Biodiversity in new starter cultures as a response to increasing bacteriophage pressures

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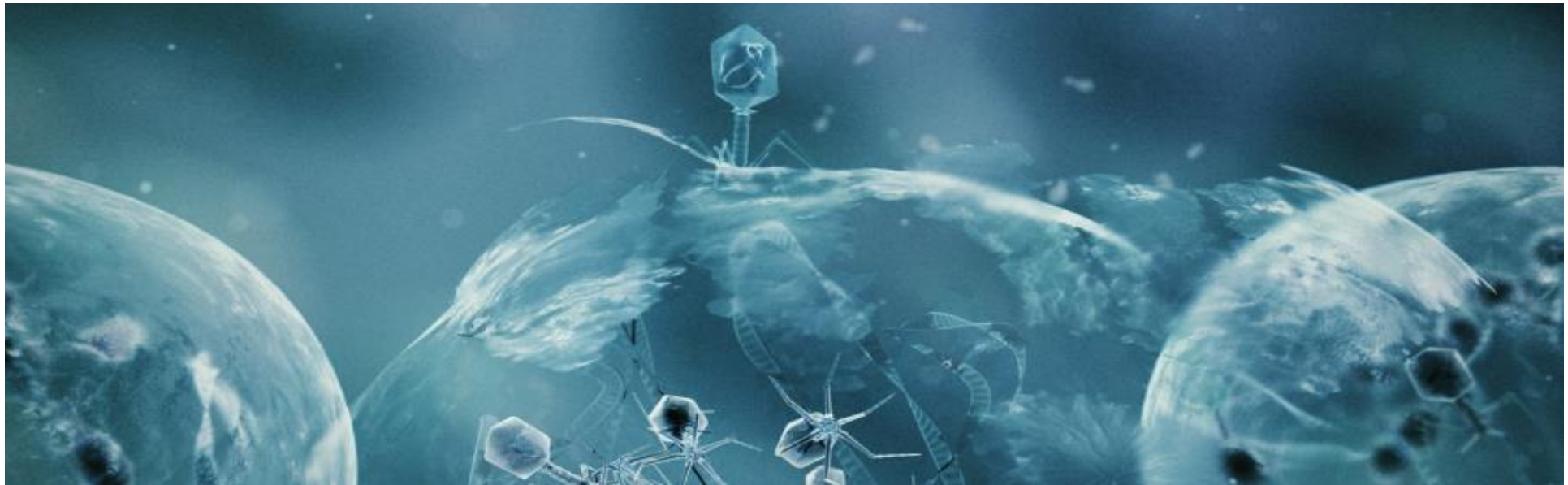
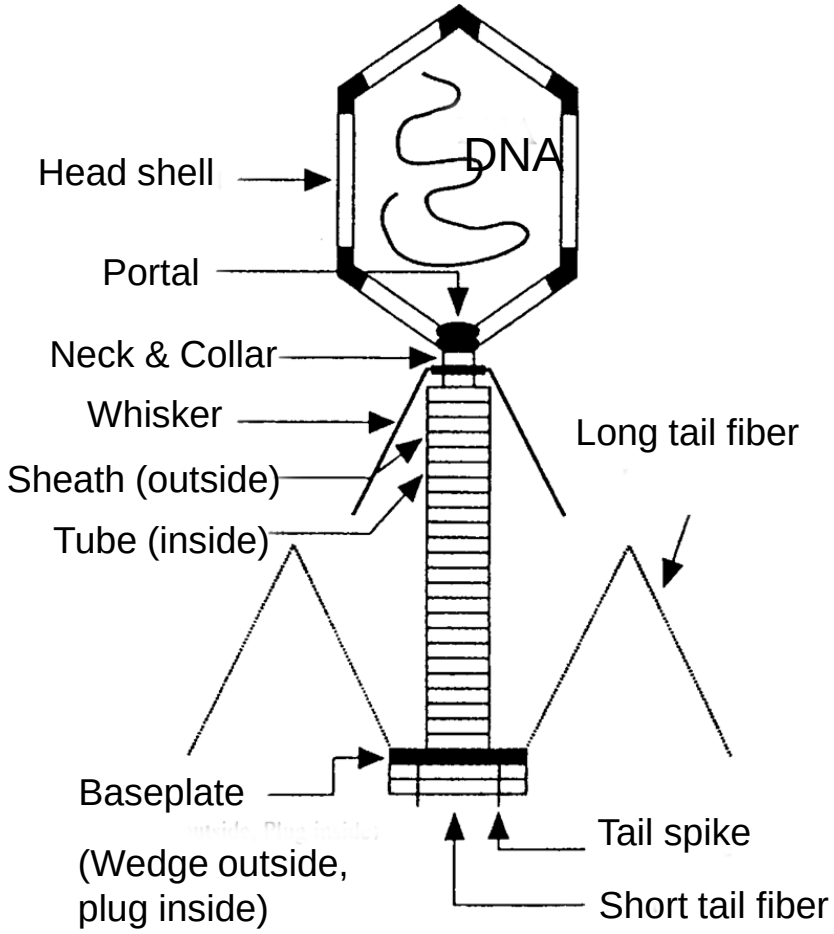


Agenda

- Bacteriophage – Know the enemy
- Bacteriophage - Impact on cheese production
- Culture development
  - A new concept

