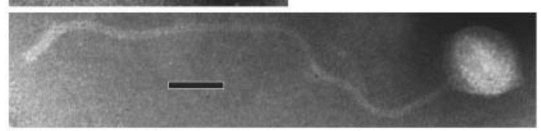
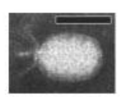
Tail tip: ‘binding proteins’

Fact 3:
Phages are very diverse

Phage diversity

Example: phages infecting *Lactococcus lactis*

- Genetically distinct types (species)
- Many variants within each species

Family / Species	Phage example	Capsid diameter (nm)	Tail width (nm)	Tail length (nm)	Electron micrograph[a]
Siphoviridae					
936	bIL170	50	11	126	
P335	ul36	49	7	104	
1358	1358	45	10	93	
c2	c2	54 X 41	10	95	
Q54	Q54	56 X 43	11	109	
P087	P087	59	14	163	
949	949	70	12	490	
1706	1706	58	11	276	
Podoviridae					
P034	P369	57 x 40	5	19	
KSY1	KSY1	223 X 45	6	32	

[a] *Bar = 50 nm*

Deveau *et al.* (2006)

Fact 4:
Phages are everywhere

It is now widely accepted that phages ... represent **the most abundant biological entities on the planet**, and total phage abundance in the biosphere has been estimated at 10^{30} or more.

Mann, 2005

Fact 5:

Phages are parasites, relying on infection of bacteria to multiply

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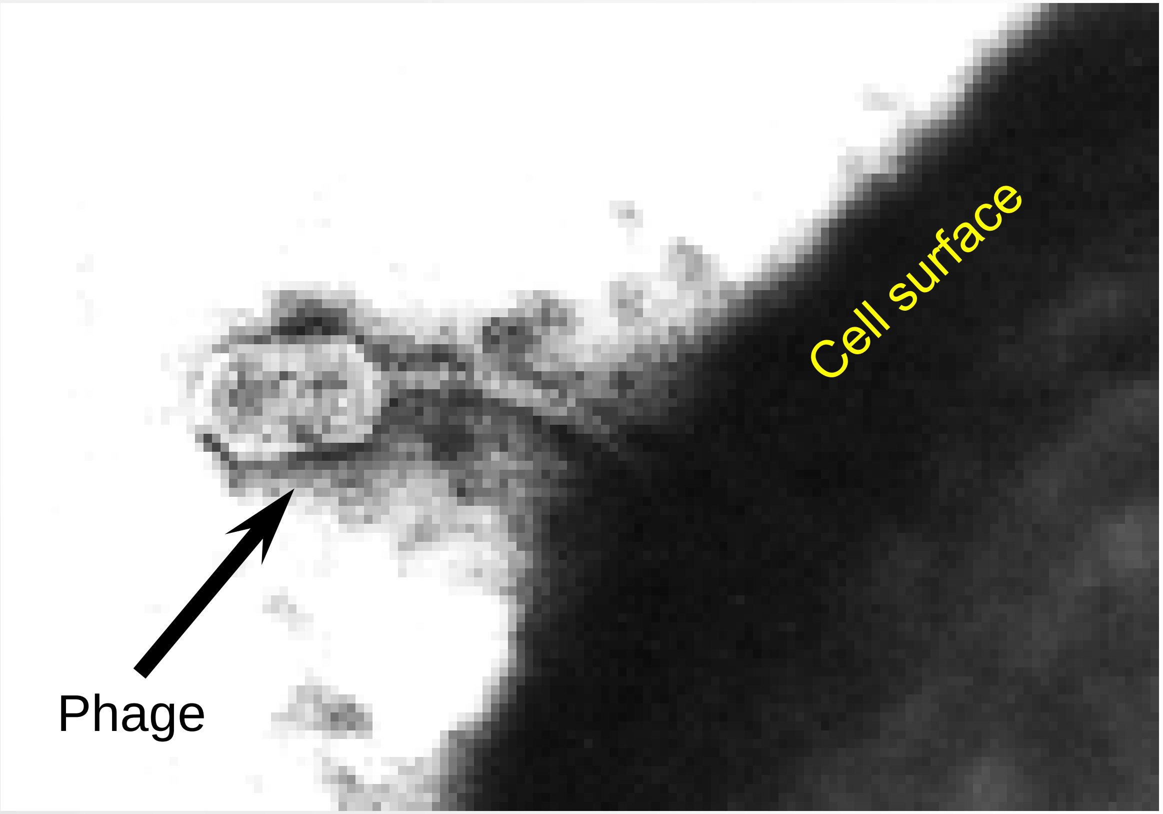
Phage Infection

