Spore Forming Bacteria-A Major Cause of Food Spoilage and Outbreaks: A Comprehensive Review

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Abstract

Spore-forming bacteria, particularly pose a significant threat to food safety as they show resistance to various physical and chemical treatments. Their presence can reduce the usability of food materials and result in spoilage. Food can be spoiled by various bacteria, including spore-producing ones such as *Clostridium botulinum* and *Bacillus cereus*. These bacteria can cause significant damage to the nutritional value and physical characteristics of food. This review illustrates the spoilage of different food products, with a particular focus on bakery items and dairy products and the outbreaks of specific bacteria that cause spoilage and the conditions conducive to their growth. According to the text notes in 1980s, the spoilage of bakery items was linked to *Bacillus subtilis* and *Bacillus mesentericus*. In the dairy industry, the text outlines the difficulties posed by spore-forming bacteria, specifically Bacilli and Clostridia that can enter the item from different sources along the prolongation of milk-process and affect product safety and quality. In fresh fruits and their juices *Bacillus coagulans* and *Clostridium butyricum* are seen. Different *Clostridium species* deteriorate vacuum-packed meats. *Moorella thermoacetica*, *Thermoanaerobacterium spp*, and *Geobacillus stearothermophilus* are mostly isolated in low-acid canned foods. *Clostridium botulinum*, *Bacillus cereus* and *Clostridium perfringens* are involve in major foodborne outbreaks and causing great destructions. Most of the outbreaks occur due to the food which is served in catering settings.

Keywords: Spore-forming bacteria, Food spoilage, Outbreaks, Food Safety

Cite this Article as: Nadeem H, Zaid M, Sajid M, Jameel N, Shahid E, Bukhari SS, Saleem A, Maqbool M, Bashir S and Toqeer SR, 2025. Spore forming bacteria-a major cause of food spoilage and outbreaks: a comprehensive review. In: Farooqi SH, Kholik K and Zaman MA (eds), One Health Horizons: Integrating Biodiversity, Biosecurity, and Sustainable Practices. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 65-72. https://doi.org/10.47278/book.HH/2025.94

A Publication of Unique Scientific Publishers

Chapter No: 25-009

Received: 08-Feb-2025
Revised: 21-March-2025
Accepted: 07-May-2025

Introduction

People need food for energy, nourishment, and health. Food has many ingredients that prestige health (Fieldhouse, 1995). Many vitamins and minerals are present in fruits, vegetables and grain that are vital for the body's systems to work properly. Antioxidants and fiber are also present in it which reduces the risk of diabetes, heart disease and cancer (Aune, 2019). Dairy items involve milk, yogurt, cheese etc. which provide calcium and other minerals to increase bone strength and promote healthy teeth (Saleem, 2023). Daily health also includes protein in food that is important for tissue growth and repair. As food is beneficial for health, so its safety is also important to sustain life and promote health. Drying, Freezing, Pickling, Salting, maturing etc. were the traditional methods follow in past to store food. Besides food preservation there are some bacteria, viruses and fungi that are still responsible for spoilage of food. Food loss has become a global issue in recent years. 88 million tons of food is dumped yearly in the European Union (Chernova et al., 2020). Spore forming bacteria are a key factor of worry for different food innovative organizations as these bacteria has proven to be a great threat in heat treated food plants.

Factors Promoting Food Spoilage

Food can be destroyed by a variety of processes, such as aggressive, anaerobic, or facultative oxygen-consuming bacteria, when spores are present (Hamad, 2012). Food contamination can be caused by bacteria like *Bacillus cereus* and *Clostridium botulinum*. Some catalysts are produced by *Geobacillus*, *Clostridium butyricum*, and *Stearothermophilus* and completely separate the food molecule, causing continuing contamination. These spores give rise to microscopic organisms that destroy the edibles' nutritional value, usability, and physical characteristics (Bibi, 2022). Figure 1 shows the factors which are helpful in promoting spoilage of food.

Spore germination and outgrowth are the cause of food degradation and important components in the non- stability and stability of cooked foods (André et al., 2017). The texture, flavor, pH, and gas generation of spoiled food vary depending on the organisms and food

matrix (plant or animal), primarily (Rawat, 2015). The overview explains the variety of food deteriorating microorganisms (including those that affect canned goods, meat and dairy items, and bread-making).

