THE UNIVERSITY OF MELBOURNE

FOOD20006

Food Microbiology & Safety

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Foodborne diseases
Toxico-Infections

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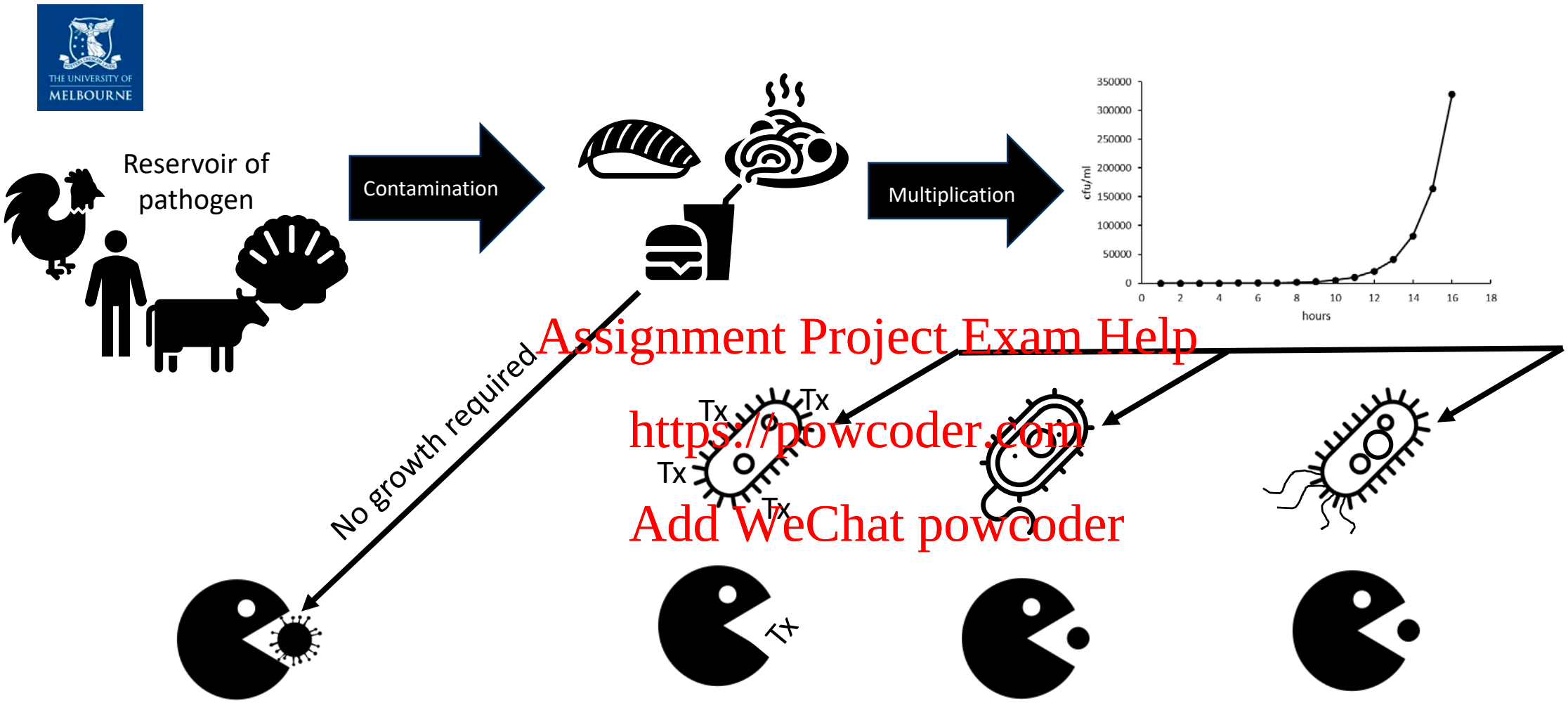
Intended learning outcomes

Understand the difference between a foodborne illness caused by a preformed toxin (intoxication), and by a pathogenic microorganism (infection) and a toxin producing infective pathogen (toxico infection)

Be able to describe the microbiological features of a toxicoinfections caused by *Bacillus cereus* and *Clostridium perfringens*



Examples of bacteria that cause toxicoinfections

Gastroenteritis caused by

Clostridium perfringens

Bacillus cereus

Gram positive, spore-formers

Vibrio cholerae

enterotoxigenic *Escherichia coli* (ETEC)

Gram negative, rods

Characteristics of foodborne toxico-infections

1. For spore-formers, ingestion of large numbers of live vegetative cells is usually necessary.
2. Vegetative cells of spore-formers do not multiply in the digestive tract but sporulate and release toxins.
3. For Gram-negative bacteria, live cells can be ingested in moderate numbers.
4. Gram-negative cells rapidly multiply in the digestive tract.
5. Many cells also die, releasing toxins.
6. Toxins of both groups produce the gastroenteritis symptoms.