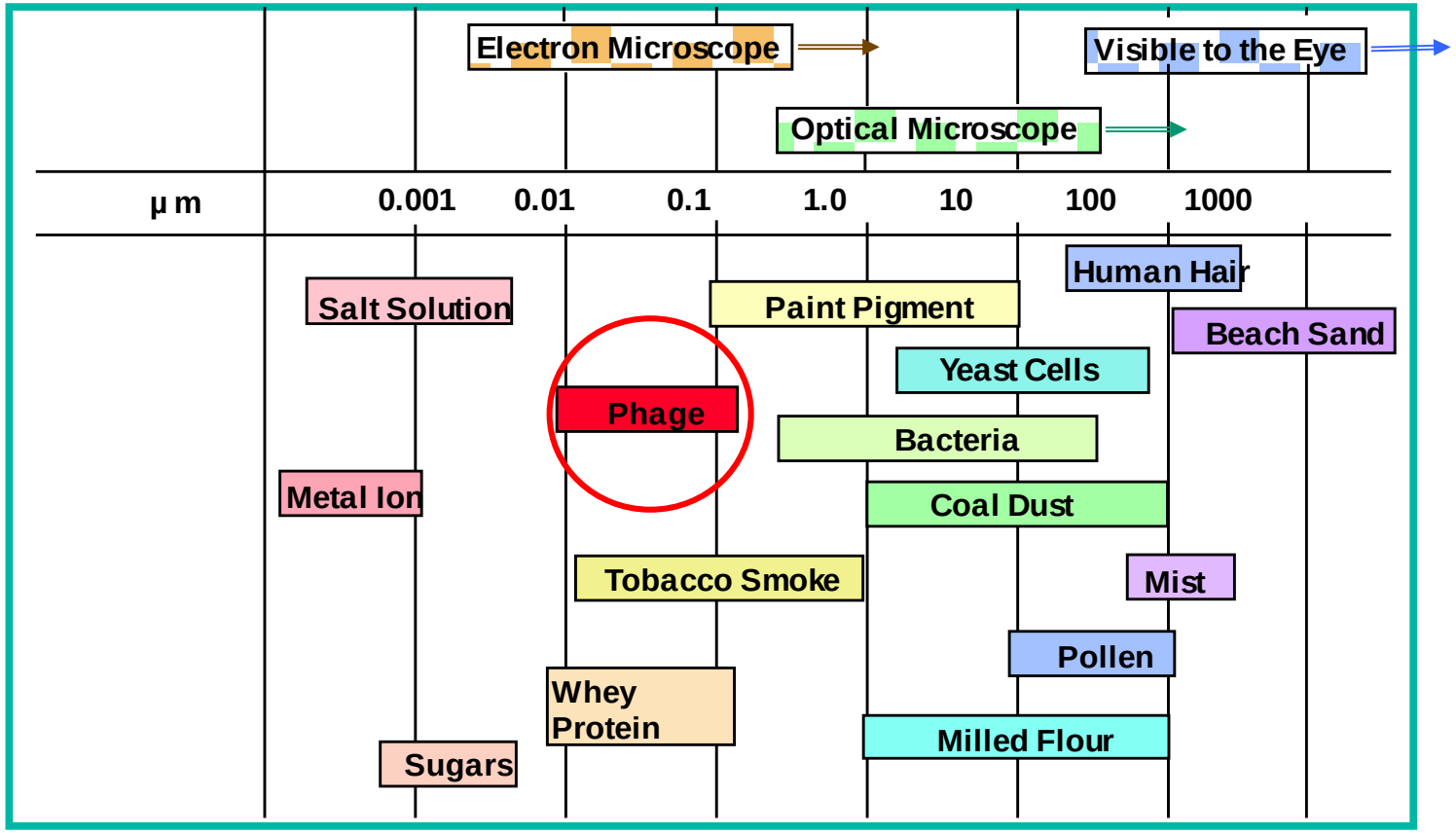
Bacteriophages

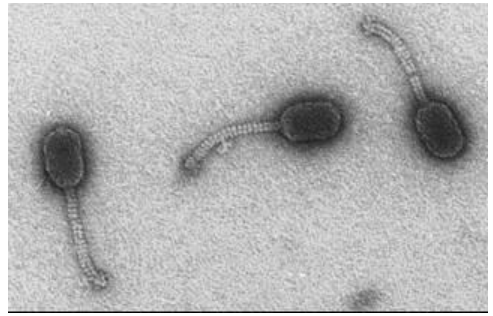
- Bacteriophages are bacterial parasites (viruses)
- They need bacteria cells to multiply!
- Mainly made of proteins (head and tail)
  Contains genetic material (DNA)
- Their structure makes them very robust and difficult to destroy



Phage Size

Relative size of common materials





Very small (10 times smaller than bacteria) and therefore not visible to the eye or in a normal microscope

but … they spread as fast and easily, as smoke !!

The cycle of bacteriophages

Bacteriophage multiplication cycle → death of bacterial cell → fermentation problem

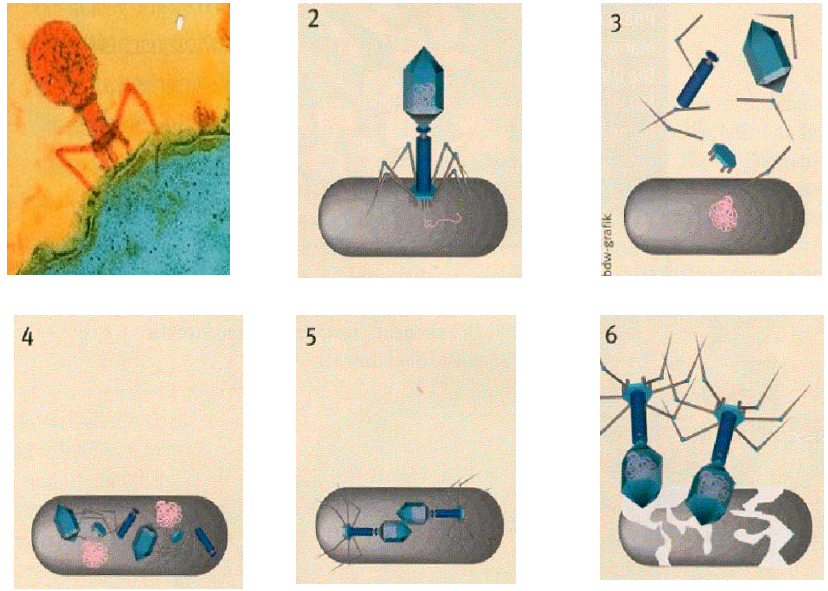


Bild der Wissenschaft; 4.2002; p21-22; Ute Eberle

1. Attachment to cell membrane
2. Injection of genetic material (DNA)
3-5. Reproduction and assembling of new bacteriophages in the cell
6. Lysis of the cell and release of new bacteriophages