Spoilage of Different Food Products

The list given below names the different food products that are spoiled by various spore forming bacteria.

Bakery Items

During early 1980s, different species of *Bacillus mesentericus* and *Bacillus subtilis* were being found in bakery products (Saranraj & Sivasakthivelan, 2015). Discolored crust, bad odor and a spongy cookie are the usual symptoms of spoilage. The presence of the *Bacillus spp.* in the raw material of food is what spoilage includes. Bread is the food that is most frequently consumable item on the world. Production of considerable amount of bread is through different pastry shops (Pereira et al., 2020; Rahman et al., 2022). Extensive production methods are rarely followed and we accepted the sweet smelling readily available organic products which is when microbes start spoiling the bread. Hence, causing the food spoilage state known as Rope (Rosell et al., 2015; Sharma et al., 2021). Fruity sweet smell basically describes the rope. It takes around 5 days to create Rope (Cizeikiene et al., 2013). In simple terms Rope is when enzymes debase the pieces of bread which is carried out infrequently by *Bacillus pumilis, Bacillus cereus* and *Bacillus licheniforms* and a few unknown bacteria. Warm and moist conditions are a requirement of by and large Bacillus spores and they can be withdrawn from pastry kitchen climate. spoilage microscopic organisms can be explained by increasing the amylase and protease creation status of rope and bread (Valerio et al., 2012; Saranraj & Sivasakthivelan, 2015; Pacher et al., 2022). Rope development requires a few key factors such as 35-45°C, high moisture area which is easily found in stuffed bread and a high pH 5.3.