The lytic phage life cycle

Phage life cycle, step 1: Phage adsorption to a bacterial cell

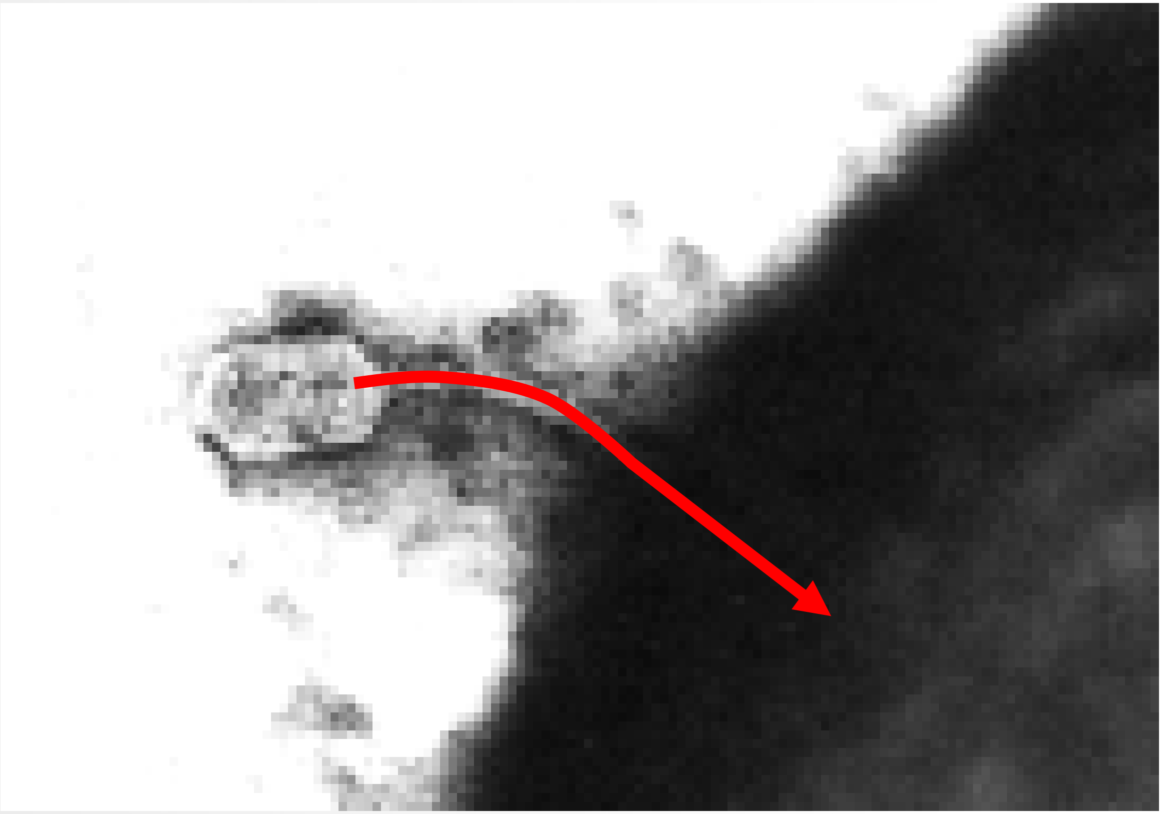


Parada *et al.*

Hamilton *et al.*

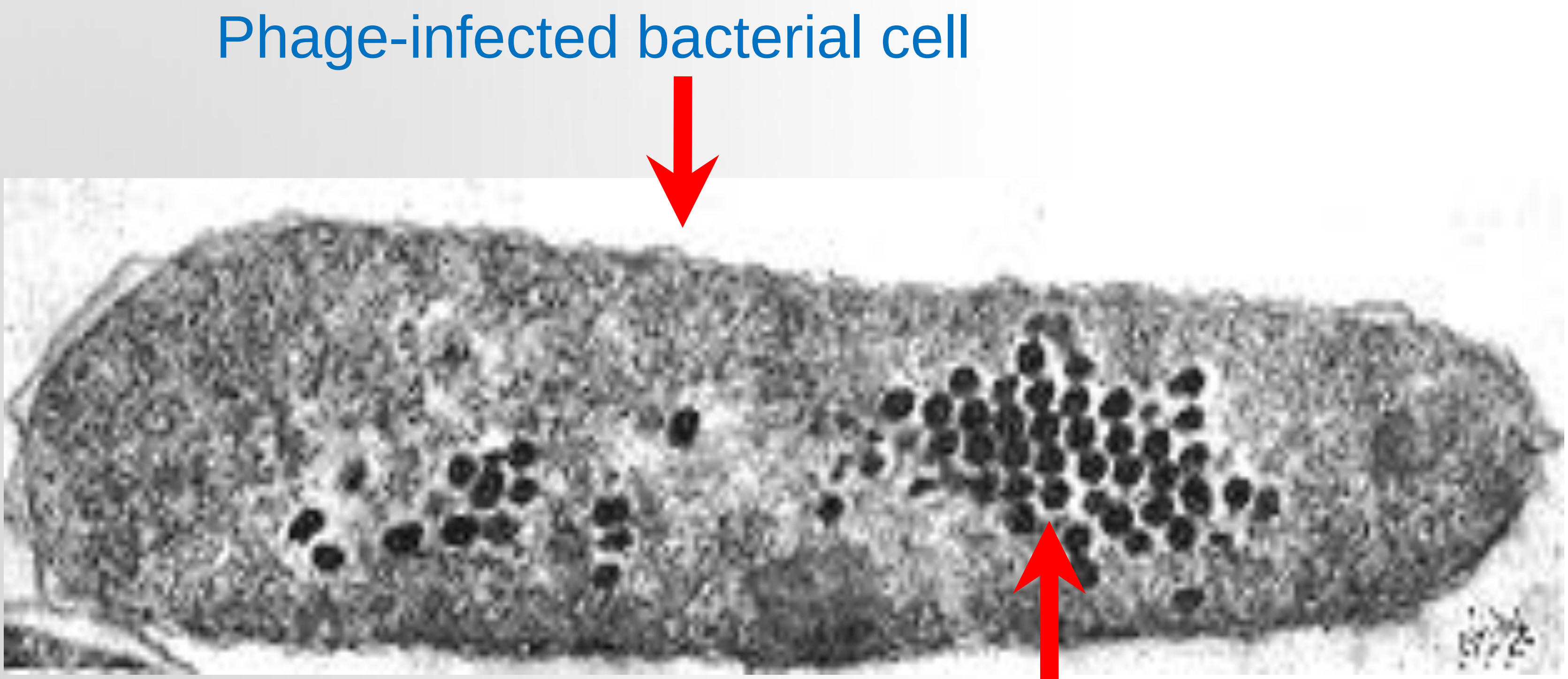
Phage life cycle, step 2: DNA injection

- Phage DNA enters cell
- Phage genes become active

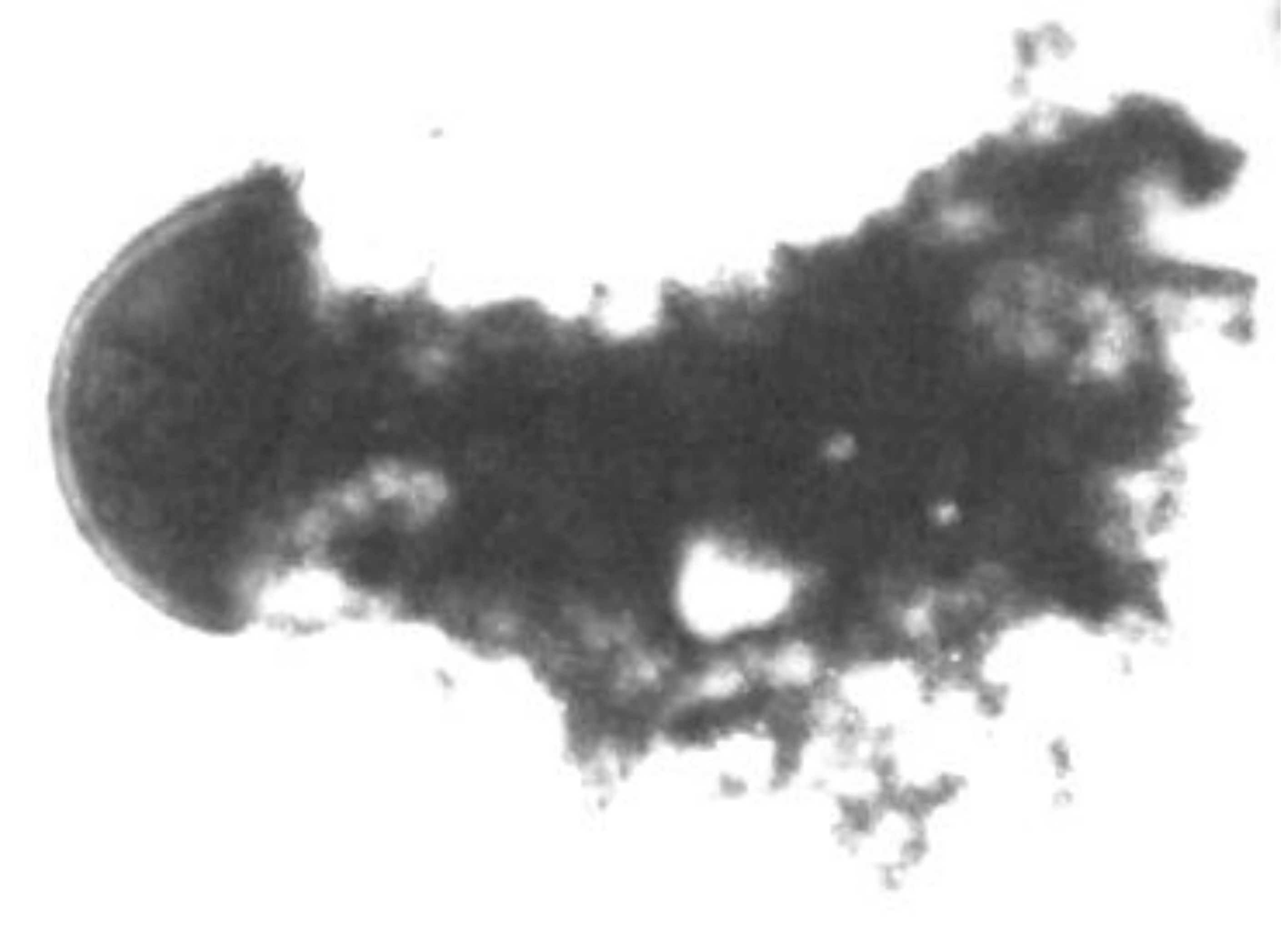


Parada *et al.*

Phage life cycle, step 3: Making phage components and assembling phage particles



Phage life cycle, step 4: Cell lysis



Neve, 1996

Phage life cycle - summary

Adsorption

DNA injection

Shut-down of bacterial functions

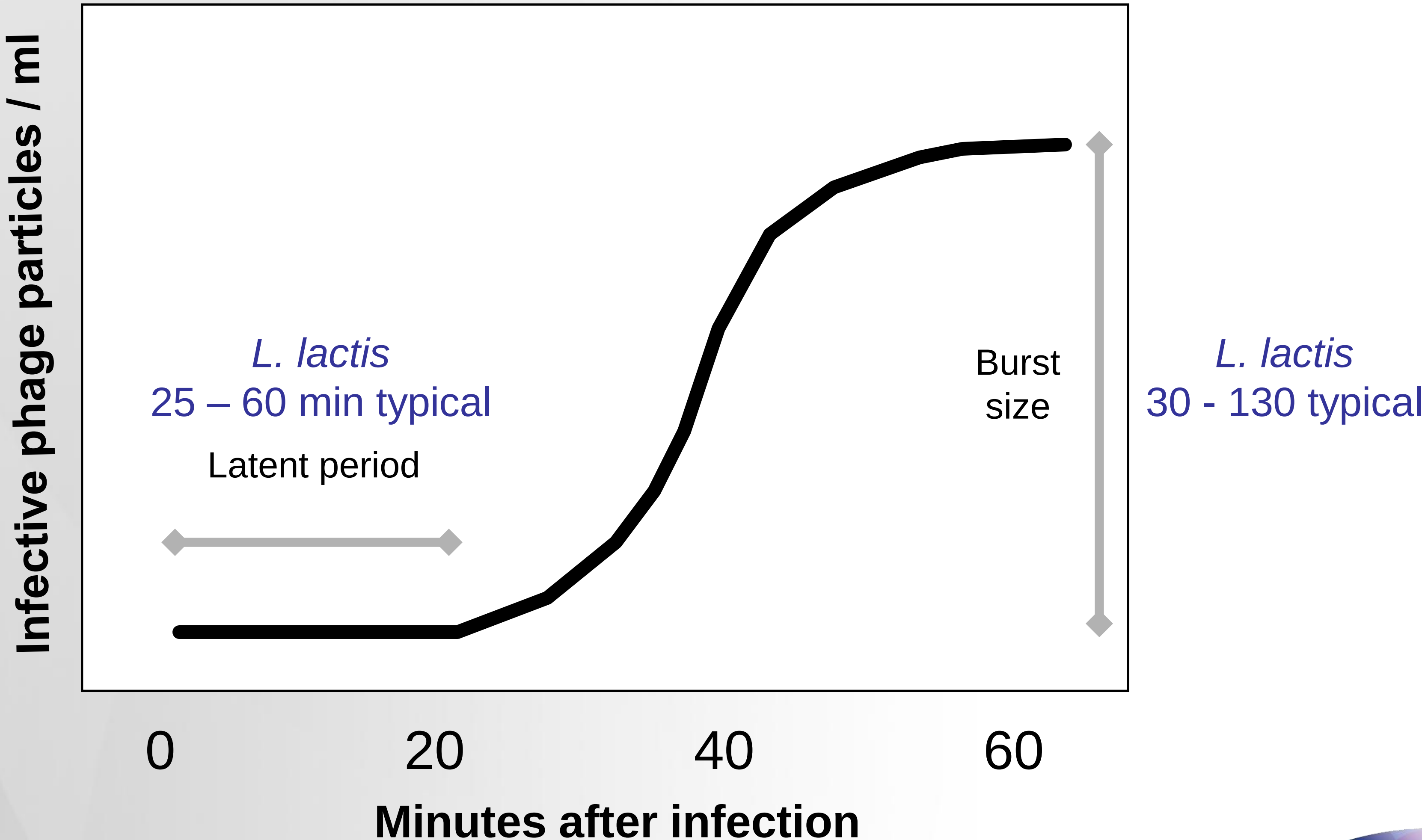
Synthesis of phage components

Assembly of phage particles

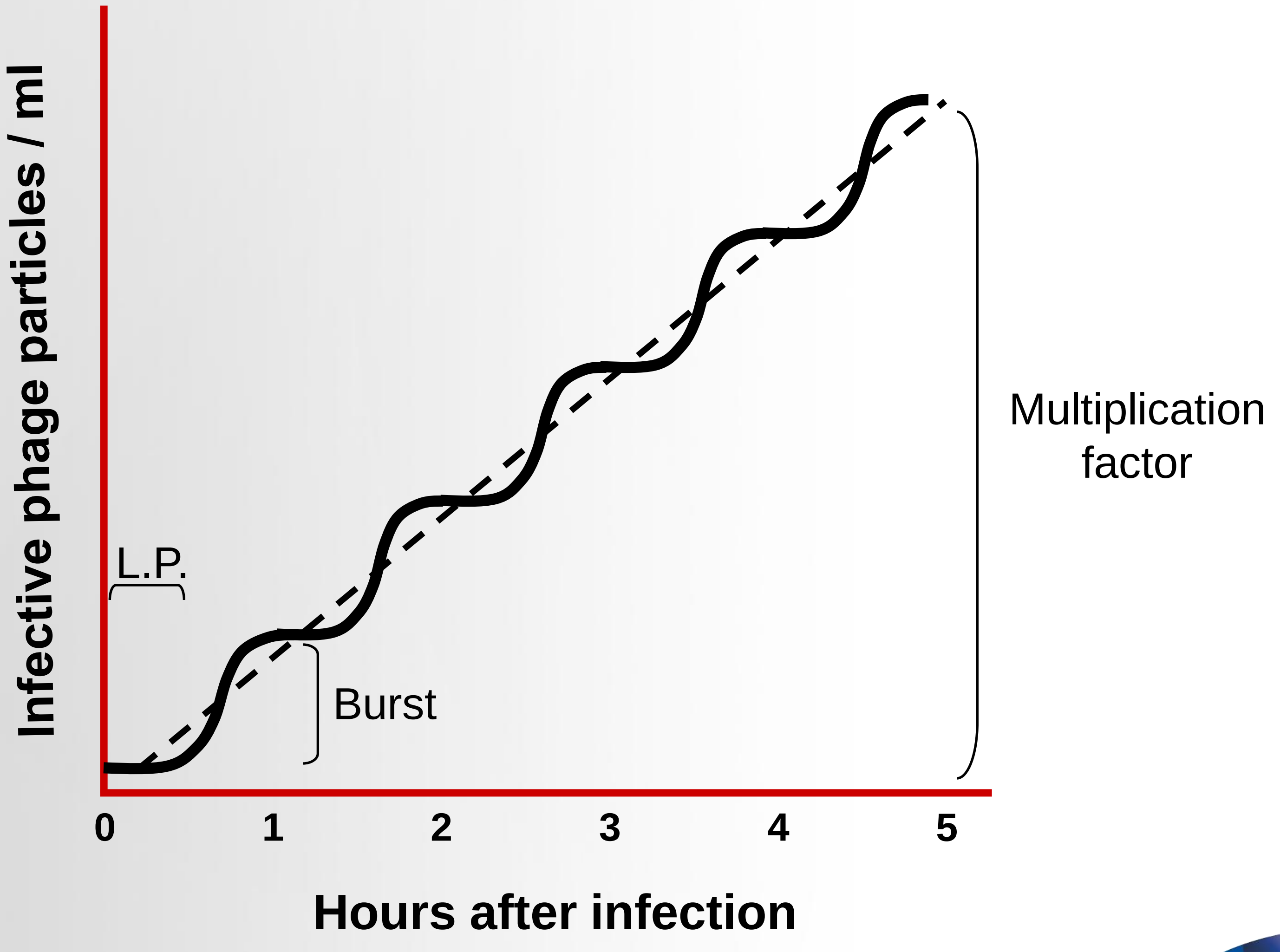
Breakdown of cell wall

Release of infective phage particles

Phage replication



Phage replication potential