Clostridium perfringens

- The cells are gram-positive, motile rods, spore-formers
- vary in size and can form short chains
- *C. perfringens* is anaerobic but can tolerate some oxygen.
- vegetative cells are sensitive to low heat treatment (pasteurization)
- the spores are extremely heat resistant
- In the presence of suitable substrates, H_2S is formed during growth.
- can grow very effectively in many protein foods.
- The temperatures of growth of vegetative cells and germination of spores and outgrowth range between 10°C and 52°C.
- The optimum growth occurs at approximately 45°C.
- cell multiplication can be very rapid, in approximately nine minutes.
- Does not grow well at pH < 5.0, in NaCl concentrations > 5%, A W < 0.93, and in 500 ppm nitrite.

C. perfringens toxin

There are five types of *C. perfringens* (A, B, C, D, and E) based on the production of four types of extracellular toxins (alpha, beta, epsilon, and iota).

Type A strains are predominantly involved in foodborne toxico-infection.

C. perfringens enterotoxin (CPE) associated with the foodborne disease, is a heat-labile protein

CPE is an intracellular protein produced by the cells during sporulation in the intestine and released.

The enterotoxin is produced in the digestive tract during sporulation.

The environmental parameters for the production of enterotoxin are directly related to the sporulation environment.

CPE toxin

CPE binds to intestinal epithelial cells, inserts into the membrane, and the toxin changes membrane permeability, resulting in loss of water, Na+, and Cl–.

CPE also causes epithelial cell death and leads to damage in microvilli, epithelial sloughing, and necrosis, further triggering fluid and electrolyte loss.

The symptoms appear 8–24 hours following ingestion of a large number of viable cells (≥5 ×

10^5/g) through a food.

The main symptoms are diarrhea and abdominal pain.

Symptoms generally disappear within 24 hours.

It is considered a mild disease and is seldom reported.

Culture of *C. perfringens*

C. perfringens requires some amino acids so it grows well on meat and animal products.
In the microbiology laboratory media must contain all the nutritional requirements and plates are incubated anaerobically.

TSC agar
Meat peptone, soy peptone and yeast extracts provide essential nutrients and vitamins for the development of clostridia. Sodium metabisulphite and ferric ammonium citrate act as an indicator of sulphide reduction, indicated by black coloured colonies.

Some strains of *C. perfringens* may produce an opaque zone around the colony due to lecithinase activity, but this is not considered to be universal for all strains after overnight incubation , and both black, lecithinase positive and black, lecithinase negative colonies should be considered as presumptive *C. perfringens* on TSC Agar and confirmatory tests carried out.

Bacillus cereus

Bacillus species are widely distributed in nature and food.

The *Bacillus cereus* group consists of several species including *Bac. cereus and Bac. anthracis*

The cells are Gram-positive motile rods, which form central endospores

Vegetative cells are sensitive to pasteurization.

