WEBINAR ON PRESENCE AND CONTROL OF SPORE-FORMING BACTERIA

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Spore Forming Microbes in Spices

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Outline

- What are spore forming microbes?
- Why are they important in foods?
- Specific issues with spices
  - Foodborne illnesses
  - Spoilage
- Control measures
- Summary

What are spore forming microbes?

- Some, but not all microbes produce spores
- Spores are a survival mechanism for microorganisms, preserving the DNA of the cells
- Spores are ***much more*** resistant to environmental stressors than vegetative cells

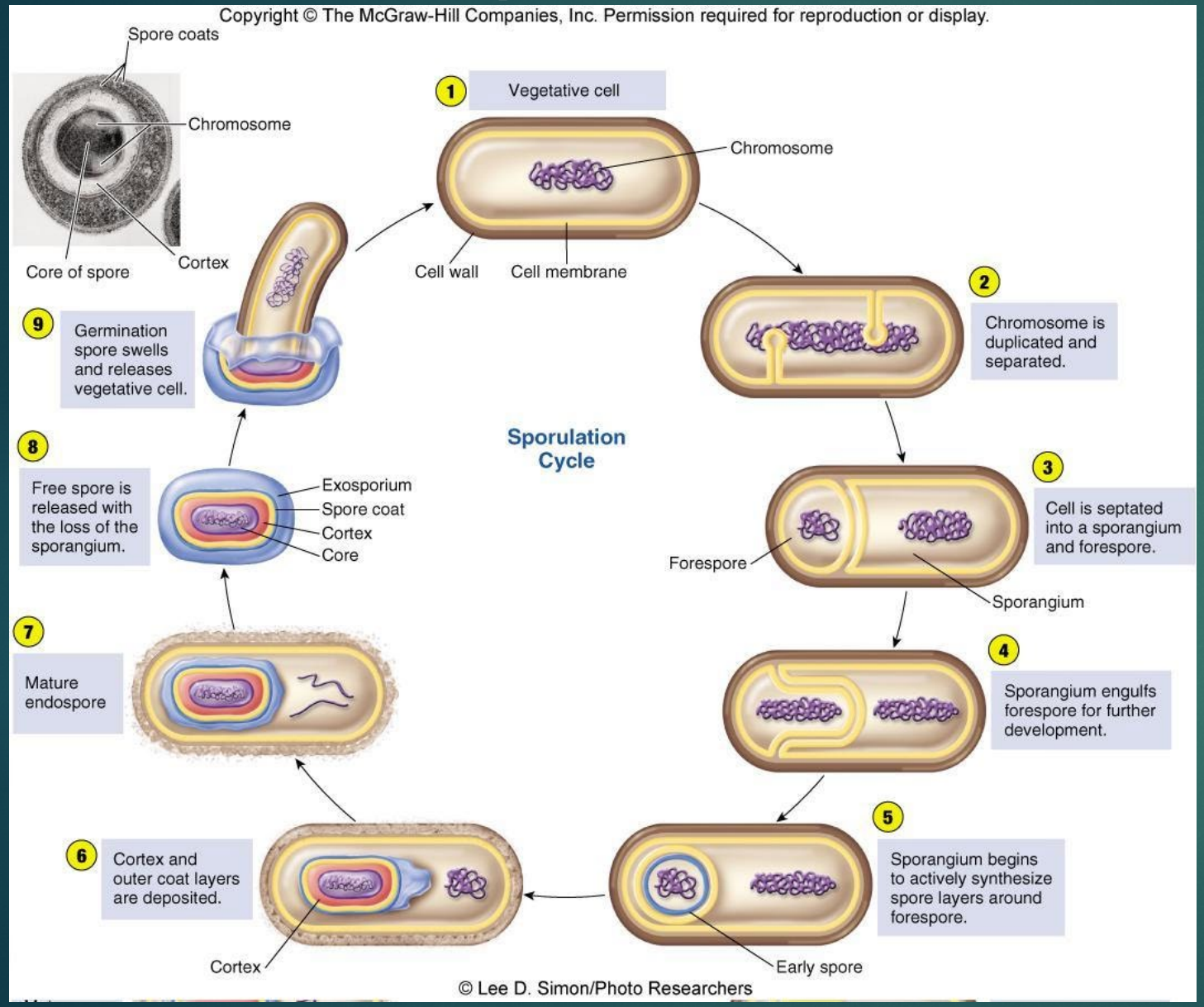
What are spore forming microbes?