Phages multiply rapidly

...but some multiply faster than others

- some phages do not multiply rapidly
- some phages multiply very rapidly
- also depends on host strain, temperature, *etc*

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Bacteriophage infection in cheese and yoghurt manufacture

Phages, cultures
and phage control strategies

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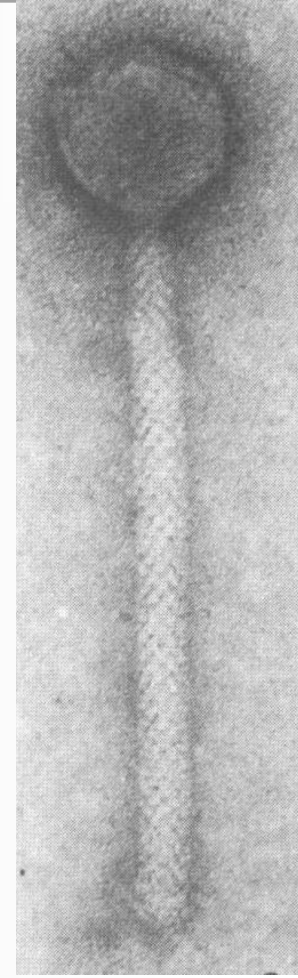
Lactococcus lactis
phage '936' species

50 nm

Lactococcus lactis
phage 'c2' species

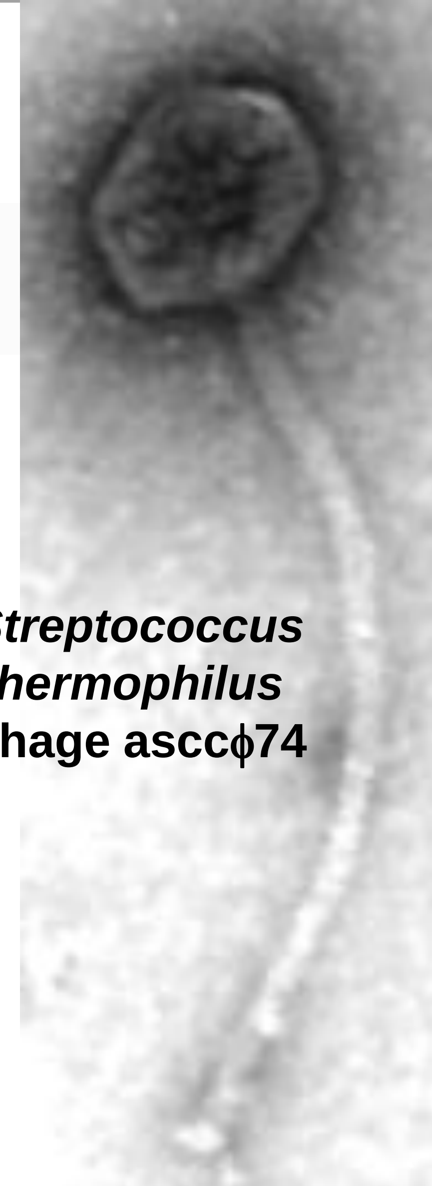
L. lactis
phage
asccϕ28

Kotsonis et al (2008)



Lactobacillus helveticus
phage hv

Séchaud et al (1992)



Streptococcus thermophilus
phage asccϕ74

Other phages: Powell et al, unpublished

Practical issues:
Phages and starter cultures

What are the consequences of phage infection?

