Chapter 8
Aerobic, Endospore-Forming Bacteria from Antarctic Geothermal Soils

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8.1 Introduction: Taxonomy of *Bacillus* Species and Related Genera

The term ‘aerobic endospore-forming bacteria’ is used to embrace *Bacillus* species and related genera, for which the production of resistant endospores in the presence of oxygen remains the defining feature. They are also expected to possess Gram-positive cell wall structures (but staining reactions, even in young cultures, may be Gram-variable or frankly Gram-negative), and may be aerobic or facultatively anaerobic. These characters have formed part of the definition of the group for many years, but some exceptions have emerged. *Bacillus infernus* and *B. arseniciselenatis* are strictly anaerobic, and spores have not been detected in *B. infernus, B. subterraneus*, and *B. thermoamylovorans*.

Molecular taxonomic methods have had a huge impact on the classification of these organisms, and the number of taxa, including thermophiles, has increased greatly. The 1986 edition of *Bergey’s Manual of Systematic Bacteriology* (Claus and Berkeley 1986) listed 40 valid *Bacillus* species, of which only four were true thermophiles. These were *Bacillus stearothermophilus*, *B. acidocaldarius*, and *B. schlegelii*, each with strains reported from geothermal soils (see Table 8.1), and *B. thermoglucosidasius*. “*Bacillus caldolyticus”, “B. caldotenax”,* and “*B. caldovelox”* (Heinen and Heinen 1972) were isolated from naturally heated waters and were listed as ‘*Species incertae sedis*.’ These still await validation.

Since that 1986 edition of *Bergey’s Manual* and up to late 2006, 229 further species have been newly described or revived among *Bacillus* and the 11 genera derived from it (subsequently two of these new genera were merged, so there are now 10 new genera recognized). Furthermore, 20 new genera containing 59 species have been proposed to accommodate novel aerobic endospore formers not previously assigned to *Bacillus*. Overall, then, there have been proposals for 30 new genera and 288 new

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Table 8.1 Thermophilic, aerobic endospore-forming bacteria from geothermal sources

Genus and Species of Organism (and Number of Species in Genus)	Original Reference	Original Source[a]	Other Sources[a,b]
Geobacillus (17)			
G. stearothermophilus	Donk 1920	Canned corn and beans	Food, milk, water, soil, hot compost, sugar beet juice, hot spring, **geothermal soil, Southern Urals** (Golovacheva et al. 1965), hydrothermal vents
G. gargensis	Nazina et al. 2004	Hot spring	
G. tepidamans	Schäffer et al. 2004	**Geothermal soil**, **Yellowstone, USA**, beet sugar factory	
G. thermodenitrificans	Ambroz 1913	Soil	Uncultivated soil, sugar beet juice, hot compost, **geothermal soil, South Sandwich Islands** (Logan et al. 2000), hydrothermal vents
G. thermoleovorans	Zarilla & Perry 1987	Soil, muds, sludge	Uncultivated soil, hydrothermal vents, petroleum reservoirs, hot springs
"G. thermoleovorans subsp. *stromboliensis"*	Romano et al. 2005	**Geothermal soil, Eolian Islands, Italy**	
G. vulcani	Caccamo et al. 2000	Hydrothermal vent	
Geobacillus spp.			Deep-sea hydrothermal vents, sea mud
Bacillus (134)			
B. aeolius	Gugliandolo et al. 2003	Shallow marine vent	
B. coagulans	Hammer 1915	Evaporated milk	Soil, canned foods, tomato juice, gelatin, milk, medical preparations, silage, **geothermal soil, Southern Urals** (Golovacheva et al. 1965)
B. fumarioli	Logan et al. 2000	**Geothermal soil, Antarctica**	Gelatin
B. infernus	Boone et al. 1995	Deep terrestrial subsurface	

(continued)

Table 8.1 (continued)