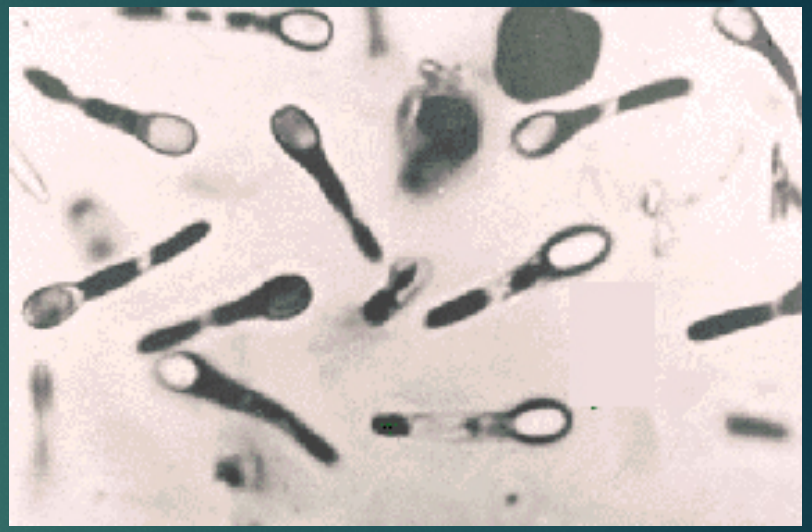
- Bacteria
  - *Clostridium*
  - *Bacillus*
- Fungi

Life Cycle – Bacterial Spores

- Vegetative bacterial cell exposed to environmental stress
- Cell produces spore
- Vegetative cell dies releasing spore
- Spore remains dormant until favorable growth conditions occur
- Spore germinates and becomes a vegetative cell

Bacterial Spores





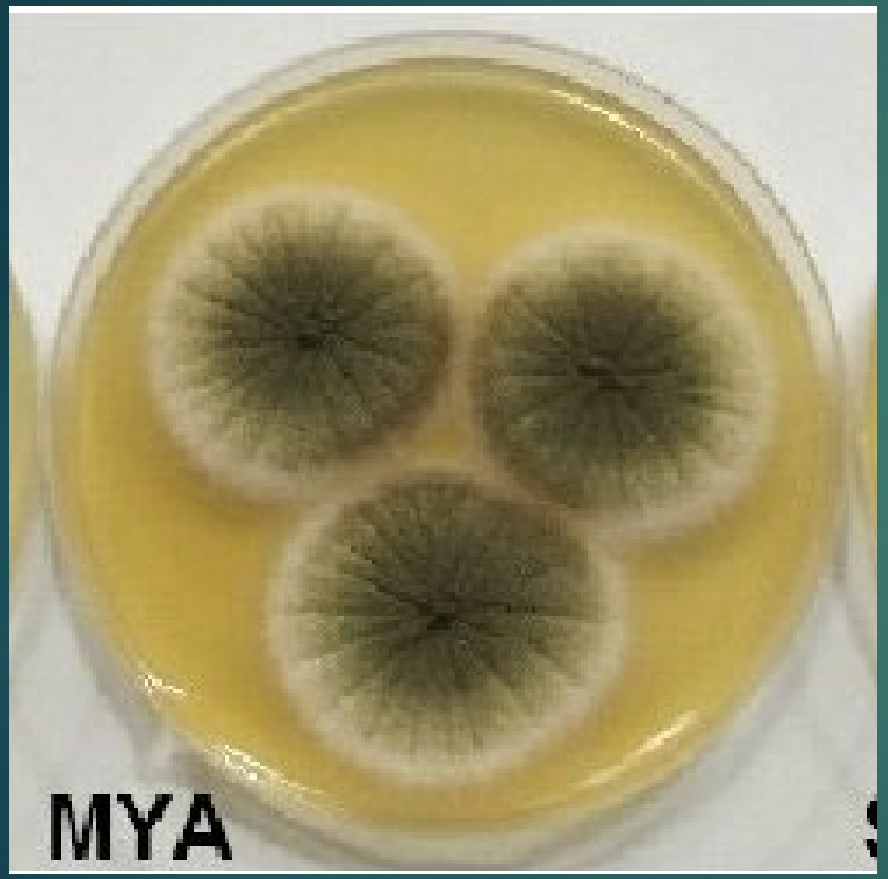
https://smartdogs.files.wordpress.com/2008/10/cdiffspores.jpg

http://classconnection.s3.amazonaws.com/1744/flashcards/859209/jpg/spore-formation.jpg