Spores can survive the high heat treatment used in cooking

B. cereus growth and culture

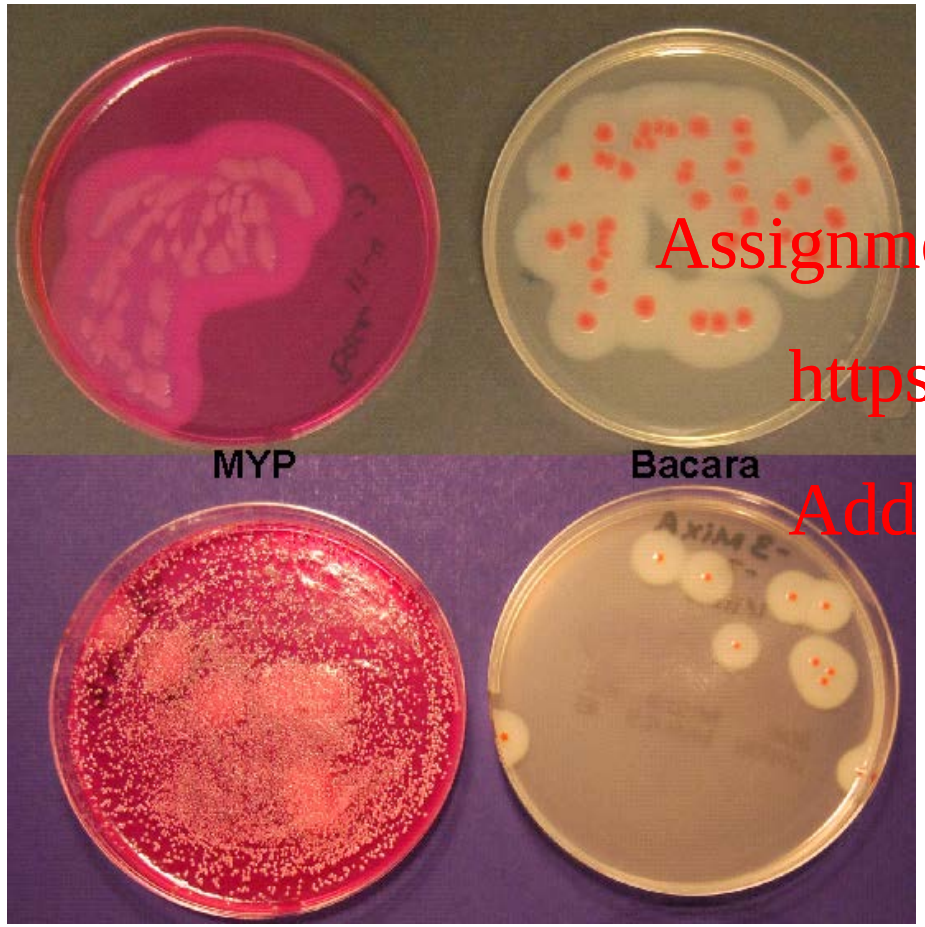
Bacillus cereus is an aerobic spore-forming bacterium that is commonly found in soil, on vegetables, and in many raw and processed foods.

The cells can multiply in a temperature range of 4°C–50°C with the optimum at approximately 35°C–40°C.

Other parameters of growth are pH of 4.9–9.3, A W of 0.95 and above, and NaCl concentration below 10%.

Most *Bacillus spp*. in the *Bac. cereus* group are motile except *Bac. anthracis*, and they form large colonies on agar plates.

Choice of medium



Colonies of *B. cereus* grown on MYP are pink and lecithinase positive, but other bacteria are not inhibited and can interfere with isolation of *B. cereus.*

Colonies of *B. cereus* grown on Bacara are pink-orange and are lecithinase positive, but other organisms are inhibited. Bacara is a chromogenic medium with a specific nutrient base and antibiotic specific mix allow extremely high selectivity

The laos around the colonies are due to lecithinase

Bacteriological Analytical Manual (BAM)
https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam

https://www.fda.gov/food/laboratory-methods-food/bam-chapter-14-bacillus-cereus

B. cereus food poisoning may occur when foods are prepared and held without adequate refrigeration for several hours before serving, with *B. cereus* reaching >10^6 cells/g.

B. cereus produces different toxins depending on the environmental conditons

- Emetic toxins cause vomiting
- Diarrhoeal toxins cause diarrhea

	Bacterial Growth		Emetic Toxin Production		Diarrhoeal Toxin Production	
	Optimum	Range	Optimum	Range	Optimum	Range
Temperature (°C)	30–40	4–55	12–15	12–37	32	10–43
pH	6.0–7.0	4.9–10.0	-	-	8.0	5.5–10
Water activity	-	0.93–0.99	-	-	-	-

FSANZ Bacillus cereus .pdf

2 disease syndromes

B. cereus causes two types of foodborne illness – emetic and diarrhoeal syndromes.

Emetic syndrome is an intoxication

- caused by ingestion of a toxin that is pre-formed **in the food** during growth by *B. cereus*
- symptoms of nausea, vomiting and abdominal cramping occur within 1–5 hours of ingestion, with recovery usually within 6–24 hours
- short incubation period and recovery time

The diarrhoeal syndrome is caused by enterotoxins produced by *B. cereus* **inside** the host.