Genus and Species of Organism (and Number of Species in Genus)	Original Reference	Original Source[a]	Other Sources[a,b]
B. schlegelii	Schenk & Aragno 1979	Lake sediment	Geothermal water, mud and ash, glacier ice, air, **Antarctic geothermal soil** (Hudson et al. 1988)
B. thermantarcticus	Nicolaus et al. 1996	**Geothermal soil, Antarctica**	
B. tusciae	Bonjour & Aragno 1984	Geothermal pond	
***Alicyclobacillus* (11)**			
A. acidocaldarius	Darland & Brock 1971	Hot acid springs, **acid geothermal soil, Hawaii**	**Antarctic geothermal soil** (Hudson & Daniel 1988), gelatin
A. acidocaldarius subsp. *rittmannii*	Nicolaus et al. 1998	**Geothermal soil, Antarctica**	
A. hesperidum	Albuquerque et al. 2000	**Geothermal soil, Azores**	
A. vulcanalis	Simbahan et al. 2004	Hot spring	
Alicyclobacillus sp.			**Antarctic geothermal soil** (Bargagli et al. 2004)
***Aneurinibacillus* (5)**			
An. terranovensis	Allan et al. 2005	**Geothermal soil, Antarctica**	
***Anoxybacillus* (10)**			
Anox. amylolyticus	Poli et al. 2006	**Geothermal soil, Antarctica**	
Anox. ayderensis	Dulger at al. 2004	Hot spring	
Anox. gonensis	Belduz et al. 2003	Hot springs	
Anox. flavithermus	Heinen et al. 1982	Hot spring	
Anox. kestanbolensis	Dulger at al. 2004	Hot spring	
Anox. voinovskiensis	Yumoto et al. 2004	Hot spring	
***Brevibacillus* (13)**			
Br. levickii	Allan et al. 2005	**Geothermal soil, Antarctica**	
***Caldalkalibacillus* (1)**			
C. thermarum	Xue et al. 2006	Hot spring	
Sulfobacillus (4)			
Sulfobacillus spp.			Geothermal waters, Yellowstone National Park, USA, Montserrat Island
***Vulcanibacillus* (1)**			
Vulc. modesticaldus	L'Haridon et al. 2006	Deep-sea hydrothermal vent	

[a] Sources of organisms corresponding to geothermal soils are indicated in boldtype.
[b] References for isolation from geothermal soil are given.

or revived species or new combinations, and yet only seven proposals for merging species or subspecies were made in that time. Nearly 50 of these new species are thermophiles, and several moderate thermophiles have also been described, but rather few have been isolated from geothermal soils (see Table 8.1).

8.2 Habitats of Thermophiles

Most aerobic endospore formers are saprophytes widely distributed in the natural environment, but some are opportunistic or obligate pathogens of animals, including humans, other mammals, and insects. The main habitats are soils of all kinds, ranging from acid to alkaline, hot to cold, and fertile to desert, and the water columns and bottom deposits of fresh and marine waters. The bacteriology of geothermal soils has received much less attention than that of hot springs and pools, hydrothermal vents, and other heated aqueous environments. There is a long history of studies on thermophilic *Bacillus*, dating from the proposal of the best-known species, *B. stearothermophilus* (now *Geobacillus stearothermophilus*), from canned food in 1920. Of the present 17 valid species in *Geobacillus*, there are reports of eight with isolates from unheated soils, six from aqueous environments associated with oil or gas fields, six from composts, five from hot springs and shallow hydrothermal vents, four from foods, including canned foods, milk, and sugar processing, and only three (*G. stearothermophilus*, *G. tepidamans*, and *G. thermodenitrificans*) from geothermal soils (see Table 8.1).

A further taxon from geothermal soil, "*G. thermoleovorans* subsp. *stromboliensis"*, awaits validation. Further, unidentified, thermophilic endospore formers have been reported from hydrothermal vents, geothermal soils, and sea muds (White et al. 1993; Marteinsson et al. 1996; Takami et al. 1997). Although the strains of White et al. (1993) were from several geothermal sites around the world, including Iceland, it is not clear which of their isolates were from heated soils. Other thermophiles are found in the genera *Alicyclobacillus, Aneurinibacillus*, *Anoxybacillus*, *Bacillus*, *Brevibacillus*, *Caldalkalibacillus, Sulfobacillus*, *Thermobacillus*, *Ureibacillus*, and *Vulcanibacillus*, but of the newer thermophilic taxa of aerobic endospore formers, fewer than 20% include strains that have been isolated from geothermal soils, and it is remarkable that over half of those were first isolated from Antarctic geothermal soils (see Table 8.1).

