Spore forming pathogenic bacteria

Paul Gibbs
Paula Teixeira
Spore forming bacteria

- Basic characteristics
  - All species produce endospores that are heat, chemical and radiation resistant to different degrees.
  - Some species cause food poisoning / intoxication.
  - Some species cause specific types of food spoilage.
  - Several species cause disease of man and domestic animals.
Bacterial spores

• Dehydrated
  • therefore heat resistant.

• Dormant core containing essentials of a new cell (e.g. DNA, etc.).

• Cysteine rich proteinaceous outer spore coat that absorbs ionising radiation and is chemically resistant.

• Germination may require heat shock and specific compounds, e.g. alanine, lactate
Sporulation

1. Division of nuclear material

2. Septation of pre-spore nucleus

3. New spore membrane around nucleus

4. Synthesis of spore cortex, UV & chemical resistance, core dehydration

5. Coat synthesis, radiation resistance

6. DPA & divalent ion uptake, spore dehydration, refractility & dormancy completed; cell lysis to release spore.
Spore forming bacteria

- Species of concern to food microbiologists:
  - *Bacillus* spp; aerobic - facultative, sporulate only aerobically
  - *Clostridium* spp; anaerobic - aerotolerant, sporulate only anaerobically
  - *Desulfotomaculum* spp.
**Clostridium species**

- Gram positive spore forming rods.
- Anaerobic to aerotolerant.
- Sporulate anaerobically only
- Species of concern:
  - *Clostridium botulinum*.
  - *Clostridium perfringens (welchii)*.
  - *Clostridium difficile*?
Clostridium botulinum

• Seven toxin types
  • A - G.
• A, B, E, F
  • associated with human botulism.
• C, D
  • Group III - associated with animal botulism only (NP)
• A, B, F
  • Group I - Proteolytic, mesophilic
• B, E, F
  • Group II - Non-proteolytic, psychrotrophic
Clostridium botulinum - botulism

- Most severe form of food poisoning.
- Illness due to ingestion of toxin.
- Incubation period
  - 8 hours to 8 days.
- Lethal dose
  - 0.005 - 0.1 µg (proteolytic).
  - 0.1 - 0.5 µg (non-proteolytic).
- Mortality rate
  - 10% (if prompt treatment).
Clostridium botulinum - botulism

- Symptoms
  - generalised muscular weakness.
  - headache.
  - dizziness.
  - visual disturbances.
  - nausea.
  - vomiting.
  - difficulties with speech and swallowing.
  - descending paralysis.
  - respiratory failure.
Clostridium botulinum - foods involved

- Meat and meat products.
- Dairy products.
- Fish.
- Vegetables.
- Infant foods.
- Canned foods.
Examples of recent outbreaks

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>No. of cases</th>
<th>No. of deaths</th>
<th>Implicated food</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>Germany and Spain</td>
<td>6</td>
<td>?</td>
<td>Dried salted fish</td>
</tr>
<tr>
<td>2015</td>
<td>Ohio</td>
<td>29</td>
<td>2</td>
<td>Potato salad prepared with home-canned potatoes</td>
</tr>
<tr>
<td>2015</td>
<td>Portugal</td>
<td>4</td>
<td>0</td>
<td>Smoked sausages</td>
</tr>
</tbody>
</table>
A recent recall…

Illnesses reported; Loblaw expands recall of organic baby food

BY CORAL BEACH | FEBRUARY 10, 2017
‘Botulinum cook’

- A heat process giving a 12 log cycle kill of the spores of the most heat resistant *C. botulinum* strain.

- Commercially
  - equivalent to at least 3 minutes at 121° C at the slowest heating point in the container.
## Characteristics of *C. botulinum*

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Toxin types</strong></td>
<td>A, B, F</td>
<td>B, E, F</td>
</tr>
<tr>
<td><strong>Proteolytic</strong></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Inhibitory pH</strong></td>
<td>4.6</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Inhibitory NaCl conc²⁻</strong></td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Minimal a_w</strong></td>
<td>0.94</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Temperature range</strong></td>
<td>10 - 48 °C</td>
<td>3.3 – 45 °C</td>
</tr>
<tr>
<td><strong>D₁₀₀ of spores</strong></td>
<td>25 min</td>
<td>&lt;0.1 min</td>
</tr>
</tbody>
</table>
Characteristics of botulinal toxin

- Proteins; MWt ca 150kDa; heavy & light chains

- Neurotoxins

- H-chain binds to neurons; L-chain internalised; blocks acetylcholine release

- Specific peptidase activities on synaptic vesicle proteins
C. botulinum - control of psychrotrophic strains

- A heat treatment of 90 °C for 10 min or equivalent lethality.
- A pH of 5 or less throughout the food and throughout components of complex foods.
- A minimum salt level of 3.5% in the aqueous phase throughout the food and throughout the components of complex foods.
- An Aw of 0.97 or less throughout the food and throughout the components of complex foods.
- A combination of heat and preservative factors which can be shown consistently to prevent growth and toxin production by psychrotrophic C. botulinum at temperatures up to 10 °C.