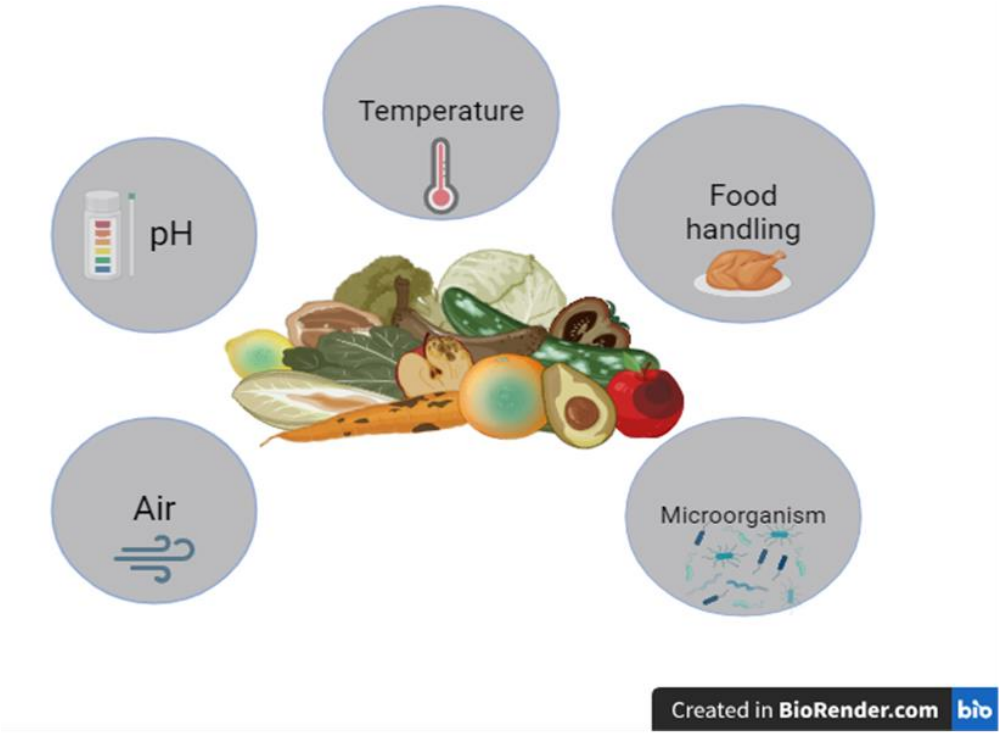

Fig. 1: Factors promoting food spoilage

Dairy Products

Due to the ability of spores to transform into dormant state and survival in harsh environmental conditions, spore-forming bacteria are the most difficult to eliminate due to their diversity in dairy chain (Rödel & Lücke, 1990). *Thermolithobacteria*, *Erysipelotrichia*, *Bacilli*, *Negativicutes* and *Clostridia* are majorly involved in this spoilage. *Bacillus* spp. and *Clostridium* spp. are everywhere in nature and may penetrate the item from various sources during the process of milk-processing, grow at refrigeration temperatures and compromise the product safety and quality. In pasteurized milk, *Bacillus* spp. and *Paenibacillus* spp. are the main psychrotolerant spoilage microorganisms (Griffiths et al., 1987; Gopal et al., 2015). In thermal treatments during extended shelf-life (ESL) *Bacillus* spp. can be present but *Paenibacillus* spp. emerged as a spoilage microorganism particularly attributed to ESL milk (Huck et al., 2007). To limitize the growth of *Paenibacillus* spp. Rysstad and Kolstad suggested that ESL milk should be preserved at ≤6 °C. In addition, some spores of *Paenibacillus* spp. can withstand temperature till 130°C (Rysstad & Kolstad, 2006; Lindsay et al., 2021). As Ultra-High Temperature (UHT) milk is purely a hygienic product, spores isolated from it mostly belong to *Geobacillus stearothermopillus* and *Bacillus sporothermodurans*. UHT milk during thermal processing prefers the growth of bacteria in raw milk (Ledina et al., 2021).

In cheeses, *Clostridium* species in phylogenic group I can cause hard cheese to crack and these are responsible for 'late swelling' spoilage (Lycken & Borch, 2006; Doyle et al., 2015). *Clostridium sporogenes*, *C. tyrobutyricum*, *Clostridium butyricum* and *Clostridium beijerinckii* are mostly seen in this spoilage (Goudkov & Sharpe, 1966).

Fresh Fruits and their Juices

Bacillus coagulans, *Clostridium butyricum* and *Clostridium pasteurianum* are isolated from fresh fruits and their juices (Dash et al., 2022) . Fruit juices mostly have low pH and high sugar this favors the growth of acid tolerant bacteria. Off flavors, cloudiness and pellicles on surface are mostly seen in this spoilage (Aneja et al., 2014). *B. coagulans*, is responsible in spoilage of tomato juice canning and can cause "flat sour spoilage". High amount of CO_2, hydrogen and strong butyric odor generates due to spoilage from anaerobic strains of *C. butyricum* and *C. pasteurianum*. From most of orange juice samples *Bacillus cereus* was identified (Rawat, 2015). *Alicyclobacillus* are the primary acidophilic spore-forming microorganism which is a major threat for fruit juice industry globally as they have the ability to contaminate pasteurized fruit juices and can withstand after pasteurization and when conditions are advantageous, can germinate again (Ağçam et al., 2018).

Shellfish (molluscs and crustacea)

The bacteria *Clostridium botulinum* type is mostly detected from marine environment (Sourri et al., 2022). Other bacteria, such as *C. perfringens* and *Bacillus cereus* are rarely seen in seafood-borne diseases. These organisms can be found in all atmospheres, mainly in soil and occasionally in an animal's gastrointestinal tract. In order to keep shellfish from spoiling, safety precautions like canning, vacuum packaging, and modified-atmosphere storage have been introduced. *C. botulinum* produces toxins at 3°C. Warm-blooded animals' in intestinal tracts frequently include *C. perfringens*, which has been proposed for use as a marker of fecal contamination in estuarine environments (Kishore & Laly, 2017). Table 1 shows the bacteria which are involve in the spoilage of different food products.

Vacuum-packed Meat

Clostridium was detected by McBride (1911) and next Sturges and Drake (1926) as a responsible factor for contamination in chilled meat. The specific specie responsible in spoilage of vacuum packed products is *Clostridium putrefaciens* by producing gas in such products (Rajkovic et al., 2020). *Clostridium bowmanii, Clostridium gasigenes, Clostridium estertheticum, Clostridium firgoris, Clostridium algidicarnis, Clostridium firgidicarnis* and *Clostridium algidixylanolyticum* are mostly seen (Sturges & Drake, 1927; Adam et al., 2013; Palevich et al., 2021). Spoilage of pressed meat shows typical signs such as bad smell, tone and surface loss of meat along with prompt swelling up (Silva et al., 2011). Based on temperature and their developmental factors, *Clostridium* is divided into two groups named as psychrotrophs and psychrophiles (Januškevičienė et al., 2012). These species are famously difficult to deal with.