Although thermophilic aerobic endospore formers and other thermophiles might be expected to be restricted to hot environments, they are also widespread in cold environments and appear to be ubiquitously distributed in soils worldwide. Indeed, Weigel (1986) described how easy it is to isolate such organisms from cold soils and even from Arctic ice. Endospores readily survive distribution from natural environments to a wide variety of other habitats, as the example of *B. fumarioli*, described in detail below, demonstrates.

Strains of *Geobacillus* with growth temperature ranges of 40 to 80°C can be isolated from subsurface layers of soils whose temperatures never exceed 25°C

(Marchant et al. 2002). That spores may survive in such cool environments without any metabolic activity is understandable, but their wide distribution and contribution of up to 10% of the cultivable flora suggest that they do not merely represent contamination from hot environments (Marchant et al. 2002). A study of some Irish temperate soils found aerobic thermophile counts of 1.5–8.8 × 10^4 colony-forming units per gram, and similar results were obtained for other temperate soils from Europe (McMullan et al. 2004), suggesting that these are part of the autochthonous flora (Rahman et al. 2004). It is possible that the direct heating action of the sun on the upper layers of the soil, and local heating from the fermentative and putrefactive activities of mesophiles, might be sufficient to allow the multiplication of thermophiles, but perhaps these organisms are capable of very low levels of activity at normal environmental temperatures (McMullan et al. 2004).

8.3 Antarctic Geothermal Soils

There was constant volcanic activity in Antarctica during the Cenozoic period, and steaming ground is to be found in a number of circumpolar islands and on the continent (Fig. 8.1). Thus, although Antarctica is largely an ice-bound continent that

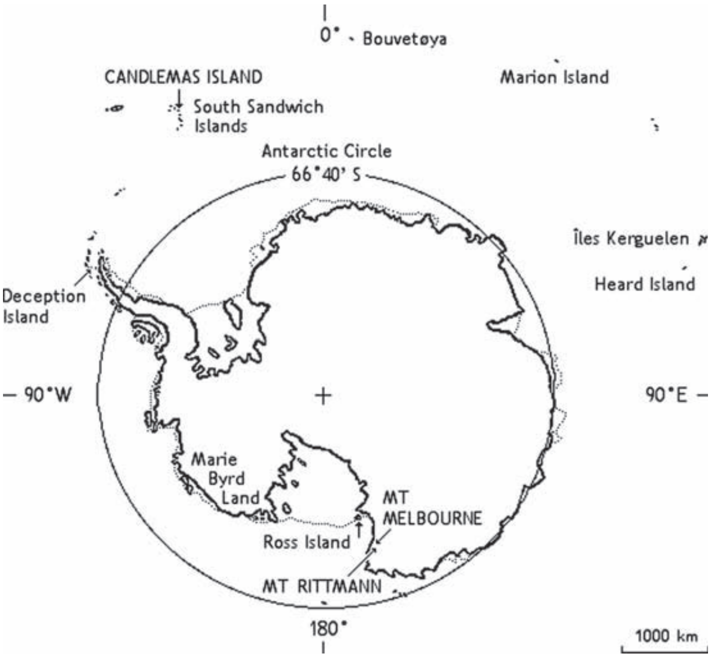

Fig. 8.1 Map of Antarctica and the sub-Antarctic islands, with geothermal sites named. Names in capital letters indicate the sites from which *Bacillus fumarioli, Brevibacillus levickii* and *Aneurinibacillus terranovensis* were isolated

relies upon solar heating during the summer to support a sparse growth of terrestrial life, several sites exist where volcanic activity warms the soil and steam emissions from fumaroles condense to maintain relatively steady water supplies that may support the growth of vegetation. All of these places are remote, and are costly and difficult to visit, and therefore no comprehensive study of the microbiologies of their geothermal soils has been made, but we are fortunate to have some information on the aerobic endosporeforming floras of five of these sites.

8.3.1 The Antarctic Continent

Three sites, Mt Erebus, Mt Melbourne, and Mt Rittmann, are the only known high-altitude localities of fumarolic activity and associated vegetation within Antarctica. The unique selective pressures of such sites make the organisms that live there of special biological interest (Broady 1993), and they can harbour unique vegetation communities which appear to have formed following colonisation by propagules from circumpolar continents (Linskens et al. 1993).