Life cycle (1-6) takes between 25-80 minutes

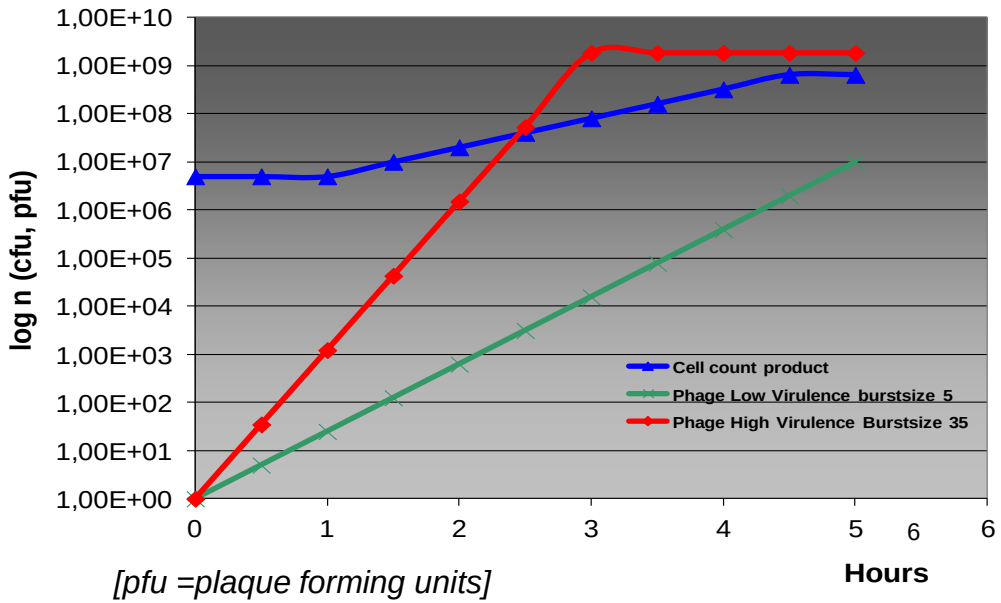
One bacteriophage can produce up to 10-400 phages (burst size) in one bacterial cell

The cycle of bacteriophages

Bacteriophages **multiply faster** than bacteria

Bacteria	Moderate Phage	Virulent Phage
1 cell → 2 cells 2 cells → 4 cells 4 cells → 8 cells 8 cells → 16 cells ….	1 ϕ → 10 ϕ 10 ϕ → 100 ϕ 100 ϕ → 1000 ϕ 1000 ϕ → 10^4 ϕ …..	1 ϕ → 100 ϕ 100 ϕ → 10^4 ϕ 10^4 ϕ → 10^6 ϕ 10^6 ϕ → 10^8 ϕ …..

