The Geobacillus paradox: why is a thermophilic bacterial genus so prevalent on a mesophilic planet?

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The genus Geobacillus comprises endospore-forming obligate thermophiles. These bacteria have been isolated from cool soils and even cold ocean sediments in anomalously high numbers, given that the ambient temperatures are significantly below their minimum requirement for growth. Geobacilli are active in environments such as hot plant composts, however, and examination of their genome sequences reveals that they are endowed with a battery of sensors, transporters and enzymes dedicated to hydrolysing plant polysaccharides. Although they appear to be relatively minor members of the plant biomass-degrading microbial community, Geobacillus bacteria have achieved a significant population with a worldwide distribution, probably in large part due to adaptive features of their spores. First, their morphology and resistance properties enable them to be mobilized in the atmosphere and transported long distances. Second, their longevity, which in theory may be extreme, enables them to lie quiescent but viable for long periods of time, accumulating gradually over time to achieve surprisingly high population densities.

Introduction: an environmental enigma

Geobacillus bacteria are rod-shaped, aerobic or facultatively anaerobic, endospore-forming microbes. Depending on the strain, the temperature range for growth can extend as low as 35 °C or as high as 80 °C. But for most isolates, temperatures between about 45 and 70 °C are required (Nazina et al., 2001). These characteristics make Geobacillus bacteria (geobacilli) attractive to the biotechnology industry as sources of thermostable enzymes (de Champdore´ et al., 2001; Karaguler et al., 2007), as platforms for biofuel production (Cripps et al., 2009; Taylor et al., 2009) and as potential components of bioremediation strategies (Markossian et al., 2000; Obojska et al., 2002; Perfumo et al., 2007). Obligate thermophiles, however, face a significant challenge outside the lab: they must survive on a planet with an average annual land surface temperature that has only ranged between 7 and 10 °C over the past three centuries (Rohde et al., 2013). It would be logical to assume, then, that Geobacillus would only be found in substantial numbers in the warmest regions of the planet, such as equatorial deserts or naturally occurring geothermal and hydrothermal hotspots. The truth, however, is rather more complicated.

As Fig. 1 illustrates, people have been able to detect Geobacillus nearly everywhere they have thought to look. (This figure is based on a survey of 146 mostly recent references describing the isolation of Geobacillus in natural or human-made environments; for full details, see Table S1, available in Microbiology Online.) One can see at a glance that these bacteria have been isolated from sources on all seven continents as well as the Pacific Ocean and the Mediterranean Sea. What may not be obvious from a two-dimensional map is that there has been much diversity in the third dimension as well. Geobacillus has been isolated from the Bolivian Andes at an altitude of 3653 m (Marchant et al., 2002) and has reportedly been detected by molecular methods in the upper troposphere at ~10 000 m (DeLeon-Rodriguez et al., 2013), although some aspects of the latter study have been questioned (Smith & Griffin, 2013). Isolates have also been obtained from the lower regions of the Earth, including oil wells at depths of 1700–2150 m (Kato et al., 2001; Wang et al., 2006), gold mines at depths of 1500–3200 m (DeFlaun et al., 2007; Rastogi et al., 2009), continental shelf sediments at 800–2060 m below sea level (Bartholomew & Paik, 1966), seawater collected at a depth of 3083 m (Liu et al., 2006) and sediments at 10 897 m below sea level in the Mariana Trench, the lowest point on the Earth’s surface (Takami et al., 2004). The ecological range of isolation sources is equally diverse. The first reported Geobacillus isolation nearly a century ago was from a can of spoiled corn (Donk, 1920). It is now recognized that fresh agricultural produce often carries Geobacillus spores, which may survive heat treatment and cause ‘flat sour’ spoilage of processed foods (Sevenier et al., 2012). Members of the genus are likewise frequent contaminants of dairy production facilities and milk products (Rückert et al., 2004; Seale et al., 2012). Hot composts have also been a common site for isolating Geobacillus strains (Strom, 1985; Takaku et al., 2004; Sevenier et al., 2012).
The most frequently reported natural sources for Geobacillus isolates have been hot springs (Canakci et al., 2007; Derekova et al., 2006; Pinzón-Martínez et al., 2010), geothermal soils (Lama et al., 2004; Meintanis et al., 2006), hot subterranean oilfields and natural gas wells (Nazina et al., 2001; Struchtemeyer et al., 2011), and hydrothermal vents (Kimura et al., 2003; Maugeri et al., 2001).

