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Bacillus species (not *anthracis*)

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Abstract

Bacteria of the genus *Bacillus* are widely distributed in nature, useful in agriculture and industry, and occasionally directly harmful to humans. The uniqueness of *Bacillus* relates to its ability to produce spores that can survive desiccation, heat, and cold and can germinate readily. The toxins that cause anthrax and food poisoning are encoded by plasmid-borne genes, allowing different species to have strikingly similar chromosomal DNA despite radically different phenotypes. *Bacillus* infections are uncommon and often associated with trauma or foreign bodies. Food poisoning caused by *Bacillus* spp. is much less common than other forms of bacterial food poisoning and almost always brief and self-limited. Treatment of *Bacillus* infections is usually straightforward once the species has been determined. Prevention of infection and intoxication is desirable, but the challenge of eradicating spores and destroying some heat-stable toxins reminds us how lucky we are that these infections and intoxications are rare.

Introduction

Bacteria of the genus *Bacillus* are ubiquitous in the natural environment, and as a result, they have a potentially important role as "contaminants," as well as pathogens, in the world of clinical microbiology (1). However, *Bacillus* species are so critical to agriculture, industry, and food safety that they deserve more attention than they currently receive. In addition, a critical concern for public safety makes it important for the clinical microbiology laboratory to recognize and report suspected *Bacillus anthracis* isolates and to be able to reliably distinguish them from clinical isolates of non-*anthracis Bacillus* spp. Finally, these organisms can, in fact, cause human infection despite the overall benign reputation of the genus.

Bacillus spp. are gram-positive or gram-variable, aerobic or facultatively anaerobic bacilli that have either rounded or squared off ends, form endospores, and tolerate extremes of temperature and moisture. They are easily cultured from soil (including wilderness, as well as cultivated land), and lake and ocean sediment (even in deep water), and they can be isolated from plants and animals. Their hardiness under extremes of desiccation and heat has been used to determine the efficacy of heat sterilization (*Bacillus stearothermophilus*) and fumigation procedures (*Bacillus subtilis*). Their phylogenetic taxonomy puts them in the phylum *Firmicutes*, class *Bacilli*, order *Bacillales*, and family *Bacillaceae*. There are no relatives in *Bacillaceae* of clinical importance other than *Bacillus* spp. The order *Bacillales* includes most of the familiar gram-positive human pathogens.

Genetics

There is still confusion about certain *Bacillus* species, as the genus is probably not monophyletic. Sophisticated genetic typing and discovery of new strains in different environmental niches (particularly at extremes of environmental temperatures) have shown a great divergence of strains types within the genus. Changes in the taxonomy of *Bacillus* species include the movement of *Bacillus alvei* into the genus *Paenibacillus* and placement of both *Bacillus brevis* and *Bacillus laterosporus* into the genus *Brevibacillus* (2). A single group, termed the *Bacillus cereus* group, includes *B. cereus*, *B. anthracis*, *Bacillus thuringiensis, Bacillus pseudomycoides, Bacillus weihenstephanensis*, and *Bacillus mycoides* based on their close genotypic similarity (3). This relatedness is reflected by the substantial amount of genetic and enzymatic heterogeneity within the species *B. cereus*. In fact, different strains within a species of the *B. cereus* group may be more closely related to other species in the group than to other strains within their own species. This

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suggests that the common ancestor of this group was most like *B. cereus* with the emergence of the other species at a later time and with *B. anthracis* developing last (4,5). Consequently, there is a very narrow range of diversity within *B. anthracis*, whose isolates occupy a tight band within the breadth of *B. cereus* group. The nearly identical DNA sequences and enzymes between *B. anthracis* and *B. cereus* may seem surprising insofar as there are great clinical and phenotypic differences between *B. cereus* and *B. anthracis*, but it reminds us how close a fairly innocuous organism and an important pathogen can be. Chromosomal DNA sequencing shows subtle distinctions within the group and indicates substantial lateral gene transfer, most likely through the acquisition of stable, integrated phages. Plasmids are also critical in determining the disease-causing potential of *Bacillus* spp. Less closely related species of *Bacillus* that may be encountered in the clinical microbiology laboratory are *B. subtilis*, *Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus*, and *Bacillus sphaericus*.