Canned Products

Food canning stores food by creating pH reliant results but due to mishandling, defective containers, improper transportation and packaging different bacteria grow in them (Yang et al., 2009). Globally fair pH is 4.6 or in Europe 4.5. Above this pH, canned items are nondestructive and botulism risk is insignificant. Canned products being under the pH of 4.6 are considered to be destructive because of acidic nature and enhancement might be satisfactory. A solitary acidophilic spore making assortment can cause rot (Oranusi et al., 2012). Canned products owe their destruction to the heat resistant microorganisms forming spores.

Table 1: Bacteria involve in spoilage of different food products.

Food products	Bacteria involve
Bakery items	*Bacillus mesentericus*, *Bacillus subtilis, Bacillus licheniformis* and *Paenibacillus* (Friedmann, 2020)
Milk	*Bacillus* spp., *Paenibacillus* spp, *Pseudomonas* spp (Cizeikiene et al., 2013).
Cheese	*Clostridium beijerincki, Clostridium sporogenes*, *C. tyrobutyricum* and *Clostridium cochlearium* (Machado et al., 2017).
Fresh fruits	*Bacillus coagulans, Bacillus cereus*, *Campylobacter jejuni*, *Clostridium butyricum* and *Clostridium pasteurianum*, *Salmonella* spp., *Shigella* (Oliveira et al., 2016).
Sea food	*Clostridium botulinum* type E, *C. perfringens* and *Bacillus cereus*, *Escherichia coli*, *Salmonella, Enterococcus*, *Klebsiella* (Balali et al., 2020).
Vacuum pack meat	*Clostridium putrefaciens*, *Clostridium bowmanii*, *Clostridium algidixylanolyticum*, *Clostridium frigidicarnis, Clostridium figoris*, *Clostridium gasigenes* (Parlapani, 2021).
Canned food	*Alicyclobacillus, Desulfutomaculum nigrificans* (Húngaro et al., 2016).

Outbreaks

In 460 B.C. the connection of food consumption and human illness was reported by Hippocrates. Some species of spore-forming bacteria like *Bacillus cereus* and *C. perfringens* are involve in major outbreaks (Maqbool et al., 2023). Food poisoning occur due to spoilage of food, is a major public health problem and is even life threatening. As declared by WHO, every year 600 million people got affected with food poisoning and 420,000 died (Oliver & Gregory, 2015). To identify the risk associated with relevant food category it is an utmost need to check the recorded data regarding foodborne outbreaks but there is a gap in this approach as many countries do not report the cases, some countries are unable to monitor foodborne illness due to lack of microbiological and epidemiological evidence and some countries that are reporting but that is not sufficient to declare an outbreak.

Spices and Herbs

Large number of outbreaks occurs due to spices and herbs because of their worldwide usage as these are the main ingredients in every food but are under reported that's why these types of outbreaks are challenging. *Bacillus cereus* and *C. perfringens* are majorly found in them. From 1973 to 2010, there were 14 illness outbreaks reported worldwide due to microbial contaminants in spices including Canada, New Zealand, France, Norway, Germany, France, U.S., Denmark and Serbia. A total of 1,946 human illnesses were reported, which included 128

hospitalizations and two deaths. 29% *Bacillus* spp. were detected as the main cause of outbreak. *Bacillus subtilis* and *Bacillus pumilus* were included with two small outbreaks of foodborne illness in 1995 and 1997. Four outbreaks of *Bacillus* spp. took place in Denmark, New Zealand and U.S. In 1995 in UK, through the consumption of lamb seekh kabab in which turmeric powder was used *Bacillus subtilis* and *Bacillus pumilus* were the causative agent. In 2010, *Bacillus cereus* was involved in white pepper outbreak in Denmark (Todd, 2020). From 1973-2012 nine outbreaks of spices contaminated with *Bacillus cereus* were reported in Europe which shows 50% of the total spices outbreaks (Van Doren et al., 2013). From January1,2005 - April1, 2015, 23 outbreaks were reported with spices and dried herbs which mostly included *B. cereus* and *C. perfringes* (Mathot et al., 2021b).