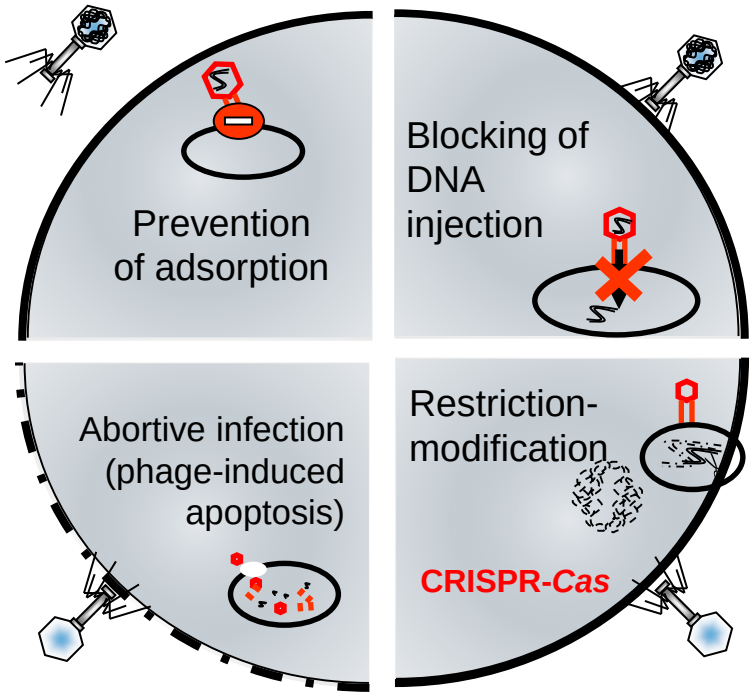
ϕ = phage particle



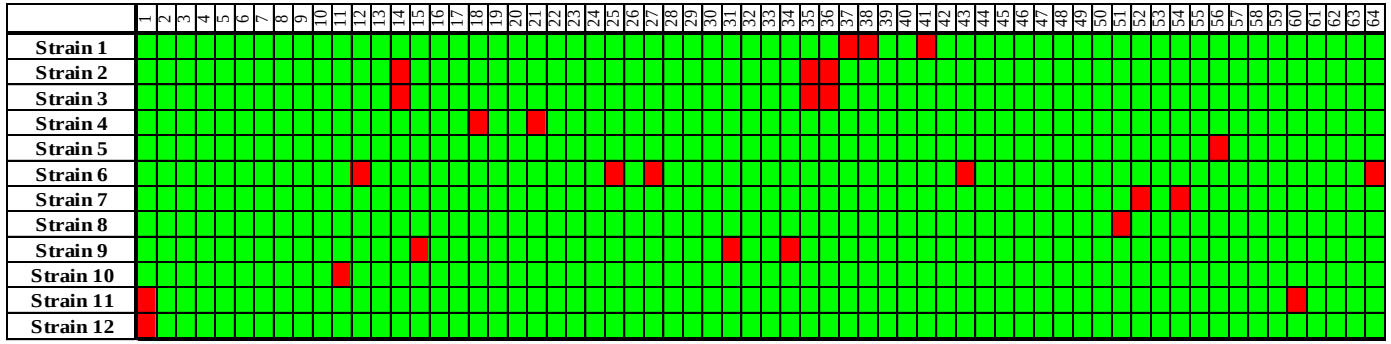
[pfu =plaque forming units]

Bacterial Defense Systems against Phages

- To resist bacteriophage attack strains have developed many different strategies
- Among the classic strategies are:
  - Prevention of absorption
  - Blocking injection
  - Abortive infection (death of the infected cell)
  - Restriction/modification (DNA-degrading enzymes)
  - ...etc



Bacteriophages are species specific



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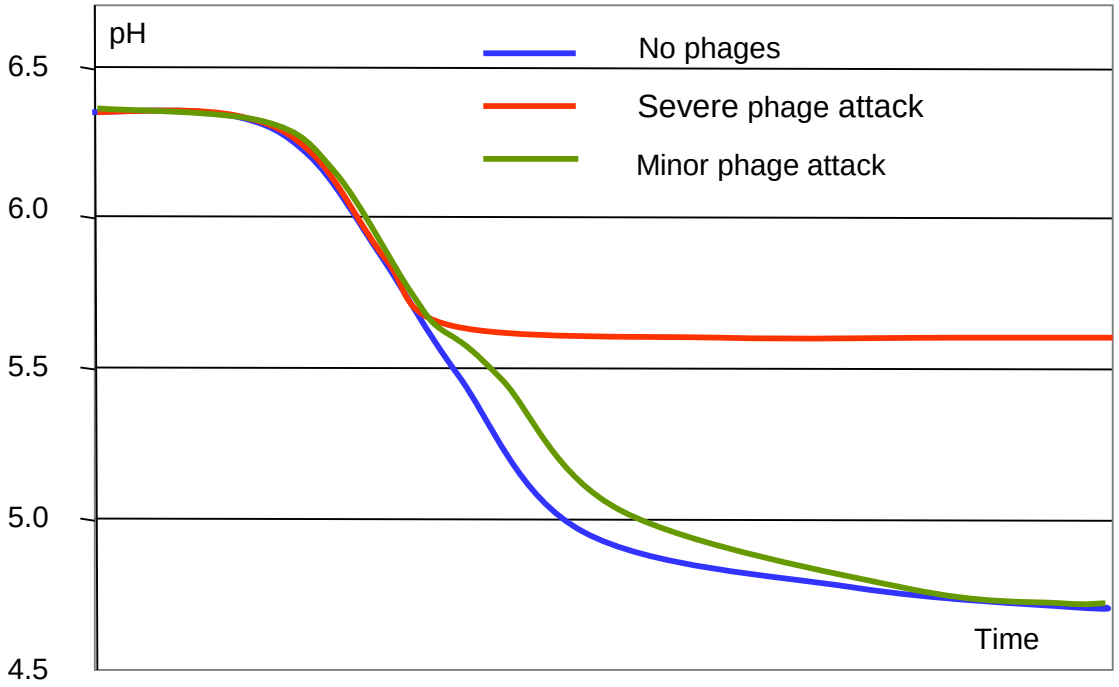
Effect of bacteriophages on the Acidification



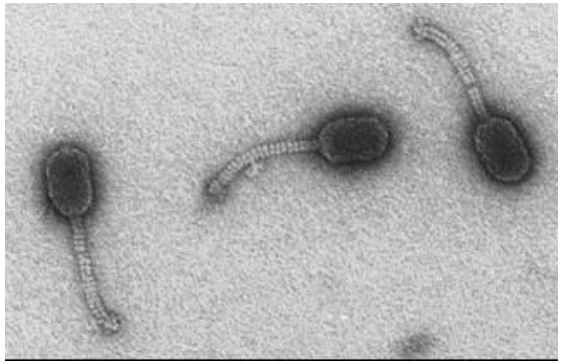
Different levels of phage virulence

- Phage A at 10^4 /ml stop the acidification
- Phage B at 10^4 /ml has no or little impact on the acidification curve

Consequences of a bacteriophage attack

- **Bacteriophages are one cause of production failures and could lead to**
  - Acidification delay or failure (loss of production)
    - Product defects
      - Low or excessive gas and flavor development
      - Texture problems
    - Yield problems in cheese production

- **Control / Management of bacteriophages is key to ensure**
  - Productivity
  - Production robustness
  - Consistent product quality

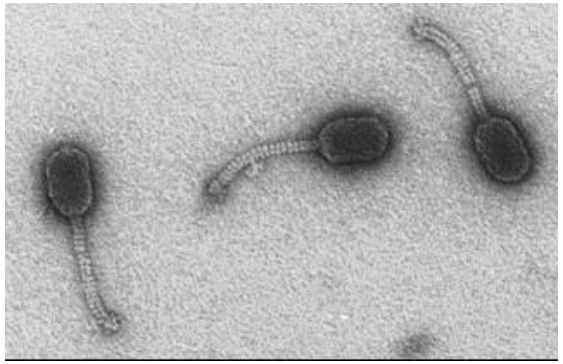


Slow Acidification = Bacteriophages?