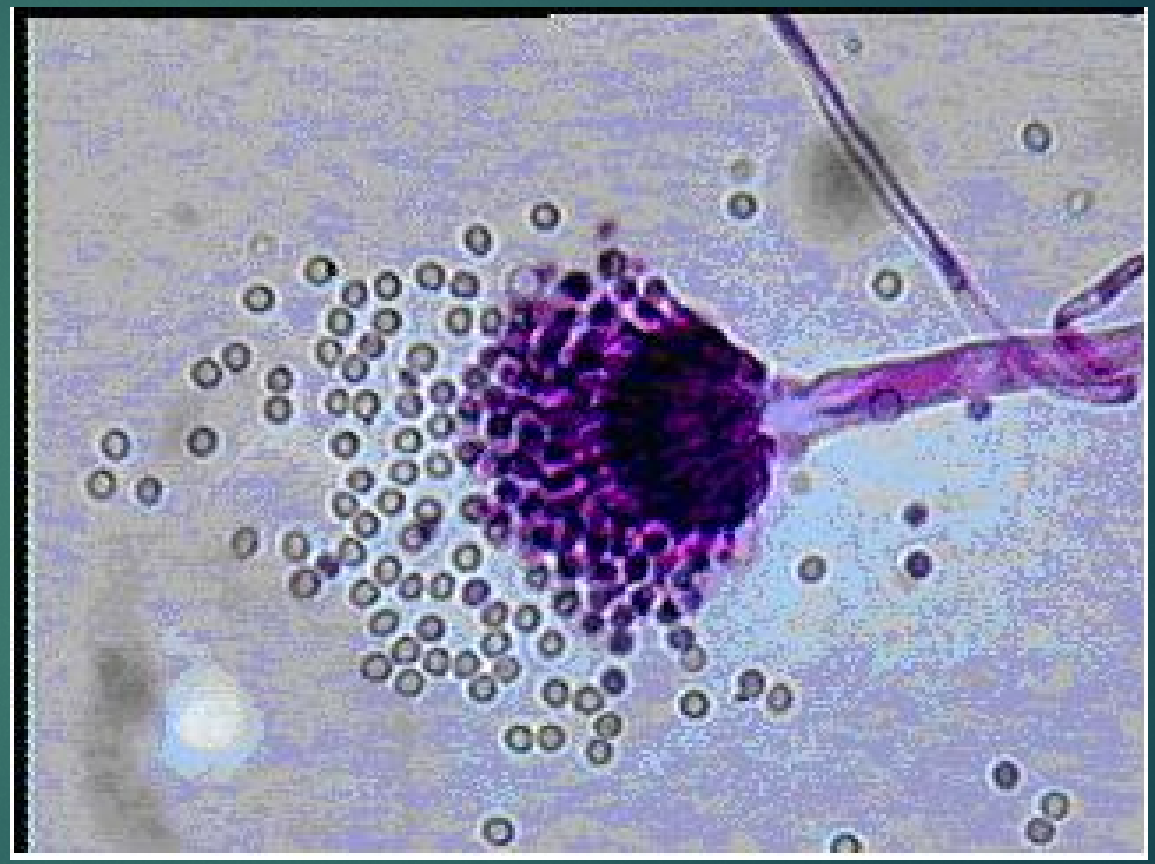
Life Cycle – Fungal Spores

- Mycelia of the mold normally develops into condiophore
- Condiophore produce spores
- Spores contain the genetic material necessary to produce new mold colony

Fungal Spores



https://www.researchgate.net/profile/Kamel_Abd-Elsalam2/publication/49655095/figure/fig1/AS:305789676867586@1449917348573/Cultural-characteristics-of-Aspergillus-niger-grown-on-potato-dextrose-agar-PDA-malt_Q320.jpg



https://aspergillusproject11.files.wordpress.com/2013/04/sporess.jpg

Effect of Environmental Conditions Temperature

- Spores require minimum temperatures to germinate
  - *Clostridium* – minimum temperature for spore germination ~10°C (J. Food Protect. (1981) 44(12):896-898.)
  - Fungi minimum temperature for spore germination <5°C (J. Stored Prod. Res. (1969), 5:127-141.)

Effect of Environmental Conditions Moisture

- Spores require moisture to germinate
  - *Clostridium* – minimum water activity for spore germination ~0.89 (J. Appl. Bact. (1967) 30(3):420-429.)
  - Fungi – minimum water activity for spore germination ~0.72 (J. Stored Prod. Res. (1969), 5:127-141.)

Effect of Environmental Conditions

- Environmental factors interact
  - Generally, as the temperature gets closer to the minimum temperature required for germination, the minimum water activity required for germination increases
  - Similar effects seen with water activity and temperature

Spore forming bacteria in spices

	Spore forming Bacteria (log CFU/g)					
	No. of samples	Mean	SD	Max	Min	Bacterial count of >1,000 CFU/g
Red pepper	59	1.91	1.51	5.61	<1.00	I3 (22.0)
Black pepper	42	2.57	1.96	6.76	<1.00	16 138.1)
White pepper	23	1.81	1.21	4.53	<1.00	5 (21.7)
Cumin	16	2.26	1.3	4.92	<1.00	4 (25.0)
Curry powder	14	4.33	1.64	6.3	1.18	10 (71.4)
Coriander	12	4.04	0.72	4.96	2.73	11 (91.7)
Fennel	9	1.5	0.56	2.3	<1.00	0 (0)
Paprika	7	1.97	1.74	4.66	<1.00	2 (28.6)
Cinnamon	7	1.16	0.36	1.78	<1.00	0 (0)
Japanese spice	7	1.53	0.64	2.65	<1.00	0 (0)
Garlic	6	1.11	0.39	1.9	<1.00	0 (0)
Garam masala	5	3.45	1.69	5.07	1.17	3 (60.0)
Clove	5	1.06	0.23	1.48	<1.00	0 (0)
Anise	4	1.95	1.43	3.9X	<1.00	1 (25.1)
Star anise	4	<1.00	0	<1.00	<:: I .00	0 (0)
Caraway	3	1.19	0.36	1.6	<1.00	0 (0)
Tunnerie	3	4.91	1.83	6.38	2.86	2 (66.7)
Fenugreek	3	3	1.7.3	4.02	1	2 (66.7)
Allspice	3	1.72	1.33	3.26	<1.00	1 (33.3)
Green pepper	3	<1.00	0	<1.00	<1.00	0 (0)