- incubation period before onset of disease is 8–16 hours and the illness usually lasts for 12–14 hours, although it can continue for several days.
- mild symptoms with abdominal cramps, watery diarrhoea and nausea

B. cereus vegetative cells

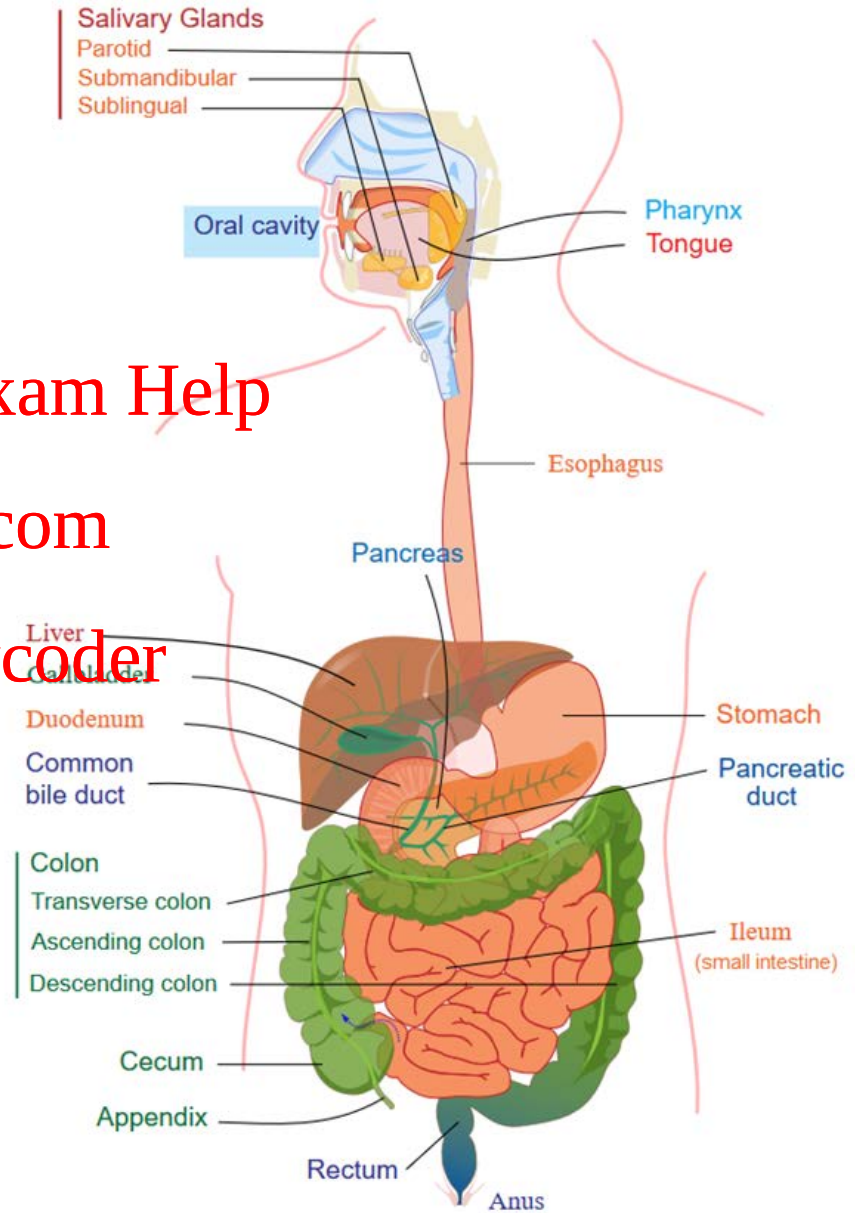
Pre-formed toxins trigger vomiting

About 25% of *B. cereus* vegetative cells can survive passage through the stomach

-survival rate of the vegetative cells is dependent on the strain) and the stomach pH

-diarrhoeal enterotoxins are unstable at low pH and are degraded by digestive enzymes

-any enterotoxins pre-formed in food would be destroyed during passage through the stomach



B. cereus spores

B. cereus spores are able to pass unaffected through the stomach

The spores contain receptors require certain low molecular weight substances to germinate

These inducers may be present in the food as well as the intestinal epithelial cells.

the spores germinate in the small intestine, grow and produce enterotoxins which cause the symptoms of diarrhoea