Yet many temperate and even cold places of the Earth have also yielded these thermophiles. There have been numerous reports of Geobacillus isolation from cool soils (Marchant et al., 2002; Rahman et al., 2004; Zeigler, 2005) and permanently cold ocean sediments (Bartholomew & Paik, 1966; Takami et al., 2004). It is not simply that geobacilli were found in unexpected locations; it is that they are found in such large numbers there. Nearly 50 years ago, researchers at the University of Southern California examined ocean basin cores collected from the North American continental slope and discovered up to 900 aerobic thermophiles per gram of wet sediment. All seven isolates chosen for taxonomic identification were Geobacillus. The authors recognized an enigma in their findings, stating that ‘the presence of obligate thermophilic bacteria in an environment having a constant temperature of about 4 °C is difficult to explain’ (Bartholomew & Paik, 1966). A decade later, another group examined soil samples collected in Reykjavik, Iceland, where bare soil temperatures at 5 cm depth averaged only about 14 °C and never exceeded 27 °C, even on a sunny July day. The researchers argued that ‘based upon normal recorded weather data, B. stearothermophilus [now Geobacillus] and other thermophilic bacteria should not be present in the soil at all’. Yet they cultured $1.6 \times 10^4$ aerobic thermophiles per gram of soil and identified all 33 selected isolates as Geobacillus (Fields & Chen Lee, 1974). More recently, workers at the University of Ulster, Coleraine, examined soils in Northern Ireland and cultured aerobic thermophiles at a frequency of $1.3–8.8 \times 10^4$ g$^{-1}$, depending on the site. They could readily isolate similar bacteria from cool soils collected in the Andes and the northern USA. Each of five isolates selected for identification turned out to be geobacilli. The researchers noted that meteorological records showed that local soil temperatures in Northern Ireland never reached the isolates’ minimum growth requirement. ‘We have to ask’, they concluded, ‘how can these organisms exist in such large numbers in an environment where they are unable to grow?’ (Marchant et al., 2002).

Herein lies the Geobacillus paradox. If the soils and sediments examined in these studies are typical of the planet as a whole, then Earth’s population of viable, culturable aerobic thermophiles, as exemplified by the genus Geobacillus, is enormous. Why have they accumulated in such numbers on a planet like ours? This review will briefly examine the role of Geobacillus in the ecosystem, the mechanism of their global dispersal and the potentially extreme longevity of their spores, in hopes of framing an answer to that question.

**How do geobacilli earn their living?**

**Lessons from compost**

One challenge after isolating a bacterium is determining whether it was actively growing in that habitat or whether it merely was transiently present, perhaps in a metabolically dormant form such as a spore. There are few reports in the research literature that unambiguously document the proliferation of geobacilli in the natural world. There is at least one habitat, however, where these bacteria are undeniably active participants in a complex, dynamic

![Fig. 1. Geographical and environmental diversity of Geobacillus isolation. Each circle represents a geographical location reported to be a source of cultured Geobacillus isolates; colour denotes type of source material. Each circle denotes a published report that may describe one or many isolated strains. For details, see Table S1.](image)
microbial community: composts. Composting is a bio-
degradation of organic matter, usually derived from plants.
A consortium of microbes, mostly located in biofilms
clinging to the surface of the organic particles, cooperates
to break down the plant polysaccharides, liberating heat as
the sugar molecules are oxidized. Over a period of weeks or
months, active compost passes from a mesophilic phase
(10–42 °C) to a thermophilic phase (45–70 °C), followed
by a second mesophilic phase and eventual maturation and
stabilization (Ryckeboer et al., 2003). Anyone who has
watched steam pour out of active compost realizes that an
enormous amount of heat can indeed be produced. It is
during that thermophilic phase that geobacilli are active.
Whether in controlled laboratory settings or in waste
recycling centres, Geobacillus species can become the
dominant culturable bacterial taxon in composts at 60–
69 °C (Blanc et al., 1997; Ronimus et al., 2003; Strom,
1985; Takaku et al., 2006). When culture-independent
methods are employed, however, a richer and much more
nuanced picture emerges in which Geobacillus and other
thermophilic members of class Bacilli still play a part,
a although a numerically minor one (Martins et al., 2013;
Michel et al., 2002; Partanen et al., 2010; Peters et al.,
2000). Yet the prevalence of geobacilli in active, hot
composts gives us a place to start in assessing the likely
role of Geobacillus in the natural environment. Earth’s
biosphere produces an estimated 100 gigatonnes of plant
biomass each year, about 90 % of which escapes consump-
tion by herbivores and enters the dead organic matter pool,
an abundant carbon source that could certainly fuel the
production of huge numbers of bacteria (Cebrian, 1999).
We can hypothesize, then, that geobacilli are likely
opportunistic decomposers of plant-derived organic mat-
ter, capable of rapid growth under transient thermophilic
conditions, but endowed with mechanisms to survive long
periods of time when growth is impossible. This picture is
admittedly developed from circumstantial evidence and
therefore requires corroboration. For this it is necessary to
take a detour into the realm of comparative genomics, in
the hope that these organisms’ secret lives are recorded in
their genes.