J. Food Protection 69 (10):2519–2523. 2006

Foodborne Diseases

- ***Clostridium***
  - ***botulinum***
  - ***perfringens***
- ***Bacillus cereus***

Foodborne illnesses associated with spices and spore forming bacteria

Case Study: Pathogens and spices

January 2016

Public Health Ontario

PARTNERS FOR HEALTH

https://www.publichealthontario.ca/-/media/documents/C/2016/case-study-spices.pdf?la=en

Foodborne illnesses associated with spices and spore forming bacteria

"B. cereus, C. perfringens and *Salmonella* spp. were the pathogens most commonly associated with spice/herb-related outbreaks."

Clostridium botulinum

- **Characteristics of the Microorganism:**
  - **Gram positive bacillus**
  - **Anaerobic**
  - **Spore forming**
  - **Commonly found in soil**
  - **produces a heat labile neurotoxin**

https://www.tehnologijahrane.com/wp-content/uploads/2008/03/clostridium.jpg

Clostridium botulinum Canned Chili

- As of August 24, 2007, eight cases of botulism have been reported to CDC from Indiana, Texas, and Ohio. The illness onset dates range from June 29 to August 7, 2007. All eight persons were reported to have consumed hot dog chili sauce made by Castleberry's Food Company. Botulinum toxin was identified in leftover chili sauce collected from this patient's refrigerator.

http://www.addictedtosaving.com/wp-content/uploads/2013/05/castleberrys-chili-hot-dog-sauce.jpg

Clostridium botulinum

- **Onset, Illness and Symptoms:**
  - **incubation 12 to 36 hours**
  - **Characterized by:**
    - **blurred vision, dry mouth, difficulty in swallowing, paralysis of respirator muscles**
    - **severe illness, recovery may take months or years**

Clostridium botulinum Canned Chili

- **Cause:**
  - **Process failure failed to control the hazard**

- **Contributing factors:**
  - **Spores in one or more of the ingredients**
  - **Can is a low oxygen environment**
  - **Cans have a long shelf life at room temperature**

Clostridium perfringens

- Spore former
- A “toxico-infection”
  - Self-limiting, non-febrile illness
  - Nausea, abdominal pain, diarrhea
  - Duration: 12-18 hours

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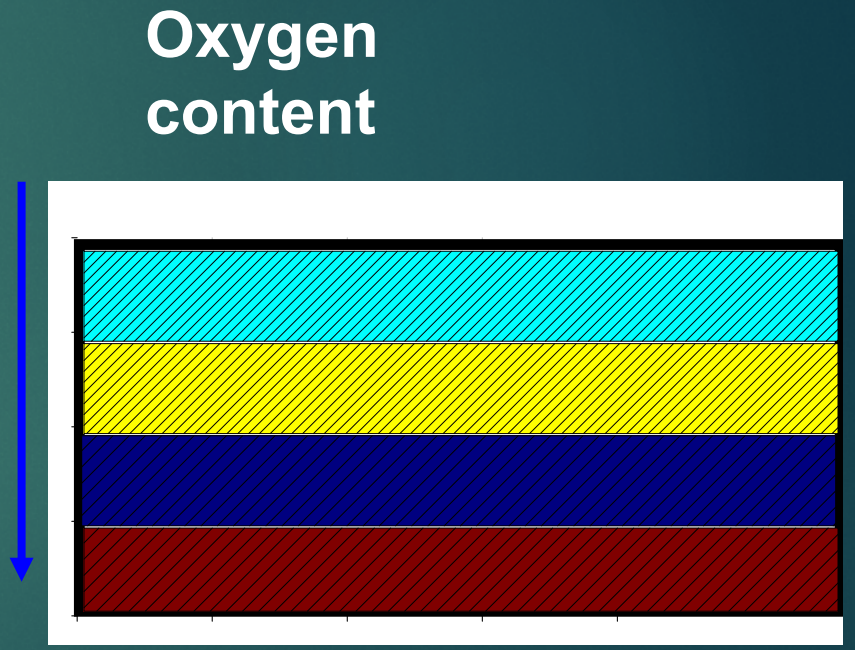
Outbreaks associated with spices and *Clostridium perfringens*