Where do phages come from?

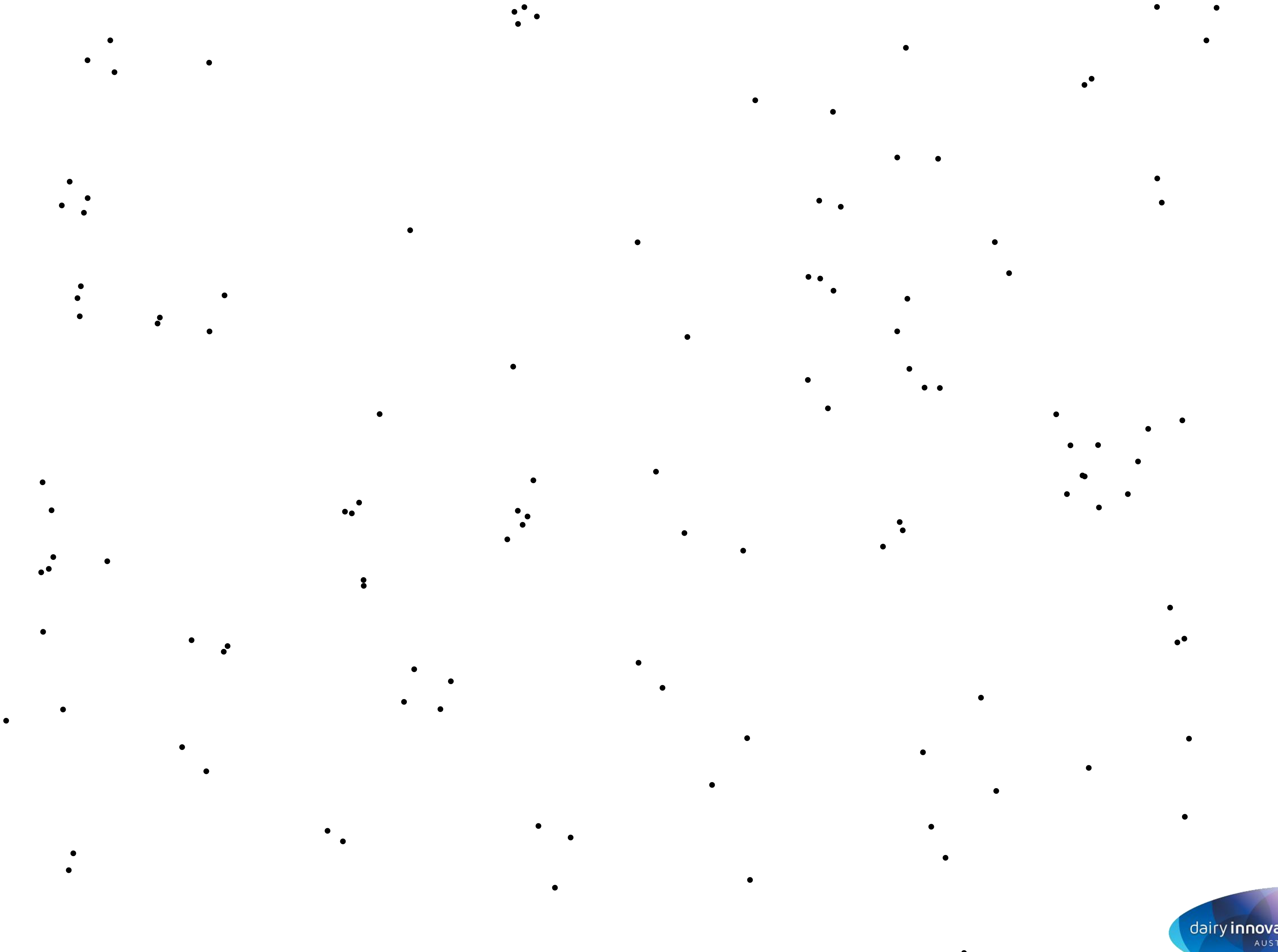
How do you manage/eliminate them?

The consequences of phage infection

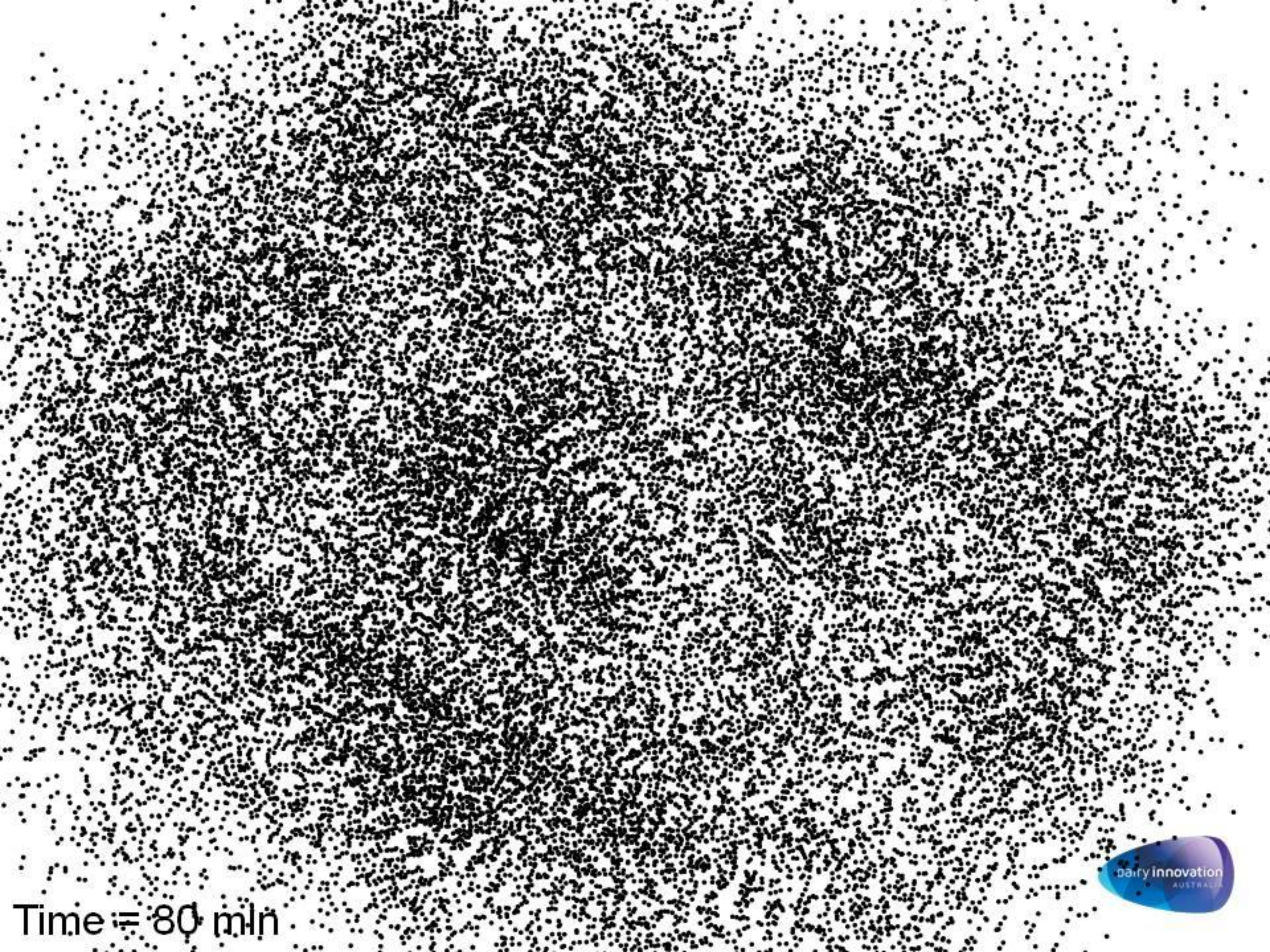
One phage particle in a vat of milk, with starter culture