Lessons from genomes
At the time of writing, there were ten publicly available
complete genome sequences for Geobacillus (see Table S2).
It is thus possible to bring the powerful tools of
comparative genomics to bear on this question. A key
concept in the field is that of the core genome, the set of
orthologous genes shared by all members of a bacterial
taxon. Additional genes found in a given bacterium that
may provide increased functionality are called its dispense-
able genome. The sum of the core genome, together with all
the dispensable genes found in at least one member of the
taxon, is termed the pan-genome (Tettelin et al., 2005).

The Geobacillus core genome, determined with the help of
the EDGAR application (Blom et al., 2009), consists of about
1665 genes. Significantly, 1409 of them (85 %) are also
found in the genome of Bacillus subtilis 168, a mesophilic
model organism that has been intensively investigated by
classical genetics since 1958 and by ‘omics’ technologies
since 1997. It is therefore possible to use the thoroughly
annotated and carefully curated B. subtilis 168 genome
sequence (Belda et al., 2013; Mäder et al., 2012) to create an
annotated Geobacillus core genome (Table S2). The
Geobacillus genome includes orthologues of 236 of the
278 genes known to be essential for viability in B. subtilis
(Commachiu et al., 2013) and 67 of the 75 genes deemed
essential for endospore production (Galperin et al., 2012).
(Presumably, some essential functions are carried out by
non-orthologous proteins in Geobacillus.) The Geobacillus
core genome includes orthologues of the majority of B.
subtilis genes involved in key cellular processes such as
DNA replication, repair, recombination, condensation and
segregation; transcription and translation; cell division
and maintenance of cell shape; membrane dynamics
and protein secretion; carbon core metabolism and ATP
synthesis; biosynthesis and acquisition of nucleotides,
amino acids and cofactors; and RNA processing. The
Geobacillus core genome also features orthologues of many
genes involved in the B. subtilis post-exponential growth
lifestyle. Besides genes encoding alternative sigma factors
and other proteins participating in sporulation and
germination, it includes genes involved in chemotaxis and
motility, biofilm formation, DNA uptake, and (at least
potentially) genetic competence. These observations imply
strongly that Geobacillus and B. subtilis share an overarching
survival strategy, inherited from a common ancestor,
featuring rapid growth when metabolizable nutrients are
readily available, followed by concerted efforts to create,
scavenge or move towards additional sources when nutrients
are exhausted, with spore formation as a last resort if these
efforts fail (Fujita et al., 2005; Fujita & Losick, 2005). Some
Geobacillus isolates lyse following nutrient exhaustion in
batch culture, a potential boon for certain biotechnological
applications (Pavlostathis et al., 2006). However, most
geobacilli in my own collection of environmental isolates
(Zeigler, 2005) do indeed form endospores on solid media
under laboratory conditions, and many show not only
motility but the ability to swarm on agar surfaces, as one
would predict from genomic analysis. But for Geobacillus,
what specialized adaptations have been overlaid on this basic
lifestyle? The remaining 15 % of the core genome is of little
help in answering this question; it seems mostly to encode
additional transporters and proteins of unknown function.
Instead we must turn our attention to the dispensable
genome for this genus. Does it feature genes devoted to the
utilization of plant biomasses? The enzymology of plant
polysaccharide decomposition has been very thoroughly
studied (Gilbert, 2010). So, what is necessary is to scan the
Geobacillus dispensable genomes for genes encoding known
enzymes for hydrolysis, uptake, and utilization of these
polysaccharides and their components.