Vehicle	Microorganism	Year	Country	Cases	Source	Details
Barbecue spice	*C. perfringens*	2011	Denmark	4	EU line list 2011	Slow cooling – detected in chilled fried chicken and in barbecue spice
Red pepper spice	*C. perfringens*	2011	Denmark	37	EU line list 2011	Slow cooling – detected in red pepper used for stew
Dried chillies	*C. perfringens*	2011	Denmark	3	EU line list 2011	Slow cooling – detected in dried chillies
Pepper	*C. perfringens*	2011	Denmark	10	EU line list 2012	Slow cooling – stew with beef (veal) and pepper; *C. perfringens* 330 ml/g detected in stew and in pepper used for stew
Pepper	*C. perfringens*	2012	Denmark	9	EU line list 2011	Fried pork with parsley sauce. *C. perfringens* detected in pepper used in sauce.

Clostridium perfringens

- Physiology
  - Anaerobe but aerotolerant
  - Maximum growth temperature: 47°C (116°F)
  - Generation time at 43°C (109°F) – 7.1 minutes



Clostridium perfringens

- ***rapid reproduction*** **of *Cl. perfringens***
- **Generation time of 7.1 minutes at 43°C (109°F)**

- **100 cells double to 200 cells, 200 to 400, 400 to 800, 800 to 1600 etc.**

- **100 cells grow to 1.6 million cells in 1 hour and 40 minutes**

Report Reveals Source of Foodborne Illness Outbreak at Iowa High School

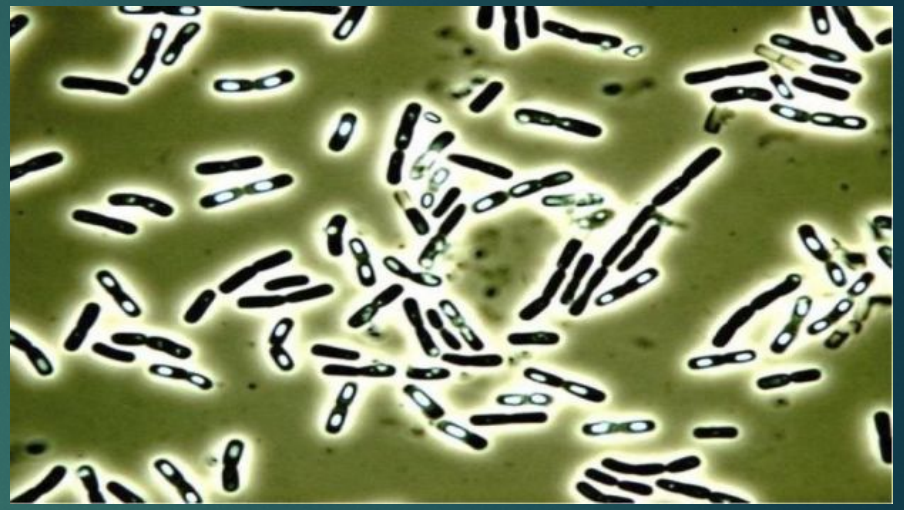
- A recent outbreak of foodborne illness following a catered staff luncheon at Roosevelt High School in Des Moines, IA, sickened at least 58 people and canceled classes for the rest of the day on Oct. 22. A subsequent investigation by state and county health officials, summarized in a report released Wednesday, Nov. 4, 2015, found that meat served at the luncheon was contaminated with *Clostridium perfringens*. The meat was reportedly purchased and brought in to be served to staff members at the school luncheon, although officials would not reveal the type of meat nor its source, quoting state regulations.
- The Polk County Health Department report noted that while C. perfringens can be killed by cooking, the spores can survive and grow into new cells. "If cooked food is not promptly served or refrigerated, the spores can grow and produce new cells. C. perfringens can grow quickly at room temperature but cannot grow at refrigerator or freezer temperatures," it stated.

Clostridium perfringens
Foodborne Illness Outbreak at Iowa High School

- What happened:
- *Cl. perfringens* spores were present in one or more ingredients
- Meat was adequately cooked
- Vegetative cells were killed; spores survived
- Meat held at too low a holding temperature
  - Spores germinated and grew
  - rapid reproduction of *Cl. perfringens*

Bacillus cereus

- Characteristics of the Microorganism:
  - Gram positive bacillus
  - spore forming
  - Commonly found in soil
  - produces two distinct illnesses
    - Diarrheal (similar to *Cl. perfringens*)
    - Emetic (similar to *Staph. aureus*; rapid onset)



https://s3-ap-southeast-1.amazonaws.com/bengkel/wp-content/uploads/2017/03/26135601/2025A83FF4724343AE8E018728F8F099.jpg

An outbreak of Bacillus cereus food poisoning--are caterers supervised sufficiently.

