



THE UNIVERSITY OF
MELBOURNE

FOOD20006

Food Microbiology and Safety

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Intended learning outcomes

The intended learning outcomes of this lecture are

- to describe the process for producing a ripened hard cheese
- to understand the use of starter cultures for primary and secondary inoculation in food fermentation.
- problems that can occur with starter cultures
- strategies to avoid problems with starter cultures

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Dairy fermentations: cheese

Cheddar cheese production

- originally from village of Cheddar, Somerset, SW England
- made by coagulation of casein in milk with lactic acid produced by LAB
- with rennin or rennet
- collecting the coagulated casein for further processing

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Australia produced 336,700 tonnes of cheese in 2016/17

About 33% milk production goes into cheese-making

Of which about 55% is cheddar cheese

About \$1.8 billion in sales on the domestic market



Cheddar Cheese production

Cheddar is an example of ripened, hard cheese

It is made from whole milk

It contains < 39% moisture, 48% fat, orange-yellow (annatto)

It is ripened to give desired flavour (numerous compounds)

Cheddar process

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Pasteurised, natural colour added, cooled and starter cultures are added

Incubated 30°C until acidity decreases then rennin/rennet added

Allowed to coagulate (30 min), then cut into cubes

“Cooked” at 38 °C, whey drained away, cheddaring to lose whey

Milled, salted, put in form, pressed 16hr to drain whey

Dried for 5 days, packed, cured at 4.4°C for 2-12 months



Cheddar Cheese: starter culture

Mixed strains of *Lactococcus lactis* ssp. *cremoris* or *lactis*.

Leuconostoc may be added in some cases for flavour

The starter culture is usually added as frozen concentrates

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Growth parameters

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- 30°C, mesophilic, cells grow, convert lactose to lactic acid
- lactic acid production usually about 60 min
- at this level, coagulation by rennin/rennet occurs quickly
- then incubated for further 30 min for coagulation
- during curing, cells die and release enzymes into the cheese

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Cheddar cheese rennin vs rennet

Rennin and rennet are often confused but they play a similar role in cheese making

Rennin is an enzyme mixture found in the stomach of cows, sheep and goats

Rennin curdles milk by transforming the soluble milk protein caesinogen into insoluble casein.

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In young animals who are feeding on milk, rennin is an important first step in digestions milk.

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Traditionally, rennin from animals was used for cheese making

Today, most cheese is made with enzymes produced by microorganisms

Aspergillus oryzae is one of the fungi that commercially produce rennet for industrial cheese manufacture



Production of cheeses using a secondary culture, e.g. mould

Table 32.1 Microorganisms involved in the manufacture of cheeses and fermented milks

Product	Principal acid producer	Intentionally introduced secondary cultures
Cheeses		
Colby, Cheddar, cottage, cream	<i>Lactococcus lactis</i> subsp. <i>cremoris/lactis</i>	None
Gouda, Edam, Havarti	<i>Lc. lactis</i> subsp. <i>cremoris/lactis</i>	<i>Leuconostoc</i> sp., Cit ⁺ <i>Lc. lactis</i> subsp. <i>lactis</i>
Brick, Limburger	<i>Lc. lactis</i> subsp. <i>cremoris/lactis</i>	<i>Geotrichum candidum</i> , <i>Brevibacterium linens</i> , <i>Micrococcus</i> sp.
Camembert	<i>Lc. lactis</i> subsp. <i>cremoris/lactis</i>	<i>Penicillium camemberti</i> , sometimes <i>B. linens</i>
Blue	<i>Lc. lactis</i> subsp. <i>cremoris/lactis</i>	Cit ⁺ <i>Lc. lactis</i> subsp. <i>lactis</i> , <i>Penicillium roqueforti</i>
Mozzarella, provolone, Romano, Parmesan	<i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> , <i>Lactobacillus helveticus</i>	None; animal lipases added to Romano for picante or rancid flavor
Swiss	<i>S. thermophilus</i> , <i>Lb. helveticus</i> , <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i>	<i>Propionibacterium freudenreichii</i> subsp. <i>shermanii</i>
Fermented milks		
Yogurt	<i>S. thermophilus</i> , <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i>	None
Buttermilk	<i>Lc. lactis</i> subsp. <i>cremoris/lactis</i>	<i>Leuconostoc</i> sp., Cit ⁺ <i>Lc. lactis</i> subsp. <i>lactis</i>
Sour cream	<i>Lc. lactis</i> subsp. <i>cremoris/lactis</i>	None

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Penicillium camemberti and ***Penicillium roqueforti*** are filamentous fungi (moulds)

Secondary fungal culture



Camembert cheese



Blue vein cheese



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First stages similar to cheddar, with bacterial starter culture

Second inoculation with filamentous fungus – *Penicillium camemberti* on the surface. Aerobic growth to form the white rind.

Second inoculation with filamentous fungus – *Penicillium roqueforti*, and cheese is spiked to allow in air (aerobic fungus) > veins



Starter culture PROBLEMS

1. Strain antagonism

- mixed cultures – either different growth rates, or secretion of inhibitors (acids, bacteriocins)

2. Loss of desired trait

- e.g. loss of plasmid borne gene

3. Cell death and injury

- mainly mishandling of purchased cultures (temperature abuse, etc.)