To this end we are greatly assisted by the work of Y.
Shoham and colleagues with a xylanase-secreting strain
with potential industrial applications, *Geobacillus stearothermophilus* strain T-6 (Khasin *et al.*, 1993). The genome of strain T-6 has a hemicellulose utilization island of 76 kb, divided equally between two subclusters providing functionality for utilizing xylan/xylose and arabinan/arabinoxylan (Shulami *et al.*, 1999, 2011). The region also contains several mobile elements, suggesting that the island could have been assembled by horizontal gene transfer. Both the xylan and the arabinan subclusters contain sensing systems that can alert the cell to the presence of the units that make up these hemicelluloses; glycohydrolases that can remove side chains from them and cleave them into oligosaccharides; ABC transporters that can import the oligo- or monosaccharides into the cell; suites of sugar metabolism genes; and master transcriptional regulators. Nine of the ten completed genomes contain at least some portion of this island, and seven contain nearly all of it (see Table 1 for a summary and Table S3 for details). Recently, a galactan utilization operon has also been identified in strain T-6, enabling the bacterium to break down a key component of pectin, type I galactan (Tabachnikov & Shoham, 2013). The entire 9.4 kb cluster is to break down a key component of pectin, type I galactan (Tabachnikov & Shoham, 2013). The entire 9.4 kb cluster is also found in orthologous form in the genome of *Geobacillus thermoleovorans* CCB_US3_UF5. Examination of the finished genomes also reveals other likely genes and clusters devoted to plant degradation. Orthologues of a well-studied neopullulanase (Hondoh *et al.*, 2003) are encoded within an apparent pullulan or starch utilization cluster found in eight of the ten genomes (Table 1 and Table S3). A previously studied system (Lai & Ingram, 1993) for the uptake and utilization of cellulbiose, a disaccharide produced from partial cellulose hydrolysis, is found in four of the genomes, while a five-gene cluster highly similar to a well-characterized *B. subtilis* glucosamannan utilization operon (Sadaie *et al.*, 2008) is found in nine of the genomes.

In summary, most *Geobacillus* isolates are equipped with a battery of sensors, transporters and hydrolytic enzymes specifically adapted to extract energy and metabolic building blocks from plant biomass. Interestingly, this generalization holds for *Geobacillus* isolates taken from seemingly unrelated sources: *Geobacillus thermodenitrificans* NG80-2 from a hot deep-subsurface oil reservoir in China (Wang *et al.*, 2006), *G. thermoleovorans* CCB_US3_UF5 from a hot spring in Malaysia (Muhd Sakaff *et al.*, 2012), *Geobacillus* sp. GHH01 from cool garden soil in Germany (Wiegand *et al.*, 2013) and *Geobacillus kaustophilus* HTA426 from cold sediment at the bottom of the Mariana Trench (Takami *et al.*, 2004). While some *Geobacillus* strains have been isolated from oil-contaminated soils and deep oil reservoirs, and some have been shown to metabolize long-chain hydrocarbons, it is also true that nearly all of these environments have been disturbed by human activity, and at least some of these isolates have been introduced in subterranean oil and gas fields by the drilling and extraction processes (Struchtemeyer *et al.*, 2011). Further, as mentioned above, the oil reservoir isolate NG80-2 possesses many clusters of genes involved in plant degradation. For these reasons, further work will be required to establish whether subterranean hydrocarbon deposits compose a significant ecological niche for *Geobacillus*.