Reason for fermentation problems = Bacteriophage in most cases

But don‘t forget!

- **Inhibitors**
  - Antibiotics
  - Disinfectants
  - Preservatives



- **Other factors affecting culture activity**
  - Mixing / excessive aeration
  - Old culture / wrong storage
  - Wrong fermentation temperature

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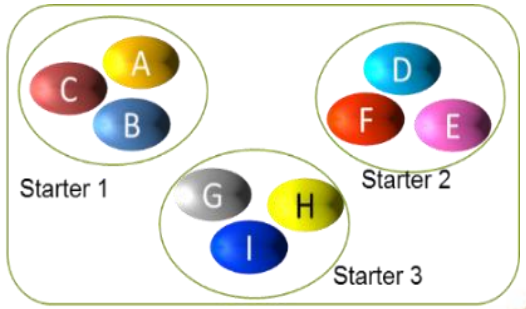
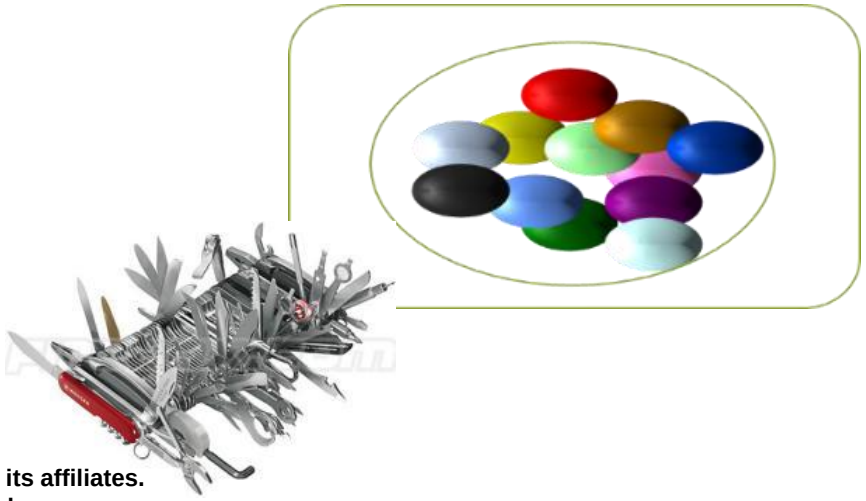
The traditional culture offer

- DEFINED STARTER CULTURES
- 1 to 4 strains
- Use of robust phage strains
- High cheese process consistency
- Suitable for cheese technology with a short process time
- Phage rotation program defined with the customer

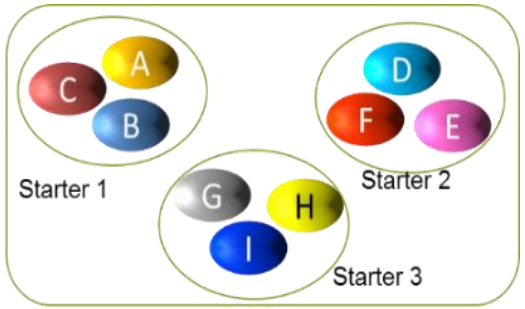
- COMPLEX STARTER CULTURES
- Interesting biodiversity but difficult to control
- Robustness of phage strains is not known
- Difficulties in obtaining cheese process consistency
- Suitable for cheese technology with a long process time
- No real phage alternative





The new concept: multiple defined cultures

- DEFINED STARTER CULTURES



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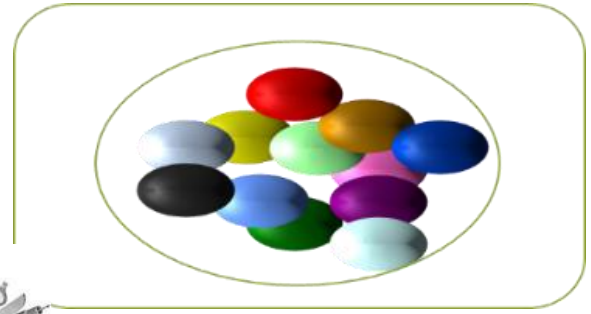
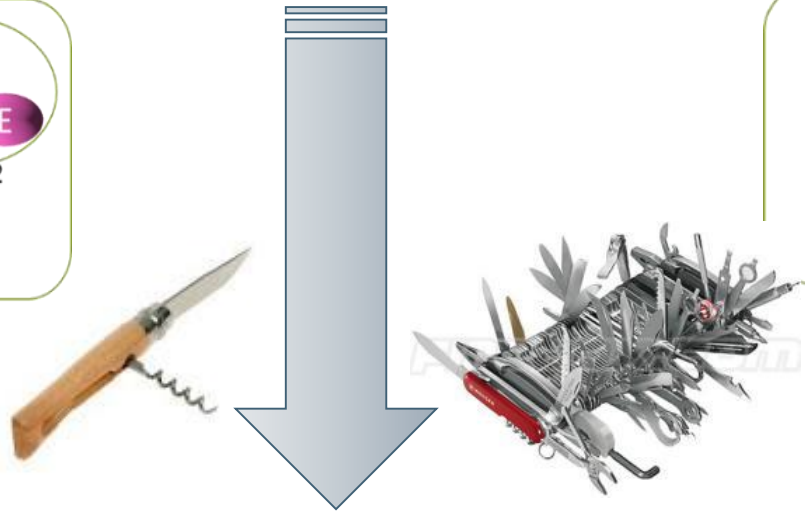
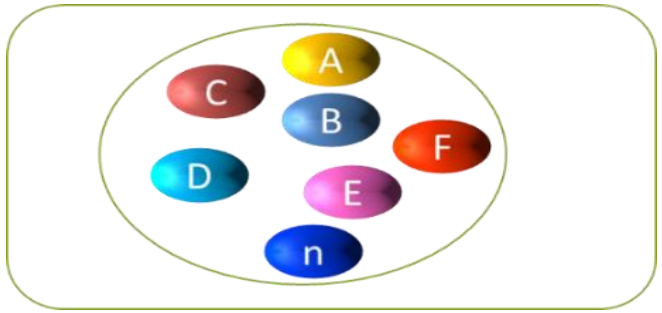