Time = 0 min

Time = 0 min

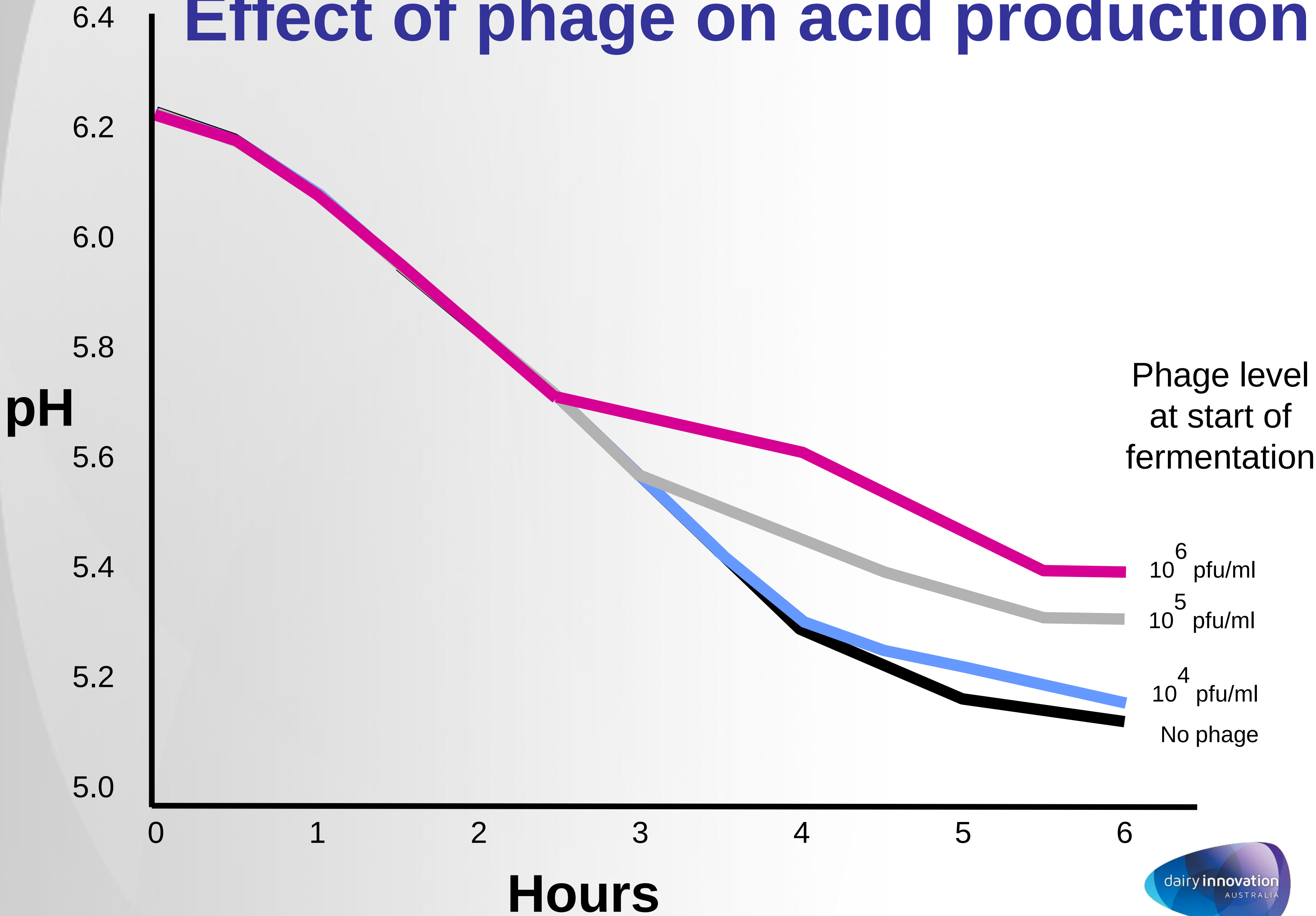


Time = 40 min


Time = 80 min

Time = 120 min

Effect of phage on acid production



Consequences of phage infection

Lysis of starter bacteria

- Lower starter numbers, altered strain balance

Reduced rate of lactic acid production

- Extended production time
- Disrupted production schedule, lower throughput

Failure to meet product specifications

- pH, residual lactose, moisture content

Effects on cheese maturation

- Early lysis of starter?
- Starter metabolism reduced or altered?
- Increased growth and altered composition of adventitious microflora?
- Altered flavour?

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What is the worst potential effect of phage?

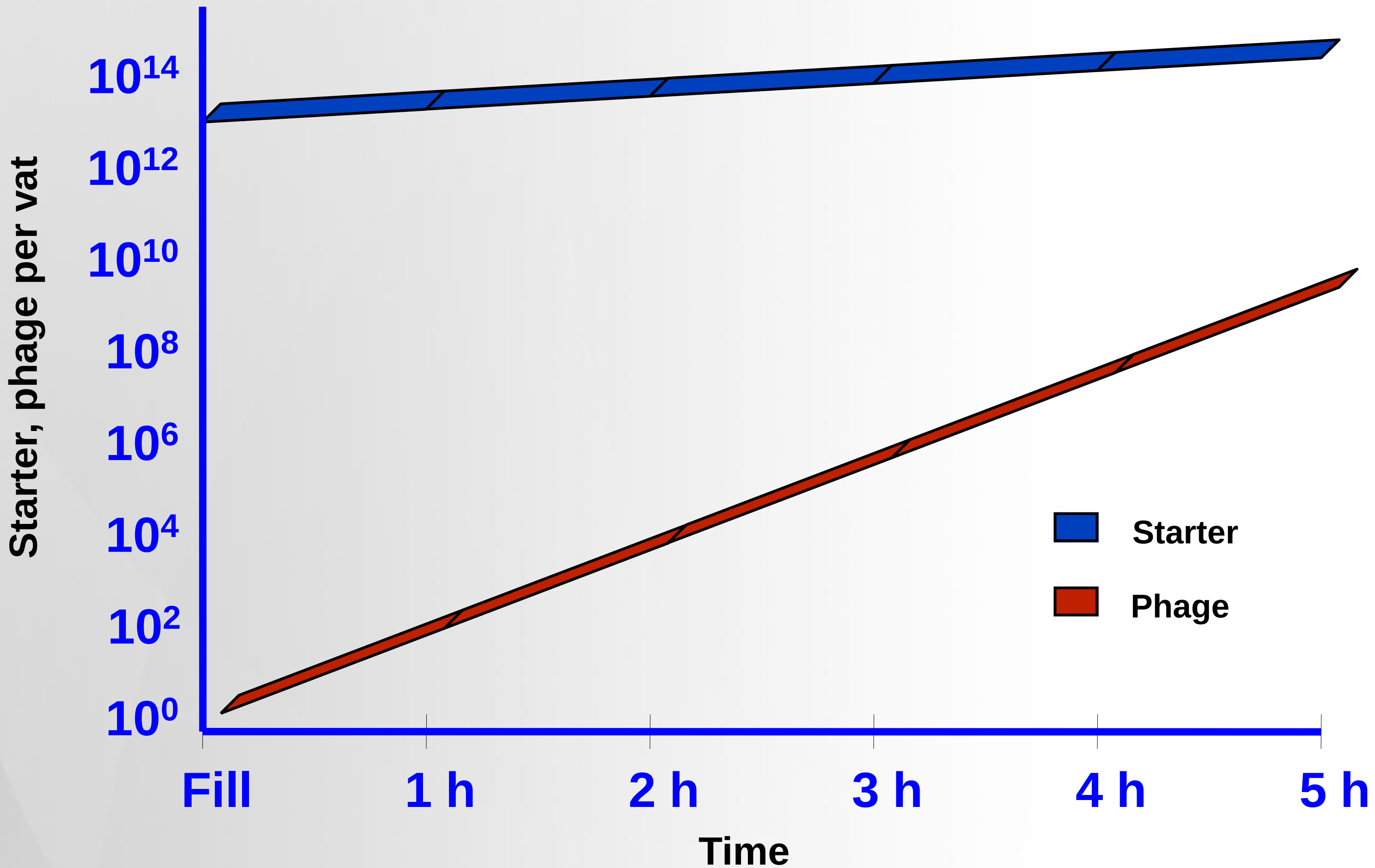
Example

A hypothetical single-strain starter

A 10,000 litre cheese vat

Start with 1 phage particle in the first fill ……..