Mounts Erebus and Melbourne represent two of the four provinces of the McMurdo Volcanic Group, which is one of the most extensive alkali volcanic provinces in the world. These two volcanoes were named in 1841 by James Clark Ross after his expedition ship *Erebus,* and the British Prime Minister Lord Melbourne. It is worth noting in passing that some of the expedition's specimens were submitted to the eminent botanist Christian Ehrenberg, in Berlin, for microbiological examination. It was Ehrenberg who had proposed *Vibrio subtilis* (now *Bacillus subtilis,* and the type species of the genus) in 1835.

Mt Erebus (3,794 m; 77° 32′S; 167° 8′E) is the most active volcano on the Antarctic continent, and the largest of four cones on Ross Island. Its cone rises 200 to 300 m above the summit plateau and it is bordered by a side crater, which is the rim of a filled-in older crater or caldera, to the SW. The floor of the main crater has an inner crater, whose floor bears a fumarolic ridge. The walls of the main crater also bear fumaroles, and discontinuous lines of fumaroles and ice towers radiate from the crater rim and across the plateau. Patches of warm ground lie on the rim of the main crater, within the side crater, and on the plateau. An area of warm ground to the NW of the main crater, with surface ground temperatures that may reach 75°C, is an Antarctic Specially Protected Area (ASPA) called Tramway Ridge; the ice-free ground here is terraced, and the steep sides of the terraces bear the main crusts of vegetation.

Mt Melbourne (2,733 m; 74° 21′S; 164° 42′E) is situated in the centre of a relatively young (probably 2–3 × 10^6 years old) volcanic field that has been formed by a large number of small, individual eruptive centres (Broady et al. 1987). Located on the southern rim of the main summit crater of Mt Melbourne is an ASPA called Cryptogam Ridge (Fig. 8.2). This is a deglaciated site with soil temperatures typically reaching 40–50°C at depths of a few centimetres; it supports a unique community including algal and bryophyte species unknown elsewhere in Antarctica

Fig. 8.2 Mount Melbourne, Northern Victoria Land, Antarctica, looking towards Wood Bay in the Ross Sea. The deglaciated Antarctic Specially Protected Area called Cryptogam Ridge is seen in the foreground. The icy hummock in the centre of the picture is formed by condensate freezing above a fumarole

Fig. 8.3 Mount Rittmann, Northern Victoria Land, Antarctica, showing areas of heated soil with fumaroles, and ice towers formed from frozen fumarole condensate, interspersed with patches of permafrost

(Nicolaus et al. 1991). The flora of the geothermally heated area of the northwest (NW) slope of the mountain, lying at 2,400 to 2,500 m, is less well developed than that of Cryptogam Ridge.

Mt Rittmann (2,600 m; 73° 27′S; 165° 30′E) (Fig. 8.3) was discovered during the fourth Italian Antarctic Expedition (1988–1989). The soil surface temperature ranges from 34.4–41.5°C and there is patchy development of vegetation. The geothermally heated biosystem at Mt Rittmann has been described by Bargagli et al. (1996). It lies at an altitude of about 2,600 m, and has small fumaroles whose internal temperatures (at 10 cm depth) range between 50°C and 63°C; patches of moss grow on the warm soil which has a relatively high moisture content (by Antarctic standards) and a pH of around 5.4.

Soil samples were collected from the NW slope of Mt Melbourne and from the Mt Rittmann geothermal site by British Antarctic Survey (BAS) members of the international BIOTEX 1 expedition during the 1995–1996 austral summer, and further samples were taken from these sites and from Cryptogam Ridge during the 1998–1999 austral summer by one of the authors (NAL).

Although Mt Melbourne and Mt Rittmann lie in the same volcanic province, and their soils may appear similar in some respects (they have very low concentrations of essential nutrients such as N and P), Bargagli et al. (1996) showed that there were differences in the mineral contents of soil samples collected from Mt Melbourne (higher Cu and Zn) and Mt Rittmann (higher Cd and Pb), and Pepi et al. (2005) reported that mossy and unvegetated soil samples from the NW slope of Mt Melbourne had higher iron contents than did soils from Cryptogam Ridge and Mt Rittmann. For other major elements, soils from the NW slope of Mt Melbourne also had the highest contents of Na and Al; soils from Cryptogam Ridge had the highest content of Mg; and soils from Mt Rittmann had the lowest contents of Ca, Al, and Fe. For trace elements, soils from the NW slope of Mt Melbourne also had the highest contents of Cd, Cr, Pb, and Zn; soils from Cryptogam Ridge had the highest contents of Cu and Hg; and soils from Mt Rittmann had the lowest contents of Cu and Hg. Thus organisms in these soils may be constrained by low concentrations of essential nutrients and, in some cases, relatively high concentrations of toxic minerals.