ACMSF; 1992
C. botulinum - plating media

- Horse Blood Agar.
- Reinforced Clostridial Agar.
- Egg Yolk Agar.
C. botulinum - isolation from foods

- **Enrichment**
  - **self enrichment**
    - vacuum pack & incubate at 5-30 °C for >10 days if food will support good growth of the organism.
  - **enrichment media**
    - Robertson’s cooked meat medium.
**Clostridium perfringens**
Type A food poisoning

- **Incubation period**
  - 8 - 22 hours.

- **Symptoms**
  - diarrhoea, severe abdominal pain, nausea (occasionally)

- **Infective dose**
  - $>10^5$ cells/g

- **Mortality**
  - very rare.
Clostridium perfringens - foods involved

• Meat and meat products.

• Milk and dairy products.

• Fish and fish products.
## Characteristics of *C. perfringens*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature range</td>
<td>15 – 50 °C (optimum 43 – 45 °C)</td>
</tr>
<tr>
<td>Inhibitory pH</td>
<td>5.0</td>
</tr>
<tr>
<td>Minimal aw</td>
<td>0.95</td>
</tr>
<tr>
<td>Inhibitory NaCl concn</td>
<td>5%</td>
</tr>
<tr>
<td>$D_{95}$ of spores</td>
<td>1.3 – 6.4 minutes</td>
</tr>
</tbody>
</table>
Characteristics of *C. perfringens* enterotoxin (cpe)

- Cpe formed on sporulation in intestine
- Cpe a protein of *ca* 35kDa
- **Heat sensitive (60 °C / 5 mins)**
- Resistant to some proteases
- Initial binding to plasma membrane intestinal cells;
- Interaction with plasma membrane proteins ➔ leakage cell contents
Recent outbreaks…

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>No. of cases</th>
<th>No. of deaths</th>
<th>Implicated food</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>USA</td>
<td>&gt;20</td>
<td>3</td>
<td>Turkey and mashed potatoes</td>
</tr>
<tr>
<td>2013</td>
<td>UK</td>
<td>150</td>
<td>0</td>
<td>Chicken balti</td>
</tr>
<tr>
<td>2012</td>
<td>Norway</td>
<td>&gt;43</td>
<td>0</td>
<td>Beef stew</td>
</tr>
</tbody>
</table>
Clostridium perfringens - media and methods

- Direct plating
  - if suspected high numbers.

- Most probable number technique
  - if suspected low numbers.
C. perfringens - plating media

- Egg yolk free tryptose sulphite cycloserine agar (EYFTSC).
- Oleandomycin polymyxin sulphadiazine perfringens agar (OPSP).
- Shahidi Ferguson perfringens agar (SFP).
- Neomycin blood agar.
- Sulphite polymyxin sulphadiazine agar (SPS).
C. perfringens - plating media

- **EYFTSC**
  - Selective agents - Cycloserine.
  - Indicator system - Sulphide blackening.

- **OPSP**
  - Selective agents - Oleandomycin, polymyxin, sulphadiazine.
  - Indicator system - Sulphide blackening.

- **SFP**
  - Selective agents - Sulphadiazine, polymyxin, kanamycin.
  - Indicator system - Sulphide blackening, egg yolk.
C. perfringens - plating media

- Neomycin blood
  - Selective agents - Neomycin.
  - Indicator system - Haemolysis.

- SPS
  - Selective agents - Polymyxin, sulphadiazine.
  - Indicator system - Sulphide blackening.
C. perfringens - MPN media

- Differential Reinforced Clostridia medium (DRCM).

- LS medium.