Commercial utility

Before exploring the clinical and important clinical microbiological aspects of *Bacillus*, it is useful to reflect on the important use of these bacteria and their products in everyday life. It is no exaggeration to state that *Bacillus* may be the single most diversely used organism across the planet. Complete genome sequences have been published for a number of strains of *B. anthracis, B. cereus,* and *B. thuringiensis* and at least one strain each of *B. subtilis* and *B. licheniformis*. The genome size varies from 4 to 5.5 million base pairs, suggesting both acquisition and incorporation of new DNA and the shedding of redundant genes as species evolve. The ease of growing *Bacillus* and the panoply of important enzymatic products generated by various strains is a formidable combination, but most critically, the low toxicity of these commercial strains makes the business of *Bacillus* fascinating and important.

B. thuringiensis, normally found in soil, is a natural insecticide. The bacterium creates a para-sporal crystal that is harmless to most plants and animals. However, when certain species of insects ingest the crystal encoded by the "*cry*" gene, the "protoxin" is converted by gut proteases into a powerful toxin. This toxin breaks down the lining of the insect gut, and this in turn kills the insect. Different strains of *B. thuringiensis* make crystals with differing insect toxicities, and over time, many of these have been used commercially. One way to disseminate this insecticide is to spray crops or decorative plants with living *B. thuringiensis*. Alternate ways include cloning the *cry* gene into other bacteria or directly into the plants! While this application sounds very modern, the origins of *Bacillus* bioremediation go back to France in 1938. Currently, *B. thuringiensis* products (referring to those with the *cry* gene) account for 1% of all agrochemicals sold commercially.

Some household uses of *Bacillus* products involve the commercial production of stable enzymes with substrates that include starches, proteins, and lipids. These enzymes provide benefits that include the enhancement of laundry and dishwasher detergents. While it is rare to see consumer products advertised lauding their bacterial enzymes, many of the "brighteners" touted in the most highly rated cleaners derive from *Bacillus*. The great variety of these enzymes is important because some of them work best in specific pH and temperature ranges used in commercial cleaning (including home washers and dishwashers) and in drain cleaning products that help break down the organic residue that can clog sink traps and other plumbing fixtures. These enzymes can be used in cleaning products because they are usually harmless to people even with direct exposure. There have been rare instances of hypersensitivity reactions in people who frequently use these cleaners, and some of the reactions are potentially serious (6). Larger-scale industrial applications of these enzymes can be crucial to manufacturing because, ironically, *Bacillus*, which is known for its biofilm production, can provide extracts to break down biofilms that can foul commercial equipment. Some of the most critical applications are in the food industry, where tubes and valves can easily become obstructed by their contents if not purged both mechanically and enzymatically.

Microbiology

Bacillus spp. can be isolated from any number of human clinical specimens, as well as foods submitted for analysis to a clinical microbiology laboratory. Early growth of *Bacillus* spp. in culture may not demonstrate spore formation, and the organism on Gram stain examination may even stain gram negative. Colonies from older cultures may show gram-positive rods that sporulate under aerobic conditions, which is typical of *Bacillus*. These organisms are characteristically large (3 to 9 μm), grow well at room temperature, and also at most common incubator temperatures in the laboratory. *B. anthracis* is nonhemolytic on sheep or horse blood agar, and beta-hemolysis is usually the first clue that the organism is not *B. anthracis*. Demonstration of motility is also a reason to reject the identification of *B. anthracis*. More sophisticated techniques used in reference laboratories or in centers specializing in bioterror threats include the measurement of susceptibility to the gamma phage and genetic markers for the specific pathogenic plasmids that account for the symptoms of anthrax. Most automated kits for the identification of gram-positive bacteria are good at distinguishing between *B. anthracis* and the less worrisome members of the genus. Some of the less commonly identified species, such as *B. sphaericus* and *Bacillus badius,* are biochemically unreactive and may be difficult to identify with commercial biochemical kits.

Ecology

While environmental contamination of cultures is always a possibility, it is also the case that people are frequently colonized with *Bacillus* species. We are exposed to *Bacillus* on our skin and mucous membranes, so the sites most often noted to be culture positive are skin and intestinal. Cultures of human stool show that 0 to 48% of people carry *B. cereus* in the colon without any evidence of illness (7). These strains are varied and often closely related to strains found in domestic and farm animals. Similarly, culture of food for *Bacillus* spp. often yields small numbers of organisms, and they are rarely implicated in food poisoning. Quantitative cultures can shed light on the possible significance of *B. cereus*. Finding more than 10^5 CFU/gram of food is a source of concern (8). However, lower concentrations may be found when the food being cultured was stored under optimal (refrigerated) conditions while the food consumed had been at room temperature for many hours (9).