### Table 1. Gene clusters predicted to function in plant polysaccharide degradation, as detected in the dispensable genomes of *Geobacillus* isolates

<table>
<thead>
<tr>
<th>Target compound</th>
<th>Gene cluster</th>
<th>Named genes</th>
<th>Length (kb)</th>
<th>ORFs</th>
<th>Genomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td><em>celRABCD</em></td>
<td></td>
<td>5.3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Starch/pullulan</td>
<td></td>
<td></td>
<td>≤17.8</td>
<td>4–9</td>
<td>8</td>
</tr>
<tr>
<td>Pullulan</td>
<td></td>
<td></td>
<td>0.7</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Glucosamannan</td>
<td><em>gnuRDCAB</em></td>
<td></td>
<td>4.3</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Xylan</td>
<td><em>uxu, kdg, agu, xyn, axe</em> operons</td>
<td></td>
<td>35.2</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>Xylan</td>
<td><em>xylAB</em></td>
<td></td>
<td>3.0</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Arabinan</td>
<td><em>ara, abf, abn</em> operons</td>
<td></td>
<td>≤38.3</td>
<td>11–21</td>
<td>7</td>
</tr>
<tr>
<td>Galacturonan</td>
<td><em>ganREFGBA</em></td>
<td></td>
<td>9.4</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Galactan, type I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Why are geobacilli found worldwide?

Bridges in the sky

In our search for planetary mechanisms for redistributing geobacilli, we need to give special consideration to the atmosphere. It has been said that air is ‘as alive as soil or water’ (Womack et al., 2010). And for good reason: according to current estimates, every square metre of the Earth’s surface emits on average 50–220 bacteria s⁻¹ (Burrows et al., 2009b). In total, Earth’s atmosphere includes 7.6 × 10²⁹ to 3.5 × 10³⁴ particles that contain bacteria (Burrows et al., 2009a). This distribution is not completely homogeneous. The near-surface atmosphere over the oceans has about 10⁴ bacteria m⁻³, with similar levels over deserts and tundra. Bacterial loads in air over grasslands, wetlands and crops are about an order of magnitude higher. The highest levels are found over shrubs, where bacteria may exceed 3 × 10⁵ m⁻³, and urban environments, where they may approach concentrations of 10⁶ m⁻³ (Burrows et al., 2009b). Models based on global wind patterns suggest that most bacterium-sized particles reside in the atmosphere for 2–15 days. They are usually removed from the air, or ‘scavenged’, by water, and ice-phase scavenging is much more efficient than liquid-phase. Bacteria from tropical grasslands, shrubs and deserts are more likely to participate in long-distance travel, as they can be caught up in tropical convective systems and potentially carried all the way to the upper troposphere. Interestingly, smaller particles of about 1 μm diameter fall into a ‘scavenging gap’ and reside in the atmosphere longer than particles of 3 μm diameter. The atmosphere thus regularly redistributes bacteria around the surface of the planet, although the patterns of transport are by no means completely uniform. The air can even serve as a bridge allowing intercontinental transit of microbes (Burrows et al., 2009a; Smith et al., 2012).

There have been many published studies documenting the isolation of viable bacteria from the air, beginning with the work of Louis Pasteur (Ariotti & Comtois, 1993). Unfortunately, most of these studies provide no specific data about Geobacillus. With very few exceptions, researchers using culture-dependent methods to study atmospheric microbes have incubated their samples under mesophilic conditions that preclude the growth of any aerobic thermophiles. Those who have employed DNA sequence-based methods have very rarely identified short sample sequences beyond the family taxonomic level, and there are many genera besides Geobacillus within the family Bacillaceae. Nevertheless, reports of mesophilic bacilli in the atmosphere are probably broadly applicable to morphologically similar thermophilic geobacilli as well. In general, culture-based studies have usually reported Bacillus as a major mesophilic component of bioaerosols. In air samples taken from various locations in Oregon, for example, the frequency of Bacillus among all cultured bacteria was 41.7% from forest air samples, 13.6% from coastal samples, 45.2% from urban samples and 25.8–41.7% from rural samples (Shaffer & Lighthart, 1997).