- ***Bacillus cereus*** **is an uncommonly reported cause of foodborne illness in the United States. An outbreak of *B. cereus* gastroenteritis occurred among 140 guests who had attended a catered wedding reception in Napa, CA. Investigation established Cornish game hens served at the event as the vehicle for disease transmission. Although the spores of *B. cereus* are ubiquitous, large numbers of toxin-producing organisms (more than 10^5 per gram of food) are required for illness to occur. In the Napa outbreak, bacterial multiplication was facilitated at several points during the preparation and transportation of the food. While a licensed restaurant kitchen was used, the facilities were clearly inadequate for the event.**

Public Health Rep. 1992 Jul-Aug; 107(4): 477–480

Bacillus cereus outbreak – Meat Pies, UK.

- Onset – 2 hours after consumption
- Food Item: two different meat and potato pies
- *Bacillus cereus* population in meat pie ~ 1.53 10^6 CFU/g
- Approximately 1 month later, two additional cases
- Four pies were collected
- *B. cereus* population ~ 10^8 CFU/ g

Journal of Food Protection, 79(5):781–788. 2016

Bacillus cereus outbreak Meat Pies, UK.

- What happened:
- *B. cereus* spores were present in one or more ingredients
- Meat was adequately cooked
- Vegetative cells were killed; spores survived
- Meat held at too low a temperature (<57ºC, 135ºF)
  - Spores germinated and grew
  - rapid reproduction of *B. cereus*

Food Spoilage - Bacteria

- ***Bacillus***
  - ***coagulans, stearothermophilus, thermoacidurans, laramie***
- ***Clostridium***
  - ***thermosaccharolyticum, bifermentans, sporogenes, butyricum***
- ***Alicyclobacillus acidoterrestris***

Food Spoilage - Bacteria

- May grow with (*Bacillus*) or without (*Clostridium*) oxygen
  - Cans, vacuum package
- Some can grow in acidic foods
- May produce:
  - Gas – swelling of cans or vacuum packages
  - Putrid odors

https://preparednessadvice.com/wp-content/uploads/2013/07/spam-bad.jpg

Food Spoilage - Fungi

- Molds
  - Require oxygen (strict)
- Yeasts
  - Can grow with or without oxygen

Food Spoilage - Fungi

https://vkool.com/wp-content/uploads/2014/06/moldy-foods.jpg

- Molds
  - Visible on foods
  - May produce “musty” odor or flavor
- Yeasts
  - Distinct “yeasty” odor
  - Typically produce gas (swollen package)
  - May produce alcohol

Control of Spores in Spices

- All currently used interventions are effective:
  - Gas sterilization
  - Steam
  - Irradiation

- ***HOWEVER***, spores are much more resistant
  - *B. coagulans* spores reported to be as much as 8 times more resistant to ethylene oxide than the vegetative cells

Control of Spores in Spices

Vegetative cells versus spores
Clostridium perfringens heat resistance

Cell Type	Temperature	D_{10} Value (minutes)	Time required for a 5 $\log_{10}$ reduction
Vegetative Cells	**65°C**	**0.8**	**4.0**
Spores	**90°C**	**30.6**	**153.0**

Byrne, Dunne and Bolton. 2006. Fd. Micro. 23(8): 803-808.

Control of Spores in Spices

Vegetative cells versus spores
Bacillus cereus irradiation resistance

Cell Type	D_{10} value (kGy)	Dose for 5 $\log_{10}$ Reduction
Vegetative Cells	**0.3 – 0.6**	**3.0**
Spores	**2.5 – 4.0**	**20**

Kamat, Nerkar, and Nair. 1989 J. Food Safety 10:31-41.

Summary

- Spores are commonly found in unprocessed spices
- Spores require appropriate environmental conditions to germinate and grow
- Spores in dry spices do not germinate
- *(continued)*

Summary

- Spores in spices have implications for both food safety and shelf life
  - When added as ingredients in further processed foods (high moisture foods)
- Spices processed to reduce spores must take into account the greater resistance of spores to interventions.
- *(continued)*

Summary

- **FDA's food safety priority for spices is *Salmonella***
  - ***However***
- ***FDA has mentioned potential concerns with spore forming bacteria***

Questions?

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Outbreaks associated with spices and *Bacillus cereus*

Outbreaks associated with spices, dried herbs recorded in publically available international foodborne outbreak database (PAIFOD)[8,21]

Vehicle	Microorganism	Year	Country	Cases	Source	Details
Spices	*B. cereus*	2007	France	146	Foodborne outbreaks in Europe 2007 - EFSA-ECDC report	Contaminated raw materials -spice blend in couscous dish - laboratory detection of the implicated foodstuff
Spices	*B. cereus*	2009	Belgium	7	The European Union Summary*	Curry - enterotoxin positive strain
Spices	*B. cereus*	2009	Denmark	48	The European Union Summary*	Rose-paprika
Spices	*B. cereus*	2010	Denmark	112	EU 2010 report	White pepper-temperature abuse during food preparation/storage
Spices	*B. cereus*	2011	Finland	4	EU line list 2011	Turmeric / curcuma -temperature abuse
Spices	*B. cereus*	2011	Finland	3	EU line list 2011	Jeera Ground Cumin - temperature abuse
Spices	*B. cereus*	2011	Finland	19	EU line list 2011	Turmeric / curcuma - temperature abuse, Detection of 5000 *B. cereus*/g. in cinnamon - slow cooling
Spices	*B. cereus*	2011	Denmark	30	EU line list 2011	Detection of 5000 *B. cereus*/g. in cinnamon - slow cooling
Spices	*B. cereus*	2011	Denmark	52	EU line list 2011	*B. cereus* detected in pepper - inadequate cooking
Spices	*B. cereus*	2013	Finland	4	Finland Annual Report 2013	Pathogen detected in food or component - symptoms and onset of illness pathognomonic to causative agent; storage time/ temperature abuse, unprocessed contaminated ingredient.