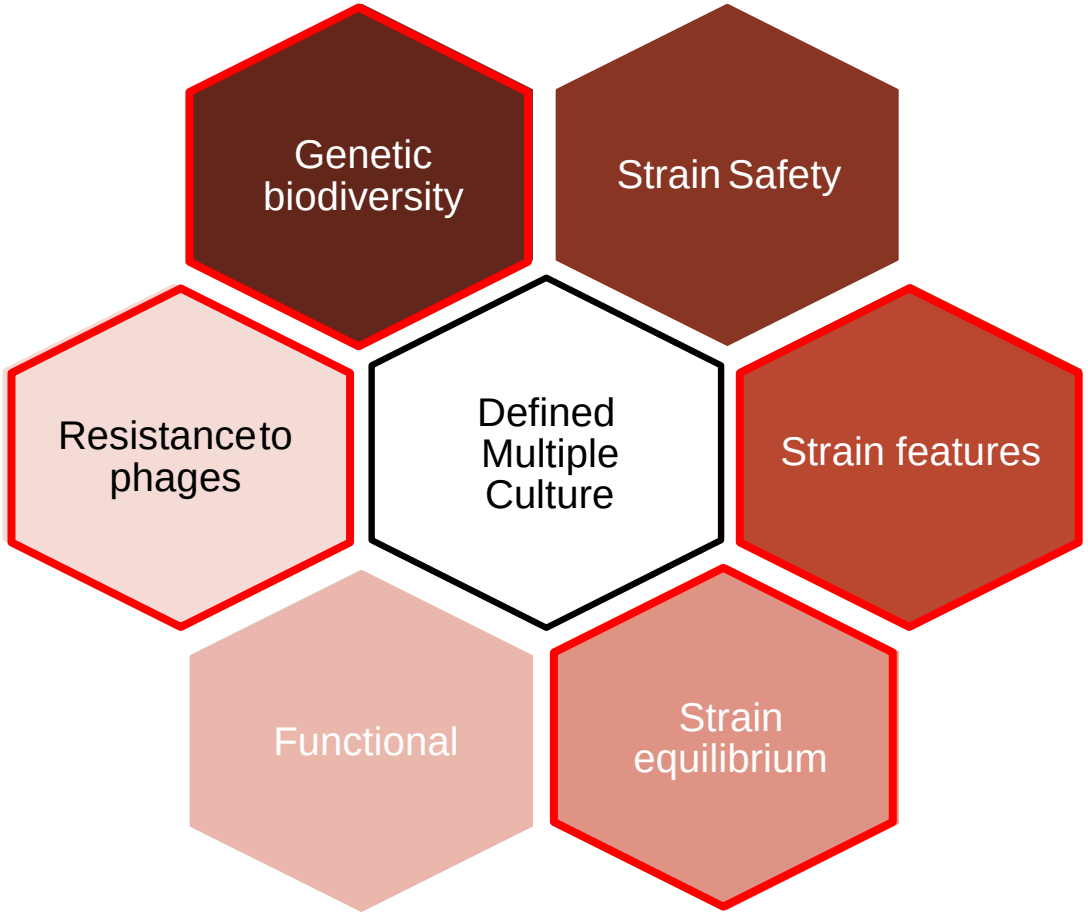


- DEFINED MULTIPLE STARTER CULTURES

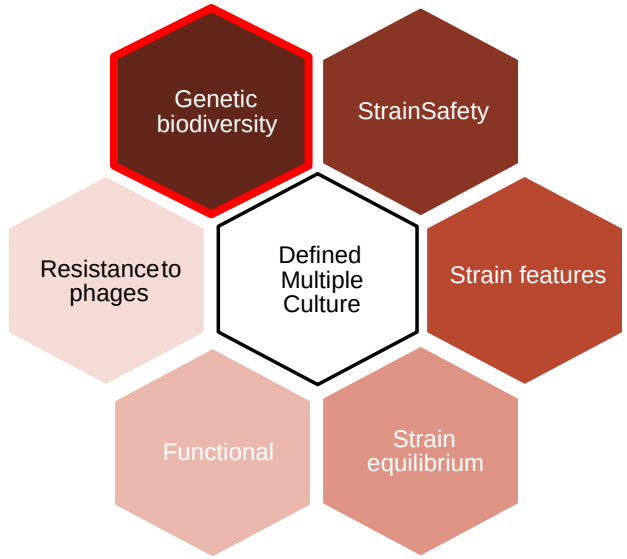


- Phage robustness -> multiplicity of strains and the quality of the strains
- Possibility of rotations -> control of the complexity
- Functionalities of culture -> controlled by the use of appropriate strains
- Reproducibility of productions