There are few clear rules as to when an isolate of *Bacillus* (other than *B. anthracis*) from a human clinical specimen is significant (10). Clearly, repeatedly positive cultures from a normally sterile body site raises a concern for true infection. Since many *Bacillus* spp. form biofilms, it is unsurprising that one of the most common scenarios for true *Bacillus* infection is in association with prosthetic devices, such as intravenous catheters or in association with trauma that leaves foreign bodies in place. Bacteremia caused by *Bacillus* is usually trivial unless there is an intravenous catheter as the source. Patients with long-term intravenous access (such as for cancer therapy) are more likely to have multiple positive cultures. These patients also respond poorly to antibiotics even though the drugs look active against *Bacillus* species by in vitro susceptibility testing assays. For people without catheters in place, the most common risk factor for true *Bacillus* bacteremia is injection drug use. *Bacillus* spores are found in the environment, and even heating drugs to the boiling point of water is not sufficient to kill them. Finally, patients with reduced resistance to bacterial infection because of profound neutropenia are at risk of bacteremia from movement of *Bacillus* from the gastrointestinal tract into the blood (11). All these reasons for bacteremia combined should be very infrequent in any given clinical laboratory.

Clinical Syndromes

Bacillus infections of the brain and surrounding structures are extremely rare except, again, when there is trauma or a catheter present (12). The most common catheters in the brain are those that drain spinal fluid. Some of these catheters are external at one end, and these are invariably temporary. Patients who require longer-term drainage have catheters that are fully internal with the non-brain end in the peritoneum or other distant body place. As is the case with device-related bacteremia, brain infection caused by *Bacillus* usually requires complete removal of the hardware.

Respiratory infections caused by *Bacillus* are also very rare. What is interesting and somewhat worrisome is an increase of recent pneumonias caused by *B. cereus* that is genotypically very closely related to *B. anthracis* (13). This organism has the capacity to cause serious, even life-threatening, pneumonia. There is no evidence for person-to-person transmission of these strains, but it does reinforce the notion that even unusual bacteria can cause infections that were unsuspected just a few years earlier.

Traumatic eye infections are perhaps the only example where *Bacillus* makes up a significant share of the microbial burden (14). The route of delivery (usually via foreign body, but sometimes in injection drug users via the bloodstream) means that spores can germinate in the eye tissue. Eye tissue has limited local defense, and bacteria that adhere to the surface of the foreign tissue can be highly inflammatory and provoke an immune reaction which may, by itself, damage the eye. In addition to giving antibiotics, the highest success rates for these patients is surgical removal of the infected debris in the eye. This can restore functional vision in some, but not all, patients. Infections of the eye related to cataract surgery or contact lens use are less common but can also be devastating (15,16).

The most underappreciated type of *Bacillus* infections involve deep skin structures and soft tissues (including traumatic and surgical wounds). Because *Bacillus* is so universal in the environment, the body has largely learned to cope with the organism. But in tropical areas in particular, *Bacillus* makes up a significant burden of infection following car or motorcycle accidents when soil is introduced into wounds (17,18). The pace of these infections can be faster than expected due to toxin production. Again, the theme of removal of foreign material and non-viable tissue applies to serious deep-wound infections. An interesting epidemic of scalp infections caused by a single strain of *B. cereus* occurred in Georgia among military cadets/college students in 2004 (19). The infections occurred shortly after the students had their military hair clipping, but the barber instruments were not found to harbor this particular source of *B. cereus* infection. The vehicle of transmission was never fully identified, but the hypothesis was that the newly traumatized scalp was prone to infection by this somewhat aggressive strain. The outcomes were universally good in this healthy cohort of young adults.

The most medically significant aspect of *Bacillus* infections involves food poisoning syndromes. Since *Bacillus* is not invasive, the food poisonings are intoxications attributed to one or more preformed toxins ingested during the consumption of contaminated food. In some instances, viable *Bacillus* organisms or spores that are ingested can germinate in the gut to produce the toxin in situ. The most common species of Bacillus associated with food poisoning is *B. cereus*. There are two distinct food poisoning syndromes described. The less common of the two is caused by a toxin called emetic toxin. As expected, the major symptoms are nausea and vomiting. The interval between food ingestion and symptoms is often a few hours and rarely more than 24 h. Patients

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seldom have other symptoms such as diarrhea and fever, and they make a full recovery. On rare occasion, patients may develop liver failure in association with emetic toxin, which is also known as cereulide (20). The key aspect of emetic toxin food poisoning is that the toxin is heat stable. Thus, even reheated foods that are culture negative for *B. cereus* can still be vehicles for food poisoning. The classic food vehicle for emetic food poisoning is fried rice. The reason is that rice is often kept at room temperature overnight before the frying process. This is to prevent the hardening of rice that occurs in the refrigerator. The frying in hot oil will kill vegetative *B. cereus* but will not affect the toxin.