Bacillus was also a dominant taxon among cultured airborne bacteria sampled from urban Beijing (Tong et al., 1993), from a border market in Thailand (Reanprayoon & Yoonaiwong, 2012) and from a North American mountain observatory 2.7 km above sea level (Smith et al., 2012). Culture-independent methods report a much higher bacterial diversity. A study of urban aerosols collected from four cities in the US Midwest reported the presence of 200–300 bacterial phylotypes, among which the order Bacillales represented a minority component (Bowers et al., 2011). A similar study of urban aerosols from two cities of the south-western US detected large numbers of Firmicutes, including members of the family Bacillaceae (Brodie et al., 2007). One interesting study that does bear directly on Geobacillus aerobiology examined the copious bioaerosols generated when hot compost piles are turned. Using a culture-independent approach, the researchers reported that Firmicutes and Actinobacteria were the most frequently detected bacterial phyla, and that Geobacillus was the most common phylotype among Firmicutes (Le Goff et al., 2010). In a study with direct bearing on the paradox that forms a theme for this review, Geobacillus has also been cultured from air samples collected above cool soils in Northern Ireland. The isolation frequency was relatively low, 1.55 c.f.u. per 1000 litres of air. The frequency of culturable Geobacillus in rainwater collected in the location was measured at 1.1 cells per 100 ml of water. These numbers are not large, but based on Northern Ireland climate records, they imply that each year about 1.4 × 10⁵ Geobacillus cells or spores are deposited on 1 m² of soil surface (Marchant et al., 2008). In summary, there is a dynamic but largely invisible interaction between microbes and Earth’s atmosphere, and Bacillus sensu lato is a minor but not insignificant part of this process. What is true for mesophilic bacilli is likely true for their thermophilic relatives, the geobacilli, as well.

Desert dust

One special focus of aerobiology has been the study of desert dust storms. These massive events mobilize on the order of a billion tonnes of soil each year, most of it originating from the Sahara and Sahel in Africa, with significant contributions from the Gobi, Takla Makan and Badain Jaran deserts in Asia. Dust storms can be very precisely monitored from Earth observation satellites. The storms can be observed crossing the entire Atlantic westward from Africa in 3–5 days and crossing the entire Pacific eastward from Asia in 7–10 days (Griffin, 2007; Kellogg & Griffin, 2006; Weiss-Penzias et al., 2006). When microbes have been cultured from mobilized African or Asian desert dust, Bacillus usually have constituted a significant proportion of the population (Griffin et al., 2001, 2003, 2006; Hua et al., 2007). As expected, when culture-independent methods are employed, a greater diversity is observed in aerosolized desert dust. Nevertheless, the Bacillaceae can still constitute one of the dominant taxa detected in DNA-based studies (Smith et al., 2013; Yamaguchi et al., 2012).
Could the geobacilli found in unexpectedly high levels in cool European soils have their ultimate origin in Saharan dust storms? Desert dust deposited in Mediterranean Turkey and Greece yielded several culturable Geobacillus isolates in a recent study. On a phylogram of 16S rDNA gene sequences, these isolates were interspersed with Northern Ireland cool soil isolates (Perfumo & Marchant, 2010). However, one should be cautious not to overinterpret these data. For one thing, they are more qualitative than quantitative; no estimate of Geobacillus loads in Saharan dust are possible from this study, because neither the air volume that contained the dust, the mass of the dust itself nor the titre of aerobic thermophiles in the dust was measured. For another, there are a priori reasons to question whether Saharan dust carries enough Geobacillus spores to account for what has been measured in Icelandic soils or in Irish soils or rainwater. Although Geobacillus strains have occasionally been isolated from desert soils (Abdel-Fattah & Gaballa, 2008; Al-Hassan et al., 2011), careful quantitative studies are generally lacking. One notable exception is a report of aerobic thermophiles isolated from Saudi Arabian soils (Abu-Zinada et al., 1981). As expected, the desert soil was found to be very poor in nutrients and moisture; it consisted of 91% sand and contained only 0.3% water and 0.2% organic matter. It was also very low in culturable thermophiles, with only 760 cfu g⁻¹ of soil. In contrast, Arabian loams and sandy clays yielded thermophiles at 20–40 times higher frequencies, roughly equivalent to what is seen in European cool temperature soils. Ten isolates were analysed further, and all were classified as Geobacillus (then named Bacillus stearothermophilus). We should be cautious about extrapolating from a single study, and research is needed to confirm these results and extend them to other desert regions. Yet it would hardly be surprising if Saudi Arabian desert microbial communities are broadly similar to those found in the Sahara and Gobi deserts. It therefore seems likely that while desert dusts may be important vehicles for redistributing Geobacillus spores, the deserts themselves should not be thought of as huge microbial incubators generating the high levels of Geobacillus observed in soils and sediments worldwide. If Geobacillus is primarily a decomposer of plant biomass under thermophilic conditions, it may be that shrubs and grasslands are the major contributors to the atmospheric load of geobacilli. This question remains open.