8.3.2 The South Sandwich Archipelago

The South Sandwich archipelago comprises 11 islands, all of which exhibit recent or continuing volcanic activity. They lie on the Scotia Ridge between latitude 56°18′ and 59°28′S and longitude 26°14′ and 28°11′W; this volcanic arc has a deep-sea trench on its convex (eastern) side descending to over 7,000 m. The fauna and flora of Candlemas Island (Fig. 8.4; map in Logan et al. 2000) are more extensive than those of other islands in the arc, and include penguins, petrels, skuas, and seals, grass, bryophytes, and lichens. The southern massif forms the largest part of the island, and is an ice-capped remnant of an extinct volcano. In the younger and actively volcanic northern part lies Lucifer Hill (232 m; 57°04′S, 26°42′W), a complex of scoria cones surrounded by a mass of five main lava flows. The oldest of these bear ash mantles, whereas the youngest is very recent. There are patches of moss around active fumaroles high on the hill, around inactive fumaroles at Clinker Gulch, and on lava soils at the base of the volcanic cone, with temperatures

Fig. 8.4 Candlemas Island, South Sandwich Archipelago, viewed from the east. Lucifer Hill, in the unglaciated northern part of the island, is seen on the right-hand side of the picture, with its summit partially obscured by cloud. (Aerial photograph kindly provided by Dr John L. Smellie, British Antarctic Survey. © British Crown Copyright/MOD.)

ranging from 85°C down to 0°C. The northern and southern parts of the island are linked by an area of low flat sand containing two large lagoons. Mossy soil samples were collected from the summit and base of Lucifer Hill by British Antarctic Survey personnel on behalf of one of the authors (NAL) during the 1996–1997 austral summer.

8.3.3 Deception Island

Deception Island (62°57′S, 60°38′W) is one of seven islands that comprise the South Shetland archipelago. It is 17-km across and ring-shaped, being the rim of a caldera that has been flooded by the sea, and it lies on the expansion axis of the Bransfield Rift that separates the archipelago from the Antarctic Peninsula. A narrow gap in the caldera rim leads to the basin of Port Foster, a perfect natural harbour. It has seen human activity since the 1820s, including sealing, whaling, and scientific research; however, there were major eruptions in 1967, 1969, and 1970, and the island is less frequented now. Several ASPAs have been established, and the whole island is an Antarctic Specially Managed Area. It was visited by a Spanish scientific expedition in the austral summer of 1989–1990, and water and sand samples were collected from four geothermal sites: a fumarole at Cerro Caliente, Irizar Lake, Kroner Lake, and Whalers Bay (Llarch et al. 1997).

8.4 Endospore Formers from Antarctic Geothermal Soils

The first report of an aerobic endospore former isolated from Antarctica was by Dr A. L. McLean, who studied the bacteriology of ice and snow during Douglas Mawson's Australasian Antarctic Expedition of 1911–1914. Little other Antarctic bacteriology was published until Darling and Siple (1941) described the isolation of 178 strains from various Antarctic environments, and found 66% of them to belong to nine *Bacillus* species. Ugolini and Starkey (1966) isolated bacteria from fumaroles of Mt Erebus, but according to Hudson and Daniel (1988) these were probably mesophiles.

The first isolations of thermophilic endospore formers were made by Hudson and Daniel (1988) from Tramway Ridge and its locality, and from the side and west craters of Mt Erebus. They found strains of *Bacillus* in most of their samples. In particular, they isolated strains resembling *B. acidocaldarius* (now in *Alicyclobacillus*) in the more acid soils of Tramway Ridge and its neighborhood (Hudson et al. 1989), *Bacillus schlegelii* (capable of utilizing thiosulfate; Hudson et al. 1988) in an acid soil near Tramway Ridge, and anaerobes resembling *Clostridium thermohydrosulfuricum* (now *Thermoanaerobacter thermohydrosulfuricus*) from most of their samples. *Bacillus schlegelii* was first isolated from the sediment of a Swiss lake, and *B. tusciae* was discovered when *Bacillus schlegelii* was first sought in geothermal environments (Bonjour and Aragno, 1984).