The other major food poisoning syndrome, diarrheal, is more common than emetic although still less common than most other bacterial diarrheas. It is estimated that for every 129 cases of diarrhea caused by *Campylobacter* spp., 95 caused by gram-negative enteric bacteria (*Salmonella*, *Shigella*, etc.), and 5.6 caused by *Clostridium perfringens*, there is 1 case of *B. cereus* food poisoning (21). The onset is about 8 to 16 h after the ingestion of contaminated food, and the illness is brief (median duration, 24 hours). The diarrheal toxin is actually a mixture of two or more proteins with molecular masses of 36,000 to 45,000 daltons. The precise mode of action is unknown, although the toxin may have sphingomyelinase activity. While the diarrheal toxins are heat labile and can be reduced or eliminated by heating food, the ingestion of spores of toxin-producing *Bacillus* spp. can lead to diarrheal food poisoning by elaboration of toxin in the upper gastrointestinal tract. Foods most commonly associated with *Bacillus* diarrheal food poisoning include meats, vegetables, and sauces (21). While the majority of isolates of the diarrheal form of *Bacillus* food poisoning are *B. cereus*, there have been outbreaks related to *B. licheniformis* and *B. pumilus*. A distinct form of food poisoning has been associated with *B. subtilis*. This syndrome is characterized by a short incubation period (median, 2.5 h), vomiting, diarrhea (in about half the cases), and various other manifestations, such as flushing, sweating, and headaches, in about 10% of the patients.

Epidemiology

Outbreaks of *Bacillus* food poisoning are challenging to document because *Bacillus* spp. are commonly found in stool and in normal foods. The quick onset of the food poisoning makes it relatively easy to target the source in a large outbreak, but the mild nature and the quick resolution of the syndrome make it difficult for patients to get to the doctor in time for a diagnosis. Most of our knowledge of these food poisonings come from large outbreaks where questionnaires and numerous cultures help pinpoint the culprit. Studies of these large outbreaks suggest that some patients have more prolonged and slightly more severe symptoms than normally described (22). It is not certain if these are the people who had the greatest toxin exposure (although that is often the case in other kinds of toxin-related food poisonings) or from in vivo production of toxin by newly germinated spores.

True outbreaks of *Bacillus* infection can occur in the hospital setting. In one medical center, *B. cereus* was an ongoing cause of positive respiratory cultures and morbidity (including two cases of true bacteremia and one fatal pneumonia) in an intensive care unit (23). This epidemic was the consequence of inadequate sterilization of respiratory circuits. No other bacterial infections occurred at higher than usual rates during the epidemic period because the degree of sterilization was sufficient to eradicate non-spore-forming bacteria, but not *B. cereus*.

The most likely scenario for the next culture of *Bacillus* in your laboratory will end with the determination that it represents a meaningless contaminant regardless of the specimen source. However, there are contaminants and there are contaminants. A Canadian outbreak of pseudobacteremia caused by *Bacillus* was determined to arise from improper storage of blood culture bottles in a closet close to the site of heavy driveway construction (24). Bottles in this closet were layered with dust, and this was felt to represent the source of contamination. Once the bottles were moved, the pseudo-epidemic resolved. In one study that compared patients for whom both bottles were positive in a set with *Bacillus* species against patients with only a single bottle positive, 29% (5/17) of the episodes with both bottles positive were associated with a subsequent positive blood culture as opposed to 3% (2/59) in patients with only a single bottle positive (25). It is often not useful to note that only a single bottle was positive for a true pathogen, but it might be useful to comment that having both bottles positive should at least raise the clinical suspicion of true bacteremia when *Bacillus* is isolated.

Faulty general hygiene and specimen-handling policies can allow an excess of *Bacillus* bacteremias. In a Japanese hospital, 29 patients were noted to have *Bacillus* sp. bacteremia (more than one-half of these were *B. cereus*) (26). However, these patients were not treated for *Bacillus* spp. and did well clinically. Review of infection control policies showed suboptimal approaches to handling the catheters (wrong disinfectant, pauses during infusion, and reuse of caps on stopcocks). When these shortcomings were corrected, the *Bacillus* spp. bacteremia pseudoepidemic ceased. False-positive cultures of cerebrospinal fluid for *Bacillus* spp. have also been reported (27).