Bacillus Cereus

B. cereus is involved in many foodborne outbreaks especially in catering settings and most of the outbreaks occur due to caterings are mostly underreported and undiagnosed which is underestimating the customer's health (Mathot et al., 2021a). In 1967-68 in Trail a large outbreak occurred on New Year's Eve in Trail, 300-450 people who attended function occurred gastroenteritis (Glasset et al., 2016). In U.S from 1998-2008, 235 out of 13,405 *B. cereus* outbreaks were reported from all 50 states, Guam, District of Columbia, Republic of Palau and Puerto Rico due to the consumption of rice (Schmitt et al., 1976). In Netherlands (2006) 5.4% foodborne outbreaks were recorded and in Norway (2000) 32% outbreaks occurred. The causes of these outbreaks were pasta salad and spaghetti leftovers (Bennett et al., 2013). From 2000-2013 *B. cereus* outbreaks 1689 cases were reported in European union due to catering services (Bintsis, 2017). On 18th December a foodborne outbreak was reported in kindergarten school of Bangkok, Thailand in 2009 due to consumption of soup 20 cases were being reported (Osimani et al., 2018; Sornchuer & Tiengtip, 2021). The reports of outbreaks caused by *B. cereus* were found similar to the outbreak in a party on January 22, 2012 in Bari, Italy. The isolation was from basmati rice and fecal specimens. 12 cases were being reported out of 13 customers (Santayakorn et al., 2016). In France from 2007-2014, 74 outbreaks were reported (Martinelli et al., 2013). In South Korea 2010, 193 adults infected by drinking underground water and eating food that was prepared in cafeteria (Glasset et al., 2016). In 2016 in U.S, customers of a Mexican fast- food restaurant were infected from refried beans (Choi et al., 2011). In 2018 Beijing China, 209 university students got affected due to consumption of Canteen food (Bintsis, 2017). 20,000 people who attended a gathering got affected in a massive foodborne outbreak in 2019 in district Selangor Malaysia (Chen et al., 2019). On 20th July 2023 in Uganda, the health ministry was being informed by the food poisoning outbreak in Mukono district at secondary school 267 cases were reported due to the consumption of lunch that was cooked at that day (Rajakrishnan et al., 2022).

C. Perfringens

C. perfringens is the most common and second most leading cause of foodborne diseases in the US causing around one million illnesses each year. *C. perfringens* is reported voluntarily by health departments of local, state level and territorial level to the US. CDC via the Foodborne Disease Outbreak Surveillance System. Many outbreaks have been reported worldwide mostly related with contaminated food and poultry meat majorly served in caterings. In just 12 years from 1998 to 2010 there were 289 confirmed outbreaks with 15208 illnesses, 83 hospitalizations and eight deaths by *C. perfringens*. Each year the reported outbreaks would range from 16 to 31 with no trend over time. The annual outbreak associated number of illnesses ranged from 359 to 2173 and the median outbreak size was 24 illnesses. Restaurants (43%) were the most common setting of food preparation; other settings included catering facility (19%), private home (16%), prison or jail (11%), and other (10%). Among the 144 (50%) outbreaks attributed to a single food commodity, beef was the most common commodity (66 outbreaks, 46%), followed by poultry (43 outbreaks, 30%), and pork (23 outbreaks, 16%). *C. perfringes* outbreaks are quite regular and cause massive morbidity although they are preventable if there is prevention of raw meat and poultry products at farm or slaughterhouse. Furthermore, if the handling and preparation of these products is enhanced it can serve as prophylaxis (Namara et al., 2024). In June 2012, nursing home manager in North East of England stated to North East health protection (NE HPU) unit about the illness of many individuals who were living there and having diarrheal symptoms. NE HPU told environmental health officers at Redcar and Cleveland Borough Council (RCBC) and an investigation started with the collaboration of both institutes, they inspected kitchen hygiene, foodstuff, interviewed staff members about the preparation of food, many samples including water and food were taken and sent for analysis, swab samples including environmental and clothes were also taken and sent to laboratories for testing 15 cases were being reported during this outbreak due to the consumption of gravy, vegetable pie and mince (Grass et al., 2013). From 2013-2017, 141 *C. perfringens* have been found in forty two foodborne outbreaks in Paris region with the total of 1,267 human cases (Acheson et al., 2016). Ten states and local health departments in US, from 2015-2018 have reported 41 confirmed outbreaks of *C. perfringens* in retail food establishments (Mahamat Abdelrahim et al., 2019). In 2016 September, Connecticut Department of Public Health reported an outbreak of gastrointestinal infection occurred among people who shared catered lunch boxes (Wittry et al., 2022). On 27 June 2019, the Hellenic National Public Health Organization was being told by general hospital in Northern Greece of eight children with gastroenteritis, the children were handball athletes who took part in PanHellenic Athletic Championship of Handball hosted in a town of 1800 residents (Dolan et al., 2016). 30 people were affected from *C. perfringens* in May 2021, several gastroenteritis cases were reported among students and staff of a high school on a Greek island due to lunchboxes which were served by the catering company (Mellou et al., 2019). Most of the outbreaks occurred due to consumption of reheated meats, meat pies, and gravies.