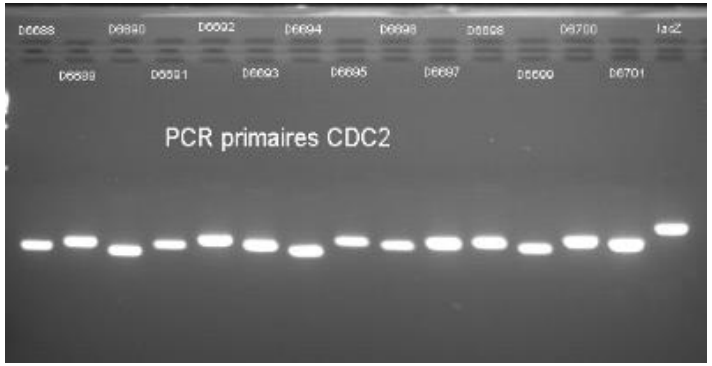
Defined multiple starter cultures



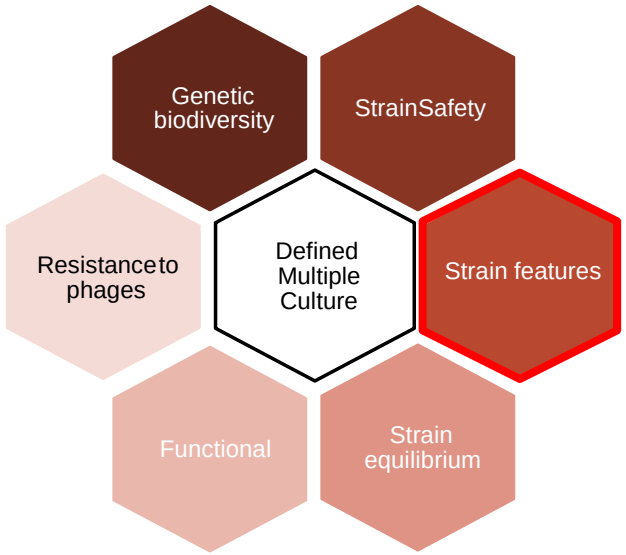
Defined multiple starter cultures



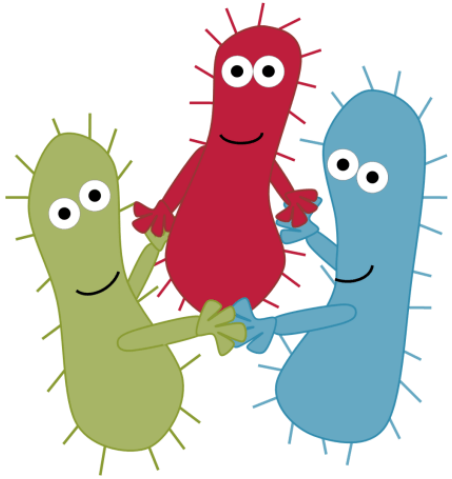
- **Genetic biodiversity**
  - Genomic data of strains have been compared
  - Strains with identical or similar genomes are eliminated
  - Develop methods for the specific detection of each strains



Defined multiple starter cultures

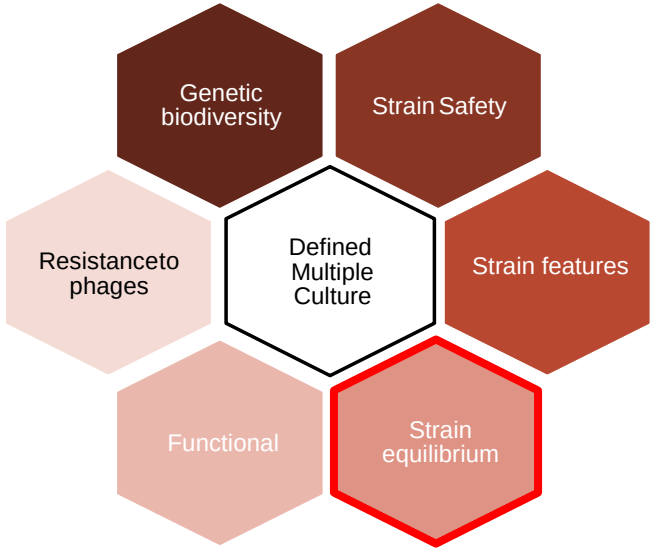


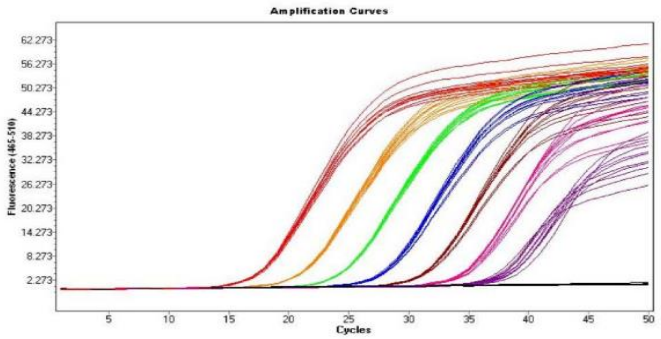
- Impact of strains on equilibrium
  - Sensitivity to physico-chemical factors
    - Optimal growth temperature
    - Lactate sensitivity
  - Competition for nutrients
    - Nutritional needs
    - Nutrient availability
    - Ability to metabolize nutrients
  - Quorum Sensing
    - Communication between strains
    - Bacteriocin

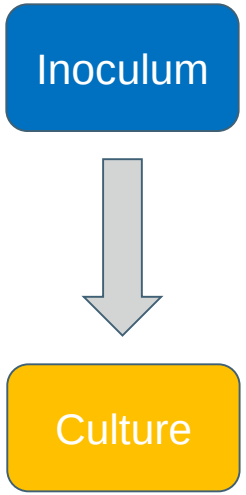


Defined multiple starter cultures

- **Strain equilibrium**
  - Upon production process
  - Risk to have some strains dominating
  - Analysis of strain ratio upon production
  - Thanks to genomics data and the development of a q-PCR method