Bacillus spp. are unlikely to become the scourge of mankind, because we have lived in peace with and actually taken advantage of this genus for a long time. In addition to their invaluable commercial properties, *Bacillus* species represent a model of safe bacteria in which to study biofilms, motility, bacterial metabolism, quorum sensing, and the roles of phages and plasmids. Because it is a spore former, it is also a great organism with which to probe not only the current technology of heat and chemical sterilization, but also the effectiveness of various hand hygiene products (28). One of our current concerns about the efficacy of alcohol-based hand-cleaning gels for *Clostridium difficile* could be more easily studied using *Bacillus*. In a similar way, flaws in our use of skin prep products for obtaining clinical isolates might be brought out during periods of excess positive cultures for *Bacillus*.

Therapy

In terms of treatment, there is no specific therapy for the food poisoning syndromes other than symptomatic measures. For deep-tissue infections, removal of prosthetic material, including infected intravascular catheters, is vital to cure (25). Most *Bacillus* sp. isolates are susceptible to vancomycin, clindamycin, fluoroquinolones, aminoglycosides, carbapenems, and, variably, to penicillins and cephalosporins. *B. cereus* is often resistant to all beta-lactams (other than carbapenems), and serious infections are best treated with vancomycin or clindamycin, with or without an aminoglycoside (29-31). The beta-lactamase of *B. cereus* and several other species of *Bacillus* is a zinc-based enzyme that is evolutionarily different from beta-lactamases in other gram-positive bacteria. Imipenem and some extended-spectrum beta-lactams, such as mezlocillin, seem to be active against almost all *Bacillus* spp. despite the presence of a beta-lactamase enzyme in *B. cereus* and *B. thuringiensis*. For strains other than *B. cereus*, various beta-lactams are active in vitro, but clindamycin is less reliably active. Vancomycin appears to be active in vitro against most *Bacillus* strains, but resistance via the *vanA* gene cluster has been reported (32). Surgical drainage and removal of necrotic debris or implanted devices are important. In endophthalmitis, pars plana vitrectomy and intravitreal antibiotics have been advocated. *Bacillus* sp. keratitis is treated topically, for example, with a fluoroquinolone. A good visual outcome is most likely when the lesion is treated early and does not involve the central part of the cornea.

Prevention

Prevention is obviously even more critical than treatment. Guidelines for the safe preparation and handling of food are available at http://www.cfsan.fda.gov/list.html. Education of commercial food vendors is of obvious importance as a way to prevent the large outbreaks that can affect multiple families or even a whole community (8). The best way to avoid *Bacillus* food poisoning is to cook foods adequately and to eat them immediately. Cooking will kill vegetative *Bacillus* spp. and destroy preformed diarrheal toxin, although not emetic toxin. If food cannot be consumed immediately, it should be refrigerated as soon as possible, as *Bacillus* sp. metabolism and toxin production are inhibited by cold temperature. Cooked rice should not be held at room temperature for prolonged periods before preparation of fried rice. Education of contact lens wearers about proper decontamination is important in preventing keratitis.

Summary

There are many layers of challenge when it comes to understanding *Bacillus* species. *Bacillus* teaches us the importance of subtle differences in phylogeny manifesting in radical differences in phenotype. The existence of anthrax is not surprising, but the grounding of *B. anthracis* in the middle of a highly variable radiation of *B. cereus* (and related species) is a reminder that bacterial identification to the species level is an ongoing process and that genotypic relatedness does not always tell the whole story of what is important to us. Strains of *Bacillus* with some properties of *B. anthracis* and others of *B. cereus* may be emerging as potential agents of uncommon *Bacillus* infections, such as pneumonia. Furthermore, there is no telling what kinds of alterations can be done in the hands of bioterrorists with good funding. For those strains of *Bacillus* that cause human infection, we may learn how to turn off the toxin production that seems to be a big part of deep infection, as well as in situ toxin formation, in some patients with food poisoning. This will demand creativity that may also apply to other infections caused by toxin-producing bacteria.

We must also find ways to distinguish environmental contamination and simple human colonization from true *Bacillus* infection. Despite the reputation of *Bacillus* as a minor pathogen, we may need to be aggressive with debridement and removal of foreign material to produce the best possible outcome for our patients. Repeatedly positive cultures for *Bacillus* are a strong clue that ignoring the organism or sticking with antibiotics alone is not working. *Bacillus* can be an elusive organism to understand and to prioritize, as it can "hide in plain sight," the veritable "Where's Waldo" of microbes.

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