4. Inhibitors in raw materials – e.g. antibiotics, disinfectant

5. Bacteriophages – infect and destroy cells

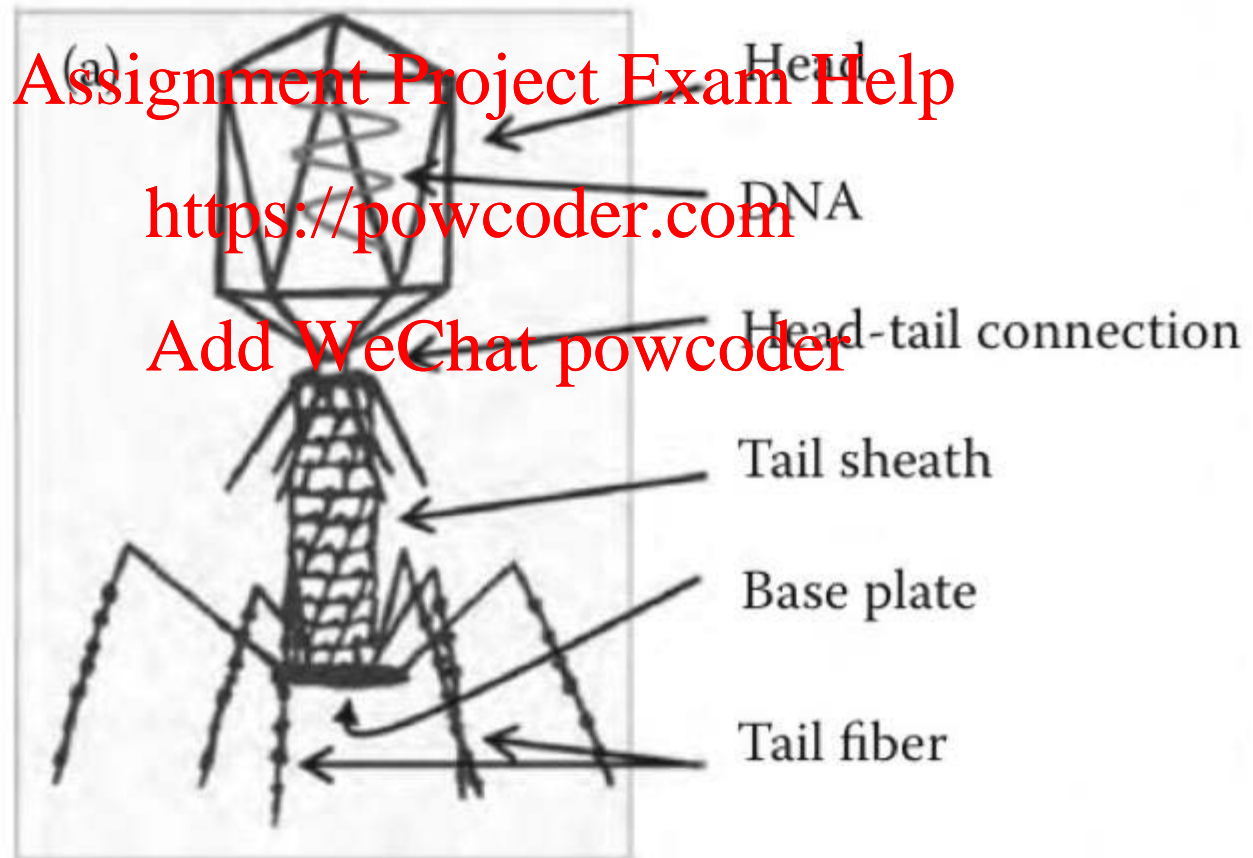
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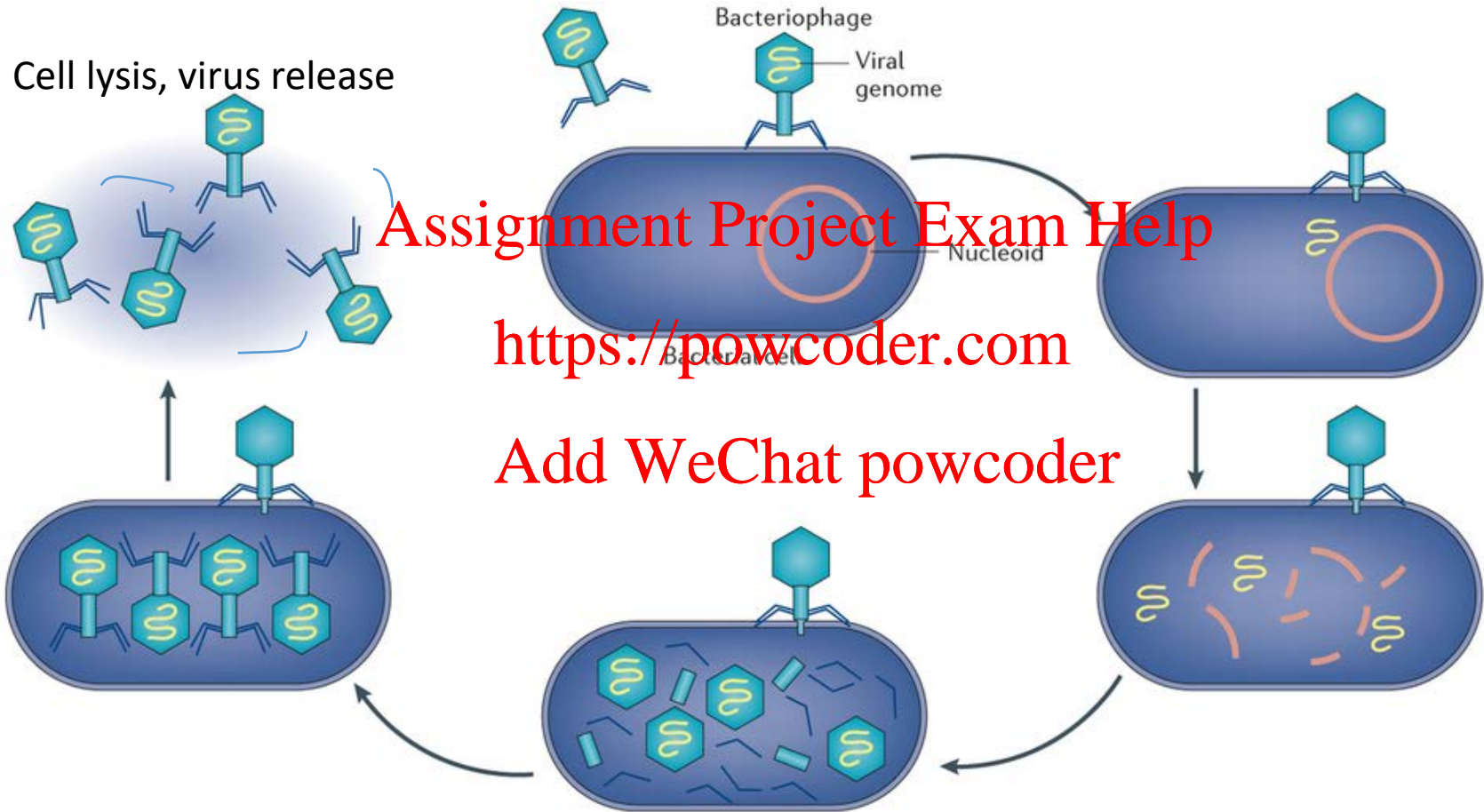
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Bacteriophages