Start with one phage particle in the vat …



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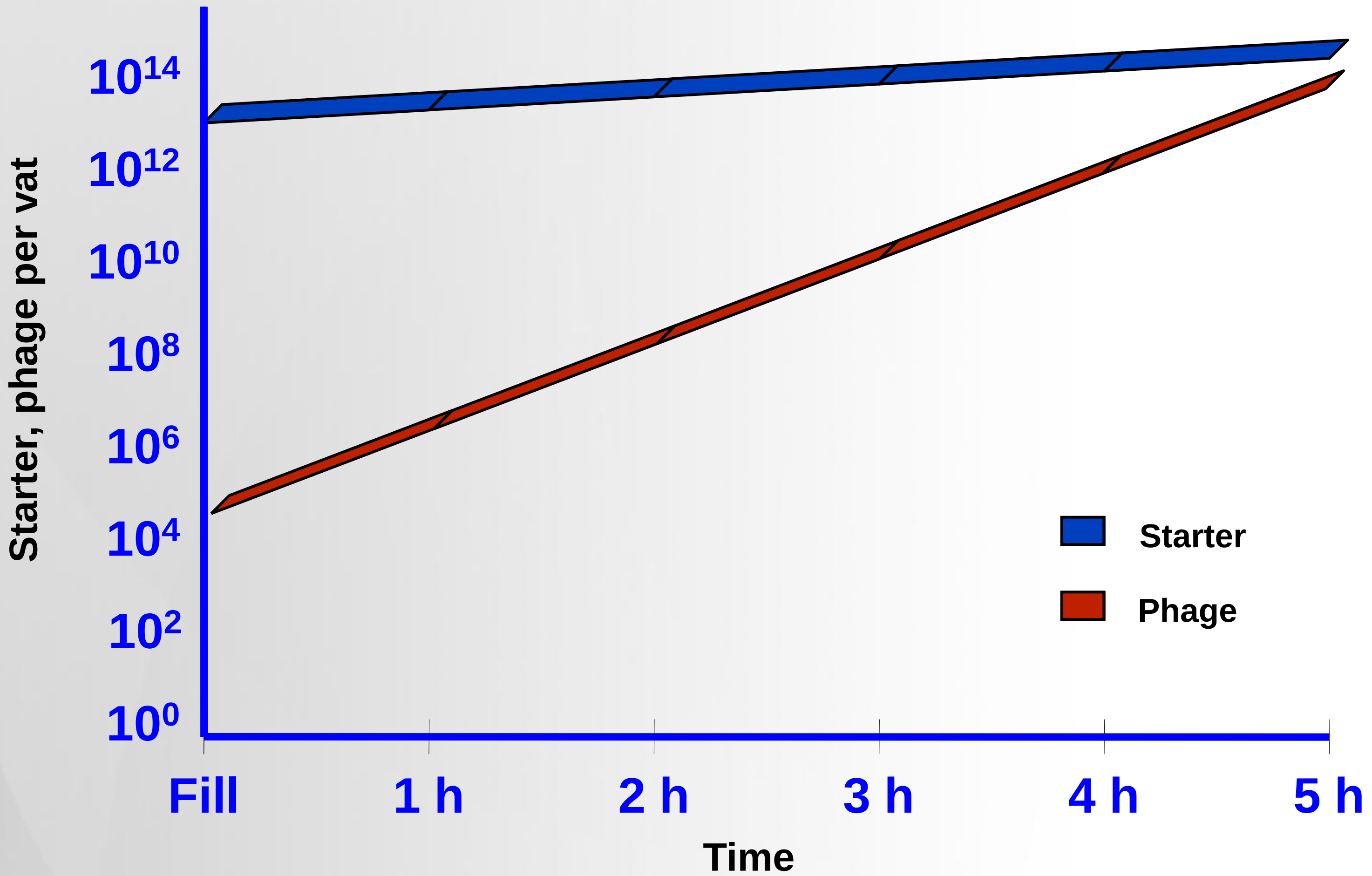
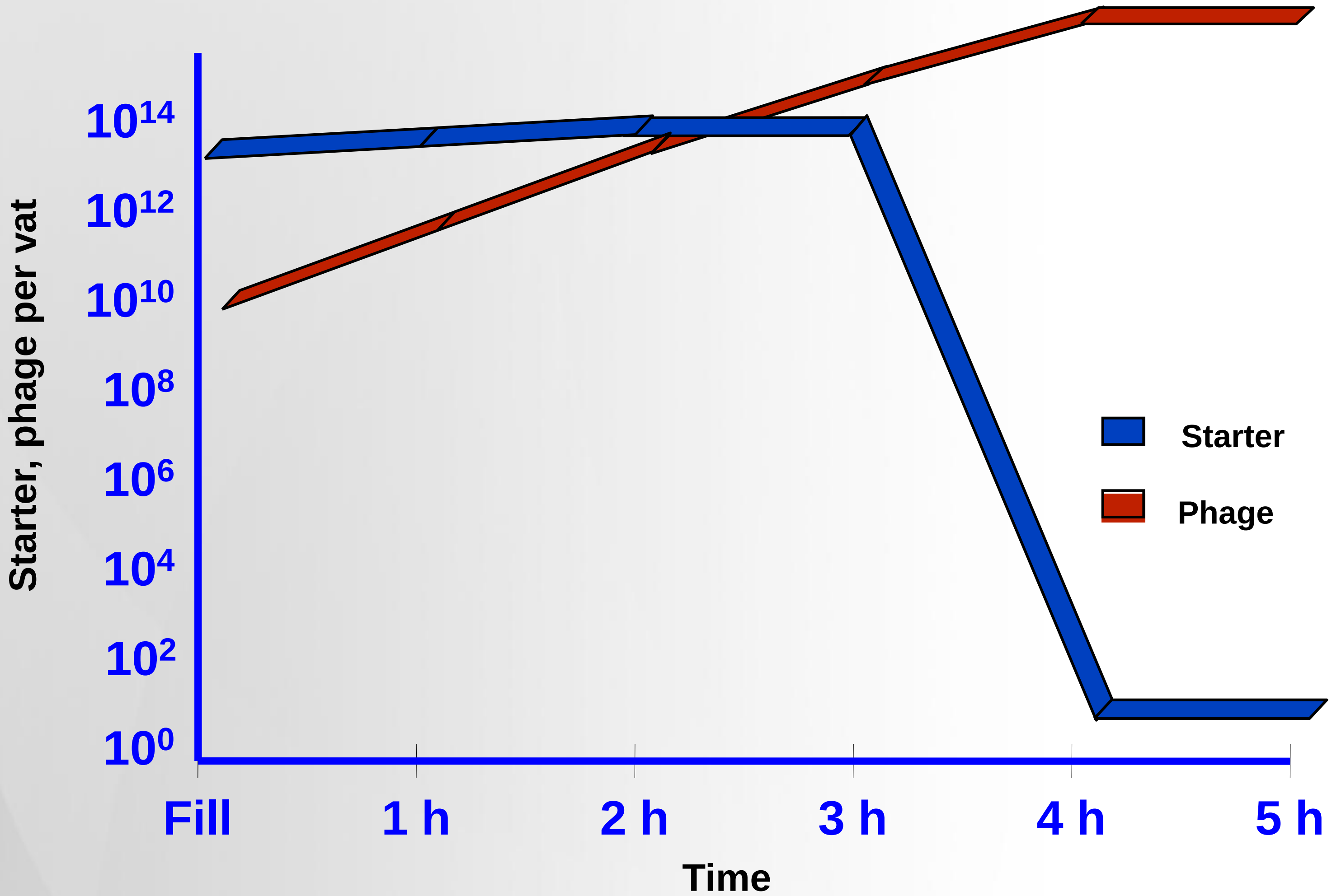
Second fill, with poor whey control



Third fill, with poor whey control



Practical issues:
Phages and starter cultures

Where do phages come from?

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Where do phages come from?