- Iron-milk medium.
C. perfringens - confirmation

- Motility (non-motile).
- Nitrate reduction.
- Gelatine liquefaction.
- Lactose fermentation.
- LEYM agar
- Lactose gelatine medium.
- Nitrate, indole motility medium
**Clostridium difficile: Foodborne Transmission?**

- *Clostridium difficile* is a major cause of illness
- Initially recognized as an hospital pathogen
- Now recognized as an important cause of severe community acquired infections
- The source of community acquired *C. difficile* yet to be established
- Foodborne being one route considered
**C. difficile in foods**

- High prevalence of *C. difficile* in animals
- Identified in food products:
  - Meat
  - Fish
  - Produce
  - Water
- No foodborne illness outbreaks have been directly linked to *C. difficile*
C. difficile in foods: research needed…

- Available data cannot be considered a true prevalence
- Low levels of the pathogen in foods may require improved detection methods
- Knowledge gaps with respect to growth ranges of C. difficile in foods
Bacillus species

- Gram positive spore forming rod.
- Aerobic and facultatively anaerobic.
- Species of concern
  - *Bacillus cereus* and closely related species e.g. *B. thuringiensis*.
  - *Bacillus subtilis*.
  - *Bacillus licheniformis*. 
**Bacillus cereus** - two types of food poisoning

<table>
<thead>
<tr>
<th></th>
<th>Emetic</th>
<th>Diarrhoeagenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation time</td>
<td>1 – 5 hours</td>
<td>8 – 16 hours</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Nausea / vomiting</td>
<td>Abdominal pain / diarrhoea</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Infective dose</td>
<td>$&gt;10^5$</td>
<td>$&gt;10^5$</td>
</tr>
</tbody>
</table>
Characteristics *B. cereus* toxins; (1) emetic toxin, cereulide

- Ring-form peptide; hydrophobic; 3 repeats of 4 amino acids; MWt 1.2kDa.
- Heat stable 121°C / 90 mins.
- Acid stable pH 2 - 11.
- Protease stable (trypsin, pepsin).
- Binds to 5-HT$_3$ receptor (vagus nerve stimulated).
- Non-immunogenic
Characteristics *B. cereus* toxins; 
(2) diarrhoeagenic enterotoxin

- Excreted with a signal peptide
- May be at least two active moieties
- Protein sub-unit of haemolysin, MWt ca 38 kDa
- Mode of action unknown - binds weakly to ileal cells
- Immunogenic
**B. cereus - foods involved**

- **Emetic**
  - farinaceous materials especially
    - boiled rice.
    - cooked pasta.
    - noodle dishes.

- **Diarrhoeagenic**
  - wide variety of foods but commonly
    - meat and vegetable dishes.
    - soups.
    - sauces.
    - puddings.
Characteristics of *B. cereus*

- **Temperature range**: 4 – 50 °C (optimum 28 – 35 °C)
- **Minimum pH growth (Stability)**: 4.3 (Diarrhoeagenic toxin 4 – 11) (Emetic toxin 2 – 11)
- **$D_{95}$ spores**: 1.2 – 3.6 minutes
- **Minimum $a_w$**: 0.95 (0.91 – rice)
B. cereus - plating media

- Mannitol egg yolk polymyxin agar (MEYP).

- Polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA)
**B. cereus** - plating media
Selective and diagnostic components

- Polymyxin B
  - Selective agent.
- Mannitol and indicator
  - Differentiation.
- Egg yolk
  - Differentiation.
- Low level of peptone / absence of meat extract
  - Encourage sporulation
  - Inhibit lecithinase production by *B. polymyxa*. 
**Bacillus cereus - rapid stain**

- Prepare films from centre of 1 day old colony or edge of 2 day old colony.
- Air-dry and heat fix.
- Stain with 0.3% Sudan Black in 70% ethanol for 15 minutes.
- Wash slide with xylene for 5 seconds. Blot dry.
- Counter stain with 0.5% w/v Safranin for 20 seconds.
**Bacillus cereus** - rapid stain

- **Appearance**
  - Cells are 4-5 µm long by 1-1.5 µm wide.
  - Square ends with rounded corners.
  - Spores stain pale to mid green.
  - Spores are central or para-central in position.
  - Spores do not swell sporangium.
  - Lipid globules are black.
  - Vegetative cytoplasm is red.
Detection of *B. cereus* toxins

- **Diarrhoeagenic**
  - RPLA, TECRA.

- **Emetic**
  - Primate feeding tests.
  - HEp-2 cell assay.
Summarizing ‘Spore forming bacteria’

- **Basic characteristics**
  - All species produce endospores that are heat, chemical and radiation resistant to different degrees.
  - Certain species cause food poisoning / intoxication, some very severe
  - Some species cause specific types of food spoilage.
  - Several species cause diseases of man, insects and domestic animals.