Are spores of (Geo)bacillus adapted for atmospheric transport?

There is an intriguing correspondence between the size of the ‘scavenging gap’ and the size of Bacillus and Geobacillus spores. Particles with a diameter of about 1 μm have the longest residence in the atmosphere and so can presumably be transported the greatest distances before falling back to earth (Burrows et al., 2009a). Strain descriptions of novel Geobacillus species rarely report spore size. But it is apparent, from both my unpublished observations and others’ published micrographs, that most Geobacillus spores are slightly ovoid, with an average diameter close to the size required to fit into the scavenging gap (Fortina et al., 2001; Nazina et al., 2001). Further, Bacillus and Geobacillus endospores are notorious for their extreme resistance to UV light, temperature changes and desiccation (Driks, 2002; Nicholson et al., 2000; Setlow, 2006; Takamatsu & Watabe, 2002), the very environmental challenges faced by airborne bacteria (Griffin, 2007). These correspondences suggest the following hypothesis: atmospheric transport has played an important role in the selective pressures that shaped the morphology and resistance properties of spores from Bacillus and related genera. Other more subtle properties of spores, such as their tendency to clump or stimulate condensation, might likewise have been shaped by this selection. Or to put it another way, Bacillus spores have evolved to remain viable during long residence times in the atmosphere, allowing them to travel great distances, perhaps to more favourable locations for growth.

Why have geobacilli accumulated in such high numbers?

In attempting to explain the paradoxically high populations of thermophilic geobacilli in cool soils and sediments, I have proposed first that these bacteria probably play minor roles in the global carbon cycle as decomposers of plant biomass, and second that geobacilli and their spores are redistributed widely, although not necessarily uniformly, around the planet through atmospheric transport. One major component of the question still needs to be addressed: how have these bacteria accumulated in such high numbers? It appears that the genus Geobacillus plays only a modest role in a large microbial community, even in environments where it is clear that geobacilli are growing and reproducing, such as hot compost. Perhaps there is some as yet poorly characterized ecological niche that serves as a Geobacillus production factory, continuously releasing enormous numbers of these organisms into the environment. But another solution is possible, one that may be more logically economical. Geobacillus could be produced by the environment only sporadically in restricted geographical locations and small numbers and then widely redistributed as spores, provided that these spores exhibit significant longevity. If so, the high populations observed in modern environments could have gradually accumulated over long periods of time. Perhaps the global population of Geobacillus has reached equilibrium, and the rate of reproduction is matched by the death rate. Or perhaps the Geobacillus population is still increasing, slowly but inexorably dusting large swaths of the planet with viable spores.

Is there evidence for such extreme longevity? There have been numerous reports of the recovery of viable bacteria from ancient preserved or fossilized materials (Kennedy et al., 1994). These reports have historically tended to be
geted with scepticism, although recent publications demonstrate that researchers have been developing increasingly rigorous methods for mitigating sample contamination concerns (Johnson et al., 2007; Sankaranarayanan et al., 2011). For Geobacillus, data regarding longevity are especially sparse. However, in the previously mentioned study of Pacific Ocean basin cores from the North American continental slope, aerobic thermophiles identified as Geobacillus were repeatedly isolated from sediments dated up to 7800 years BP in such substantial numbers that contamination is an unlikely explanation (Bartholomew & Paik, 1966). It would be fascinating to learn whether similar organisms could be isolated from much deeper, older cores, but such studies do not seem to have looked for thermophiles.