Clostridium Botulinum

This bacterium produces many toxins and shows high resistance towards much food processing treatments (Papanikou et al., 2023). From 1990-2000 outbreaks occurred in United States 263 people got sick from 160 foodborne botulinum incidents. The average number of reported cases per year was 23. Alaska, Washington and Idaho were at highest risk of occurring disease (Dahlsten et al., 2015). In 2005, an outbreak occurred in a family in Anatolia due to intake of suzme yoghurt that was buried under soil (Sobel et al., 2004). A significant outbreak (209 cases) in Thailand in 2006 was linked to eating bamboo shoots that had been improperly home-canned (Akdeniz et al., 2007). Table 2 shows different outbreaks that occurred due to *Clostridium botulinum*. Eight instances of botulism were linked to improper thermal

processing of commercial hot dog chili sauce cans in the US in 2007, which resulted in the original recall of 39 million cans and the subsequent expansion to 111 million cans. Several major outbreaks of foodborne botulism, such as those linked to commercial chilled carrot juice and commercial chicken enchiladas, have also been linked to temperature abuse of items meant to be stored refrigerated (Wangroongsarb et al., 2013).

Table 2: Outbreaks of *Clostridium Botulinum*

Year	Country	Reason	Cases/ Outbreaks	References
2010	Thailand	Food items: meat, sausages, pickled vegetables	19 cases	(Sheth et al., 2008)
2011-2012	Poland (First Major Avian Type C outbreak)	Shallow water habitat	5500 birds in 2011 and 1600 in 2012	(Wangroongsarb et al., 2014)
2011-2013	France	Poultry house settings	17 outbreaks	(Radosław et al., 2014)
2015	Ohio	Potluck meal	29 cases	(Souillard et al., 2014)
2018	France (Cattle Botulinum outbreak)	Poultry manure	Clinical signs show paralysis 2 cow died	(McCarty et al., 2015)
2018	New York	Home- canned peas	3 women affected	(Souillard et al., 2021)
2004-2020	China	Household food products	80 outbreaks	(Bergeron, 2019)

Conclusion

Hence, food spoilage from spore forming bacteria is a major topic to talk about. Seeing it from economic as well as health point of view the spore forming bacteria are causing major outbreaks in different countries alongside the destruction of food and feed industries. This review elaborates that how different spore forming bacteria spoil different food products and causing large outbreaks. To examine the possible uncultivable microbial variety of this ecosystem, additional molecular investigation is needed. Classification of food spoilage bacteria is, however, a developing field. Sterilization was once considered the appropriate method for food preservation, but today with advancements in technology and on high costumer's demand of organoleptic properties of food the food company Owners are upgrading their machinery, their strategies, by not providing the environment to spore-formers to grow and by strictly following protocols in order to fulfill the requirements of customers and to prevent from further foodborne illness outbreaks.

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