Phages occur with their host bacteria

- any source of bacteria is potential source of phages
- phages can easily spread beyond that source

Where are the natural sources of dairy phages?

- where do the wild relatives of our cultures live?
    - insects, birds, mammals, vegetables, decaying leaf litter ....

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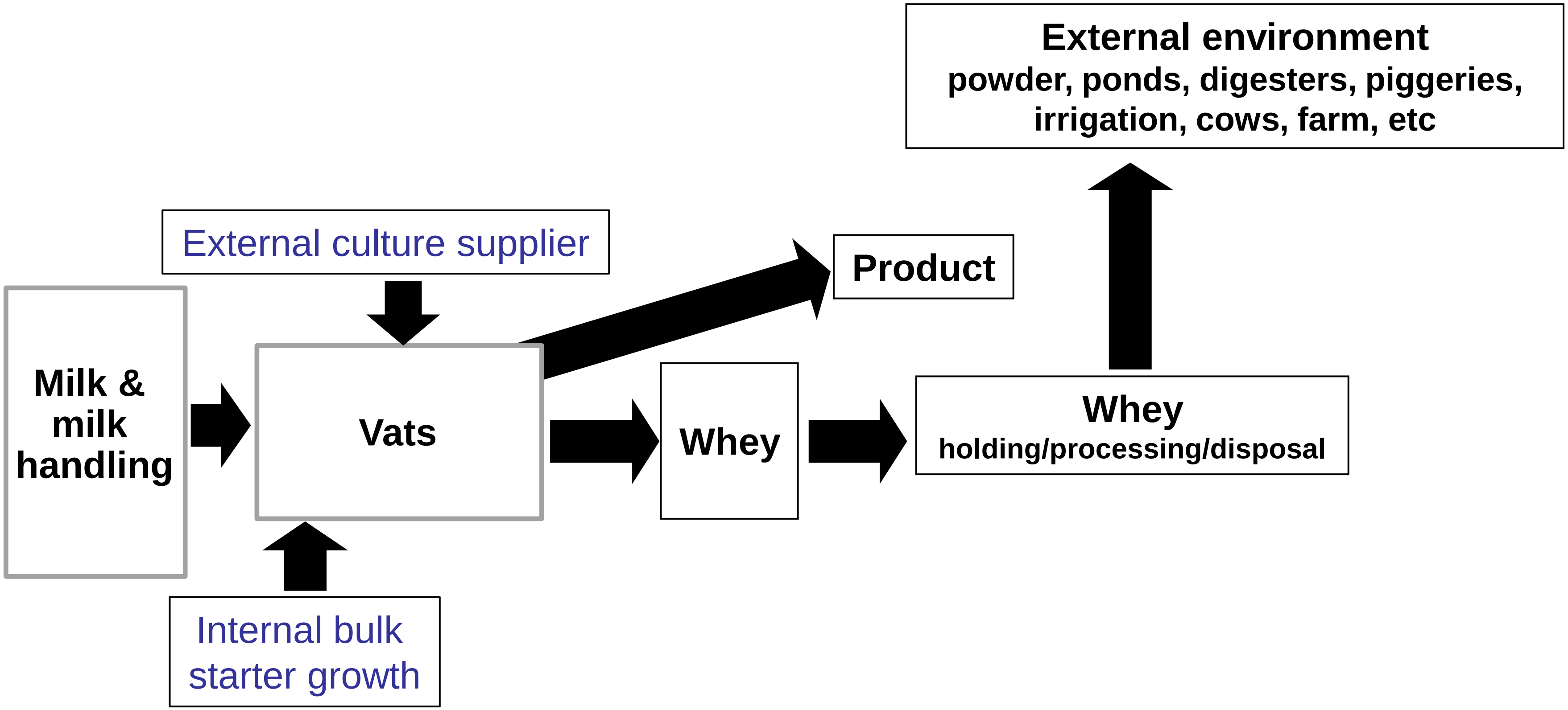
Where do phages come from?

Where are the industrial sources of phages?

- enter the system potentially via milk, air, soil, personnel and equipment movements, cultures
  - poor quality milk, air and general environment
  - phages are **not** inactivated by pasteurization

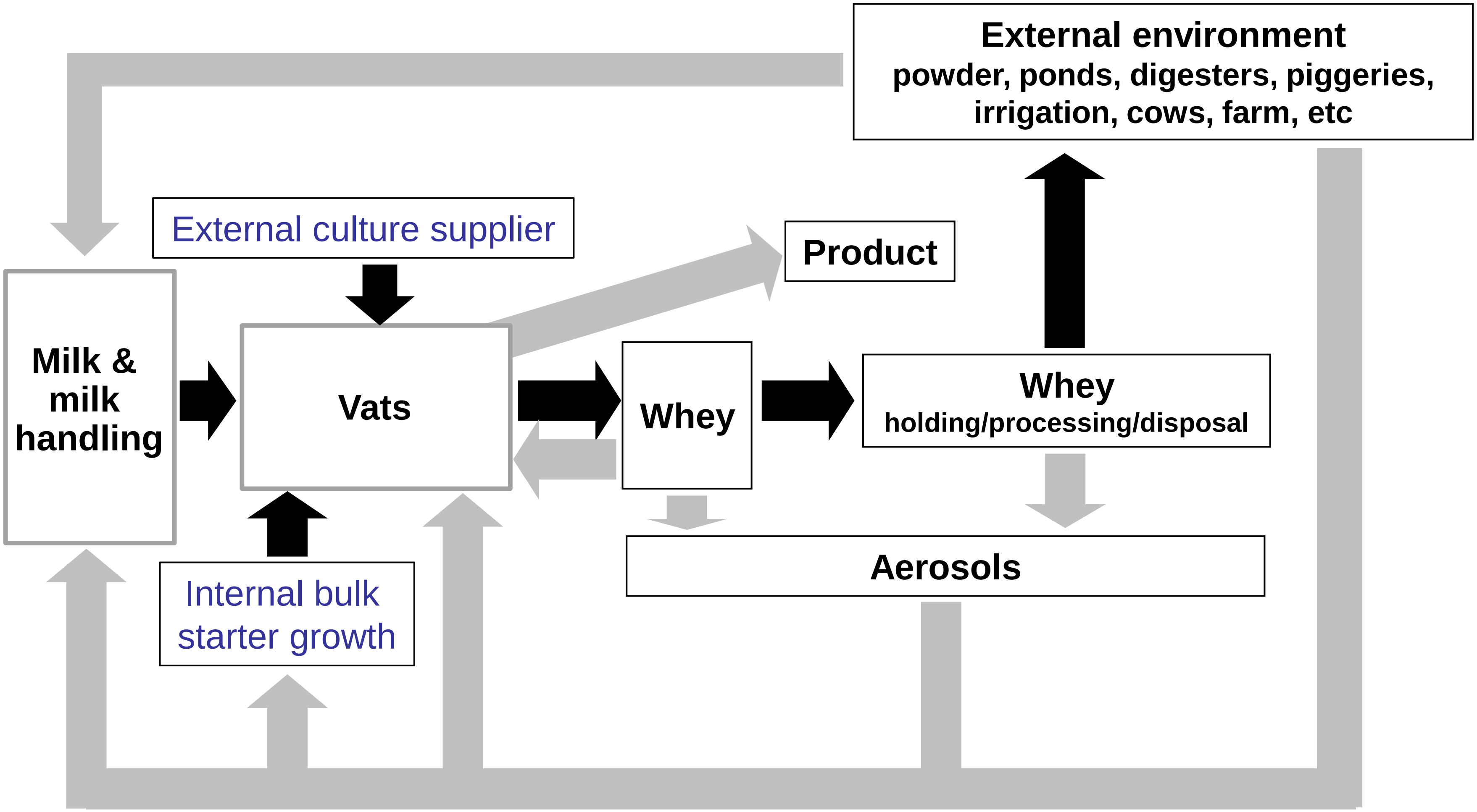
- persistence within the factory due to
  - poor whey and waste handling (procedures, design)
  - inadequate cleaning/sanitation
  - poor control of air and staff movements
  - poor culture management

Material flow in the factory ecosystem



Phages in the factory ecosystem

A tale of whey, aerosols and CIP



Practical issues:
Phages and starter cultures

How do you manage or eliminate phages?