It has been persuasively argued that chemical instability of DNA is a limiting factor for the longevity of bacteria (Lindahl, 1993). There is a hint in the literature that Geobacillus cells may be highly resistant to ionizing radiation, comparing favourably with cells of Deinococcus radiodurans in that regard (Saffary et al., 2002). This observation needs to be confirmed by repetition, and other Geobacillus isolates should be similarly tested. However, there is already a considerable body of evidence documenting a bacterial structure that clearly protects genomic DNA not only from ionizing radiation (Moeller et al., 2008) but also from many forms of environmental damage (Nicholson et al., 2000; Setlow, 2006): the Bacillus spore. It may be that the same mechanisms that protect spores from these sudden environmental crises could also dramatically extend their lifespans under ordinary conditions by protecting them from more gradual damage over time. For once we have an abundance of data for Geobacillus spores. Because they are a standard biological indicator for sterilization, their inactivation kinetics when challenged by steam, dry heat, plasma and various chemicals have been carefully documented (Klämpfl et al., 2012; Lambert, 2003; Marquis & Bender, 1985; Mosley et al., 2005). A convenient concept in sterilization is the decimal reduction value, or D-value. It denotes the time increment for a particular treatment, such as incubation at a given temperature, to reduce the number of viable spores by one order of magnitude. When samples of the same spore crop are tested at different temperatures, an interesting phenomenon is observed: the plot of the log_{10} of the calculated D-values versus incubation temperature is linear over all tested temperatures. As incubation temperature decreases, therefore, the time required to inactivate spores increases rapidly but predictably. Nicholson (2003) made clever use of these data to estimate the longevity of spores under temperature conditions typical for a warm terrestrial climate (25–40 °C). Thermal inactivation curves for the spores of B. subtilis, a mesophile, required no extrapolation, because D-values had already been experimentally determined for temperatures in this range. But Geobacillus spores require much longer incubation times for thermal inactivation to occur, so Nicholson was forced to extrapolate the available thermal inactivation curves to obtain an estimate for spore longevity at moderate temperatures. Yet the result was startling: based on this extrapolation, the longevity of Geobacillus spores at 40 °C would be 1.9 billion years, and the longevity at 25 °C even longer (Nicholson, 2003)! Of course, this estimate of extreme longevity cannot simply be accepted uncritically, as Nicholson himself discussed. Other factors besides thermal inactivation could be at work under natural conditions, and extrapolating curves is an inherently risky business. Still, this analysis at least suggests that real-world longevity of Geobacillus spores could be surprisingly high. Even if their lifespans are measured in millennia, a very modest global production of Geobacillus spores coupled with atmospheric dispersal could lead to the high populations observed in an environment where they cannot grow.

What can geobacilli teach us?

The genus Geobacillus is a taxon of humble stature. Although these bacteria may well achieve some level of importance in biotechnology, in any given environment they probably serve a minor and redundant function. If the entire genus were to become extinct in an instant, one wonders whether Earth’s biosphere would even notice. Yet consideration of their paradoxically high populations in some cool and cold environments has perhaps highlighted some important points. First, there is a need for studies surveying microbial diversity in a given environment to take extremophiles into account, especially when culture-dependent methods are used. Just because a given category of microbe would not be expected to thrive in an environment does not mean that they are not present. Second, there is a need for a broad interchange of ideas between aerobiology and other branches of microbiology. Most of the environments that bacteria occupy can interact freely with the atmosphere, and this fact needs to be taken into account if we are to have a complete and nuanced understanding of bacterial ecology and evolution. Third, the importance of the bacterial endospore in environmental microbiology deserves more focused study. Bacillus sporulation has been primarily approached from a developmental biology and genetics point of view, a focus that has yielded some impressive accomplishments. But it is likely that these organisms interact with their environment primarily as spores, rather than as vegetative cells. This phenomenon has undoubtedly had a significant impact on the evolution of these bacteria and their roles in Earth’s ecosystems. Finally, the distribution of Geobacillus in the environment requires us to regard the concept of natural selection from a somewhat unconventional angle. The accumulation of large reservoirs of viable spores in places where they are unlikely to germinate and grow suggests that ‘reproductive fitness’ sometimes can mean ensuring that reproduction happens only rarely. In this regard, the longevity of spores in the natural environment is a topic that begs for more attention and careful analysis. One might expect that if this model is...
valid, it should also be possible to find other kinds of ‘misplaced’ spores in the natural environment: strict anaerobes in aerobic environments, halophiles in mesophilic environments, and the like. These possibilities are largely unexamined. ‘Everything is everywhere, but the environment selects’ is a well-worn maxim that provided the conceptual framework for much of microbial biogeography in the 20th century but has been vigorously debated in recent years (O’Malley, 2008). I feel unqualified to weigh in on that discussion. But I hope that the continued study of Geobacillus will provide fruitful insights into the complex relationship between ecological variation and geographical distance.

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References


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