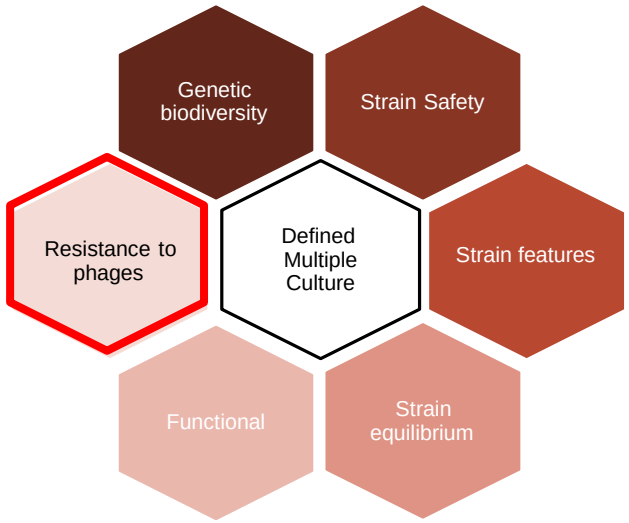




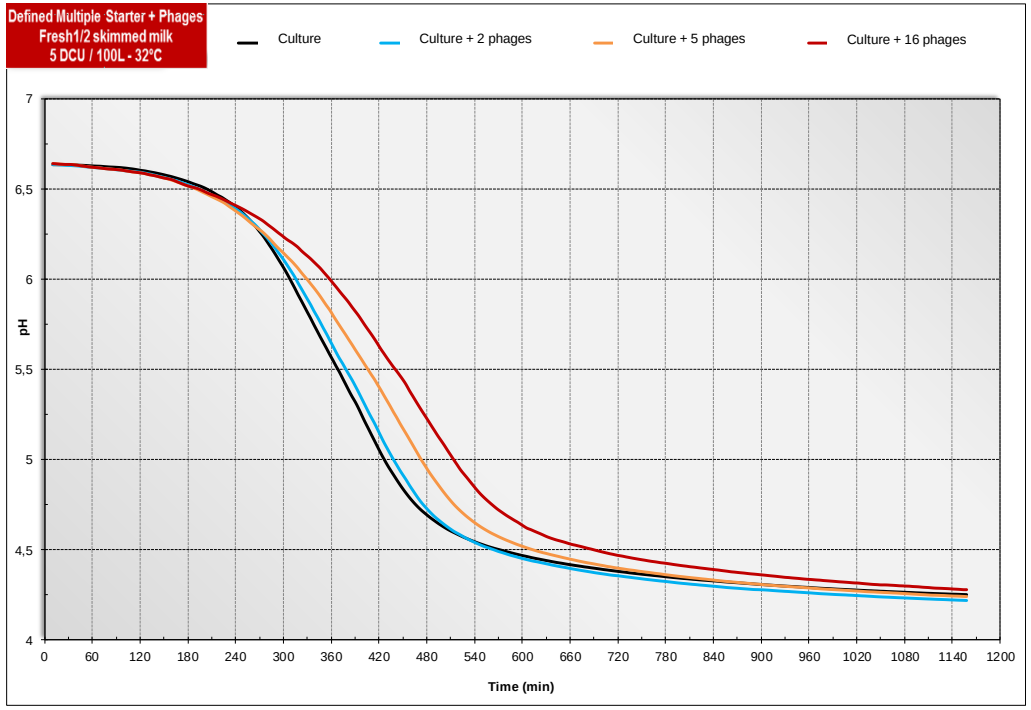


- Examples of maintained versus lost biodiversity

Defined multiple starter cultures



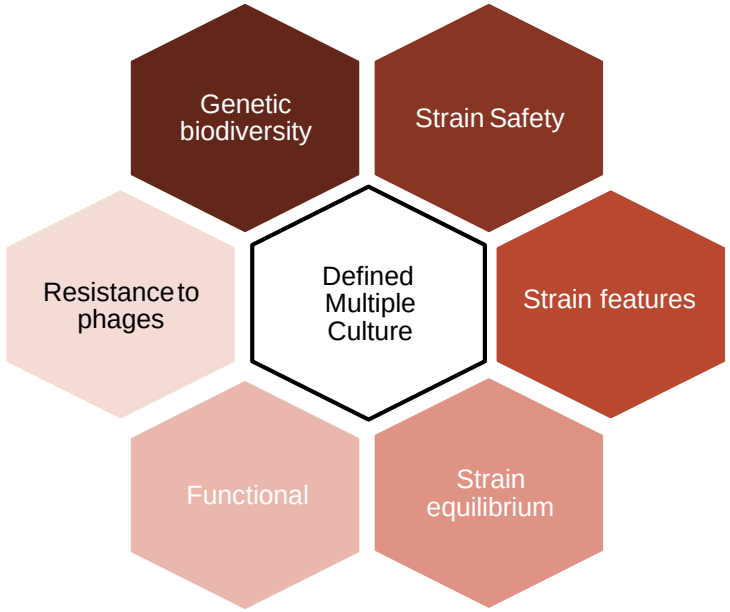
- **Resistance to phages**
  - Thanks to biodiversity, little impact of reasonable phage attack
  - No dead-vat upon massive phage attack on the contrary to simple starter cultures



A high rate of phage infection was used (10^3 pfu/ml)

[pfu =plaque forming units]

Conclusion



- Ready to use
  - Very high phage resistance
  - Reliable fast acidification time
  - Formulated for high productivity
- Easy to combine with flavor adjuncts for more intense/specific flavor
- Can be combined with other cheese cultures to accelerate acidification
- Good feedback from customers

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