Phage in the factory
Physical measures

The goal:

- prevent phages getting in
- keep phage numbers low
- remove any residual phages

Residual whey in vats, pipes, etc

- the more there is, the quicker phage numbers reach inhibitory levels

Whey control, air control

- whey contamination (1 drop of late whey > 10^8 phage)
- contain whey; prevent leaks, splashes, aerosols
- pipe, pump and valve cleaning critical
- control air movement – directional; filtered in crucial areas

Phage in the factory

Biological measures – exploiting host range differences - 1

- A phage usually only infects one bacterial species
- Within a bacterial species, strains differ
  - cell surface sugars and proteins
  - intracellular properties
    - DNA restriction, ‘abortive infection’
- A phage will infect some starter strains, not others
- A strain will be infected by some phages, not others
- Host range is a basis for strain selection and culture blend design

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Phage in the factory

Biological measures – exploiting host range differences - 2

- Strains are blended into culture blends
  - Multiple strains, different sensitivities to phages
    - undefined traditional mix or defined blend
    - not all likely to be infected by any one phage
- Blends are rotated
  - Alternating use of blends with different phage sensitivities limits opportunities for phage numbers to build up
- Replacement of blends or of individual strains
    - identify strains/blends resistant to current phages
    - use ‘unrelated’ strains, phage-resistant variants
    - conjugal transfer of resistance genes

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Phage in the factory

Biological measures – exploiting host range differences - 3

- Culture suppliers
  - phage detection
  - sensitivity/resistance testing
  - selection of resistant strains
  - composition of blends
- Culture users
  - monitor culture performance: ‘slow’ might mean phage
  - keep track of performance of different blends
  - rotate cultures and/or keep a reserve replacement culture (depending on operational scale)

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Phage in the factory

Best practice:

- use only milk with low bacterial count
- maintain a clean environment
- wash/sanitize tankers, silos, vats, pipes & valves between fills
- separate whey and product from milk and make
  - no whey in milk transport tankers
  - no shared milk/whey lines or tanks
- directional airflow (pressure differentials), HEPA-filtered inlet
- control personnel movements
- rotate selected culture blends

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Three FAQs

Are dairy phages inactivated by pasteurization?

- No. Almost all are inactivated by UHT or 90°C/30min.

Are dairy phages inactivated by sanitizers?

- Yes. Sanitizer efficacy is of course greatly reduced if milk, curd or other organic residues are present.

Are any dairy cultures GMOs?

- No. Many laboratory studies have used genetically manipulated organisms to increase our understanding of phage-host interactions, but no GMO cultures are in commercial use.

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Phage detection

Why test for phages?

Detect phages *before* problems arise

Find out which starters are sensitive

Monitor phage levels

(and degree of inhibition due to phage)

Using data

- Design multiple starters, rotations
- Replace infected strains or blends
- Select resistant variants

Who tests for phages?

Culture suppliers

- selection of strains, design of multiples and rotations
- remote testing limitations

Major factory laboratory - daily testing

- prompt information
- data on potential phage inhibition levels
- can test a range of starter strains
- combine with non-phage inhibitor tests

Where do you look for evidence of phages?

- Whey
  - Convenient to collect and assay
  - Late whey has highest phage levels
- Environmental monitoring
  - Generally not routine; sensitivity?
- Production data
  - Need careful interpretation (phage and non-phage inhibitors, other effects)

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Phage detection: activity tests

- *Principle*
  - Put a culture in milk* and see how quickly it makes acid.
    - Two identical tubes: milk + starter. Add whey to one tube.
    - Incubate.*
    - Measure acid production.*
    - If tube with whey has less acid than ‘control’ tube ….. Phage?

*Various growth media, incubation conditions and acid detection systems (pH, TA, indicator dyes, conductivity/impedance) are used.

- Phage detection most sensitive if tests done with pure single strains
  - test each component strain separately
  - get information that tells you which (if any) of the starter strains you are using are sensitive
- Tests by starter suppliers
  - note that undefined mixed-strain starters cannot be tested as separate strains: only overall inhibition can be detected
  - what tests does your supplier do?

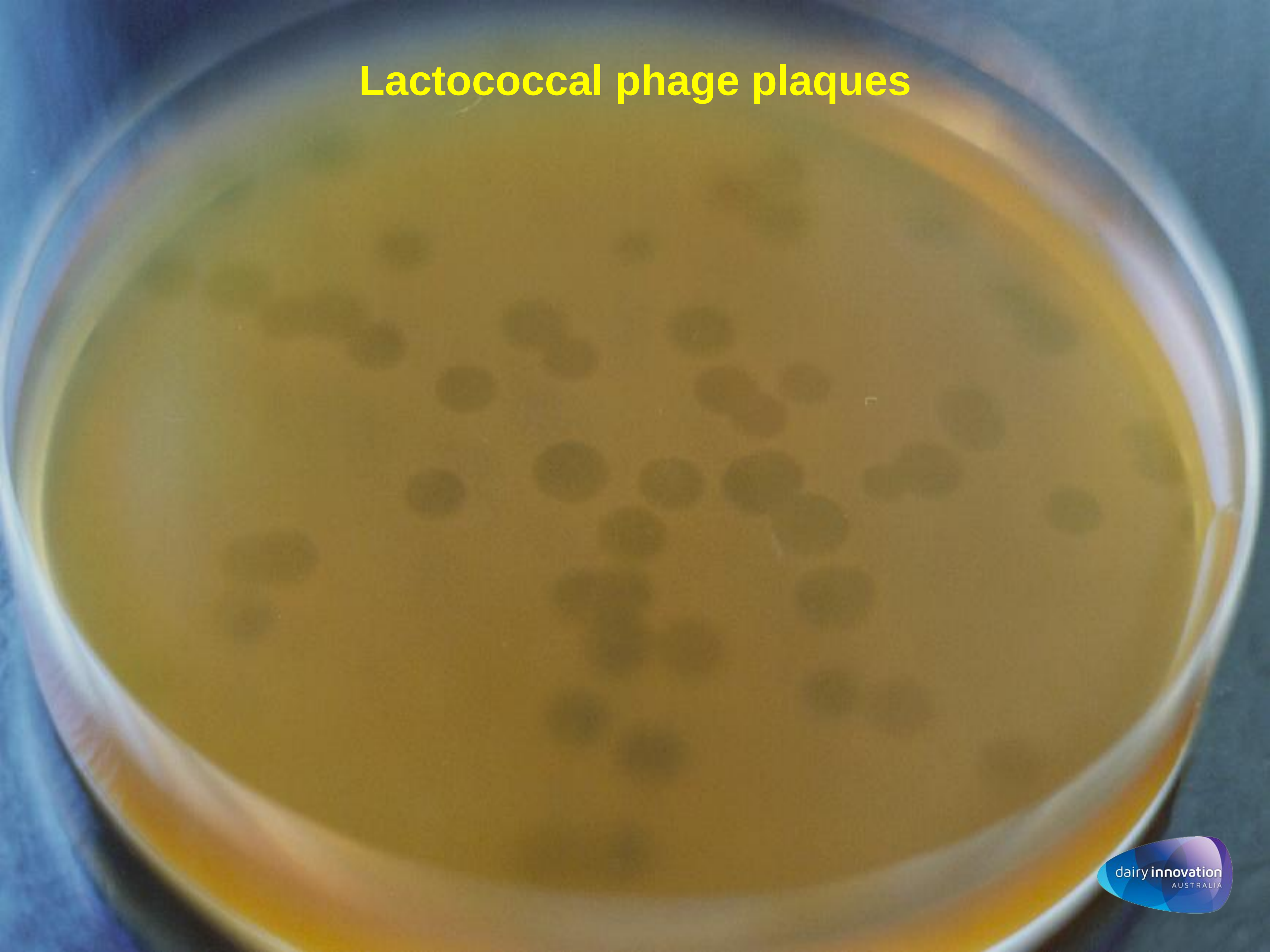
Phage detection by plaque assay

Principle

Grow a lawn of bacteria in the presence of phage particles.

A phage will infect, multiply, kill cells
 - result is a dead zone (plaque).

Lactococcal phage plaques



Understand the tests

What tests are done? How often?

Does your lab or an external lab do them?

How soon do you get the information?

What do the results mean?

What if a phage appears?

- What alternative cultures do you have?
    - Will they affect flavour, etc?
- How quickly can strains or rotations be changed?

If no testing is done, how vulnerable are you?

- Do you monitor culture activity during the make?

Phages - summary

Very small viruses infect bacteria

Stable particles

- environmentally persistent

Kill starter, multiply rapidly

Phage control

- milk quality, whey handling
- factory design, sanitation
- starter selection, multiple-strain cultures, rotation

Phage monitoring

- central to culture management at industrial scale