Hogan Lovells

Presence and Control of Spore-forming Bacteria

Webinar for the American Spice Trade Association

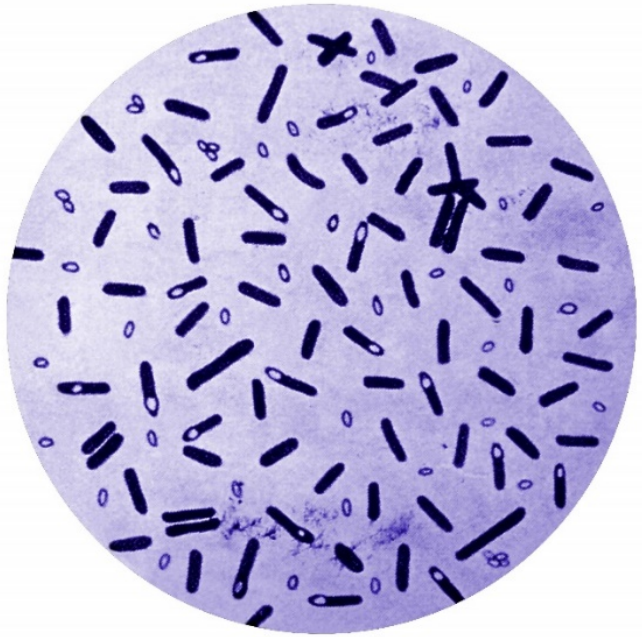
November 5, 2020

Spore Forming Bacteria in Spices

Question: How should spice manufacturers address spore forming bacteria ("spore formers") in their Food Safety Plans?

Answer: It depends.

Here's how to figure it out…



Preventive Controls for Human Food

FDA's PCHF rule requires facilities to:

1. Conduct a hazard analysis to identify and evaluate known or reasonably foreseeable hazards to determine whether there are any hazards requiring a preventive control; and
2. Identify and implement preventive controls to provide assurances that any hazards requiring a preventive control will be significantly minimized or prevented

Hazard requiring a preventive control: *"a **known or reasonably foreseeable hazard** for which a person knowledgeable about the safe manufacturing, processing, packing, or holding of food would, **based on the outcome of a hazard analysis** (which includes an assessment of the severity of the illness or injury if the hazard were to occur and the probability that the hazard will occur in the absence of preventive controls), establish one or more preventive controls to significantly minimize or prevent the hazard in a food and components to manage those controls (such as monitoring, corrections or corrective actions, verification, and records) as appropriate to the food, the facility, and the nature of the preventive control and its role in the facility's food safety system"*

Hazard Analysis

- Not all hazards are hazards requiring a preventive control
- The purpose of the hazard analysis is to do this sorting
- A hazard analysis must consist of two parts:
  1. Hazard identification
  2. Hazard evaluation

Hazard Identification

- **Hazard identification** must take into account:
  - Known or reasonably foreseeable hazards that include:
    - **Biological hazards**, including microbiological hazards such as parasites, environmental pathogens, and other pathogens;
    - Chemical hazards, including radiological hazards, substances such as pesticide and drug residues, natural toxins, decomposition, unapproved food or color additives, and food allergens; and
    - Physical hazards (such as stones, glass, and metal fragments); and
  - Known or reasonably foreseeable hazards that may be present in a food due to any of the following reasons:
    - The hazard occurs naturally;
    - The hazard may be unintentionally introduced; or
    - The hazard may be intentionally introduced for purposes of economic gain

Appendix 1

- FDA's draft Appendix 1 can assist in conducting a hazard identification
- Appendix 1 identifies *B. cereus* and *C. botulinum* as hazards for dried spices
- But **Appendix 1 is not definitive** on the issue of whether a hazard is significant enough that it's also a hazard requiring a preventive control
- Whether a hazard identified in Appendix 1 also is a hazard requiring a preventive control depends on the outcome of the **hazard evaluation**

Table 1P: Information that you should consider for potential ingredient or other food-related biological hazards for Spices and Herbs