Typical head-tail phage



Bacteriophage (phage) is a virus that infects bacteria. Very common in nature.





Bacteriophages

- Can only replicate in bacteria (not animals/plants)
- Usually very host specific, often closely related strains of same species
- Inactivated by heat, pH, disinfectants, desiccation, but phage can persist in moist environments.
- Can act very quickly (life cycle of ~ 5-10 minutes)
- Can reach enormous numbers in environment (of fermentation facility), e.g. $>10^8$ phage/ml

How to deal with this problem??



Starter cultures and bacteriophages:

- If one routinely uses pure cultures of bacteria for inoculation of fermented foods, then you run a risk of contamination with bacteriophages
- These expand in numbers with every new batch of fermented food, and contaminate the entire manufacturing plant: machinery, floors, pipes, etc.
- Typically, the inoculation of a new batch of raw material (e.g. milk), where there are high levels of bacteriophage from the machinery or work environment, will lead to lysis of the starter culture and failure of the fermentation
- The batch has to be discarded, usually at considerable cost. Imagine a 100,000 litres of milk to be discarded...

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Starter cultures and bacteriophages:

- Most starter cultures are purchased from specialist sources
- They are frozen or freeze-dried concentrates with high cell densities, and can be used directly
- They are designed to be bacteriophage resistant and to maintain consistent genetic characteristics, in order that the fermentation product is of consistent quality.

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For interest:

small <https://www.cheeselinks.com.au/product/blue-camembert-fetta-kit/>

large <http://www.cheesemaking.com.au/product-category/cheese-yoghurt-and-other-ripening-cultures/>



Bacteriophages, and how to deal with them:



- Regular cleaning/disinfection in fermenter facility
- No residual fluids (milk, etc.) in pipes/fermenter that would allow bacteria and phage to grow or contaminate next batch
- Aseptic technique, heat treat fermenter tanks/pipes before use
- Use phage resistant starter cultures, and change/rotate starter culture strains regularly
- Monitor plant for phage active against starter culture strains
- If confirm a phage damaged culture, do intensive clean



Summary

In summary;

- We have looked at cheddar cheese as an example of a dairy fermentation that results in a hard cheese and how the primary starter culture is important in influencing the characteristics of the cheese.
- Secondary inoculation with other bacteria or moulds result in different styles of cheese for instance camembert and stilton.
- Starter cultures used for either primary or secondary inoculation need to be of high quality and obtained from a reliable source.
- Bacteriophage are a significant risk to starter cultures and can result in failure of fermentation