Category	#	Subcategory	Storage Conditions	*Bacillus cereus*	*Clostridium botulinum*	*C. perfringens*	*Brucella* spp.	*Campylobacter* spp.	Pathogenic *E. coli*	*Salmonella* spp.	*L. monocytogenes*	*Shigella* spp.	*S. aureus*	*Giardia lamblia*	*Trichinella spiralis*	Example Products
Untreated, Raw Herbs and Spices	1	Dried, ground or cracked	Shelf-Stable	X	X	X			X	X						Cinnamon, Turmeric, Paprika, Pepper (Black, White, Red), Cayenne Powder, Cumin, Coriander, Mustard, Fenugreek, Horseradish, Fennel Seeds, Caraway, All-spice, Nutmeg, Ginger, Garlic (Minced or Powder), Onion (Minced or Powder), Celery Seed
Untreated, Raw Herbs and Spices	2	Dried, whole	Shelf-Stable	X	X	X			X	X						Cinnamon, Cardamom, Fenugreek, Cloves, Nutmeg, Cumin, Anise, fennel Seeds, Caraway, Chili Peppers, Saffron, Peppercorns (Black or White), Coriander, Basil, Varieties of Chili Peppers

Hazard Evaluation

- Hazard analysis must include an evaluation of the hazards identified to assess:
  - The severity of the illness or injury if the hazard were to occur; and
  - The probability that the hazard will occur in the absence of preventive controls
- The **hazard evaluation** must consider the effect of the following on the safety of the finished food for the intended consumer:

- **The formulation of the food**;
- The condition, function, and design of the facility and equipment;
- Raw materials and other ingredients;
- Transportation practices;
- Manufacturing/processing procedures
- Packaging activities and labeling activities;
- Storage and distribution;
- **Intended or reasonably foreseeable use**;
- Sanitation, including employee hygiene; and
- Any other relevant factors, such as the temporal (*e.g.*, weather-related) nature of some hazards (*e.g.*, levels of some natural toxins)

What It Means for Spore Formers In Spices

- Your hazard analysis should identify spore formers as a potential hazard in its **hazard identification** because this hazard is identified in Appendix 1
- Based on your **hazard evaluation**, you may be able to conclude that spore formers are not a hazard requiring a preventive control for dry matrixes
  - Dr. Dickson's presentation demonstrated how you could reach this conclusion:
    - Spores need certain conditions to germinate (e.g., temperature, moisture)
    - Spores cannot germinate in dried spices
- If you conclude that spore formers are not a hazard requiring a preventive control, there is no need to implement a preventive control
- If you conclude that spore formers are a hazard requiring a preventive control, then you need to implement a preventive control or provide a written disclosure to your commercial customer
- Whatever you decide, **you must document your analysis and conclusion**

Food for Thought

- What about your customers' use of the product in a wet matrix?
- Hazard analysis requires consideration of known or reasonably foreseeable use
- Though spores cannot germinate in dried spices, they could be used in foods that would provide conditions where spores could germinate
  - If you know your commercial customer will use the spices in a wet matrix (e.g., pasta sauce or dip), can you conclude spore formers are not a hazard requiring a preventive control?
  - If you know consumers will use jarred spices in a wet matrix, can you conclude spore formers are not a hazard requiring a preventive control?

What To Do When Spore Formers Are a HRPC

- If you conclude spore formers are a hazard requiring a preventive control, you have two options:
  - Develop and implement a preventive control to significantly minimize or prevent the hazard; or
  - Provide a written disclosure to your commercial customer that the spices have not been processed to control spore formers
- Requirements for written disclosure in 21 C.F.R. § 117.136
  - Disclosure must be included in documents accompanying the food, in accordance with the practice of the trade
    - Food is "not processed to control [identified hazard]" (e.g., "not processed to control for *B. cereus* and *C. botulinum*")
  - ~~*Annually obtain written assurance that the customer or downstream entity is controlling the hazard*~~
    - FDA currently exercising enforcement discretion for written assurance requirement

Practical Considerations

- Commercial customers have their own obligation to perform a hazard analysis, and they also will look to Appendix 1 for guidance about the hazards in the spice ingredients they use
  - They need to assess whether spore formers are a hazard based on how the spices are used in their product
- Even if not required by the PCHF Rule, spice manufacturers may always choose to disclose that spore formers are a hazard when spices are used in some matrixes as an extra precautionary measure
  - If something goes wrong with your customer, you will have disclosed the hazard to them
  - There are commercial considerations about making such a disclosure voluntarily

Precautionary Note

- It is possible FDA could disagree with a hazard analysis that concludes spore formers are not a hazard requiring a preventive control in dry matrixes
  - May take the view facilities must disclose that the spices are not treated to control spore formers based on foreseeable use in wet matrices
- FDA's attention to and position on this issue also could evolve with time
- Spice manufacturers may need to modify their Food Safety Plans based on input from FDA during future inspections
  - Appendix 1 also is still in draft and could evolve
- Whichever way you go, it is important for the hazard analysis in your Food Safety Plan to include a written justification for your approach

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Questions

THANK YOU!