Nicolaus et al. (1991) isolated four strains of thermophilic eubacteria from Cryptogam Ridge, Mt Melbourne, and one strain from near the seashore at the foot of the mountain, and the effects of growth temperature on polar lipid patterns and fatty acid compositions of these and other strains were compared (Nicolaus et al. 1995). One of the Cryptogam Ridge isolates was proposed as *Bacillus thermoantarcticus* (Nicolaus et al. 1996). Its exopolysaccharide chemistry has been investigated (Manca et al. 1996) and its digestion of xylan studied (Lama et al. 2004). The proposal was later validated and the name of the isolate corrected to *Bacillus thermantarcticus*. However, it should probably belong in *Geobacillus*.

Nicolaus et al. (1998) also isolated strains of *Alicyclobacillus* from Mt Rittmann, and proposed the new subspecies, *Alicyclobacillus acidocaldarius* subsp. *rittmannii*; strains had ω-cyclohexyl fatty acids, MK-7 quinones, and hopanoids characteristic of *Alicyclobacillus* species, but lacked amylolytic activity. The effects of growth temperature on lipid modulation were studied in detail (Nicolaus et al. 2002). In 30 geothermal soil samples from Mt Rittmann, these authors found only aerobic endospore formers belonging to *Alicyclobacillus*; it will be recalled (see above, this section) that Hudson et al. (1989) found strains similar to *Alicyclobacillus* (then *Bacillus acidocaldarius*) on Mt Erebus. Bargagli et al. (2004) isolated a strain tentatively identified as an *Alicyclobacillus* species from the iron-rich NW slope of Mt Melbourne, and found that it needed iron supplements in its growth media (Pepi et al. 2005).

Llarch et al. (1997) studied six isolates from sand, sediment, and water from geothermal sites on Deception Island. None of these strains was identifiable as a member of an established thermophilic species of *Bacillus*, but two strains from fumarolic water showed some relationship with *B.* (now *Geobacillus*) *stearothermophilus*. More interesting was the identification of two strains as *B. licheniformis* and *B. megaterium*, and of two others as outliers of the species *B. firmus* and *B. lentus*; all four species are known as mesophiles, but the Deception Island isolates all had optimal growth temperatures between 60 and 65°C, which considerably extend the maximum growth temperatures known for these species.

Logan et al. (2000) examined geothermal soil samples from Mt Melbourne (both Cryptogam Ridge and the NW slope), Mt Rittmann, and Candlemas Island. They used a variety of growth conditions in order to isolate aerobic endospore formers but they did not find strains of *Bacillus thermantarcticus* or *Alicyclobacillus acidocaldarius* subsp. *rittmannii*. Instead, they isolated an organism that grew optimally at pH 5.5 and 50°C on a nutritionally weak, solid medium (*Bacillus fumarioli* agar, or BFA: 0.4% yeast extract, 0.3% KH_2PO_4, 0.2% $(NH_4)_2SO_4$, and traces of $MgSO_4$ and $CaCl_2$; with 5 mg/l $MnSO_4$ to enhance sporulation). The organism subsequently grew and sporulated better on a medium of half this nutrient strength. The organism was isolated from Cryptogam Ridge on Mt Melbourne (but not the NW slope of Mt Melbourne), and from Mt Rittmann and Candlemas Island and was proposed as *Bacillus fumarioli* (Fig. 8.5). It was found on Mt Rittmann both as spores and vegetative cells, in soils whose temperatures ranged from 3.4°C to 62.5°C, the proportions of sporulated cells tending to be higher at the temperature extremes (9% at 8.3°C; 29% at 58.5°C), and lower (3% at 42.5°C) at temperatures approaching the growth optimum.

Finding *B. fumarioli* on two volcanoes 110 km apart and on Candlemas Island, which is about 5,500 km distant from Mt Melbourne, was quite striking, and it seemed remarkable that it could not be isolated from the NW slope of Mt Melbourne,

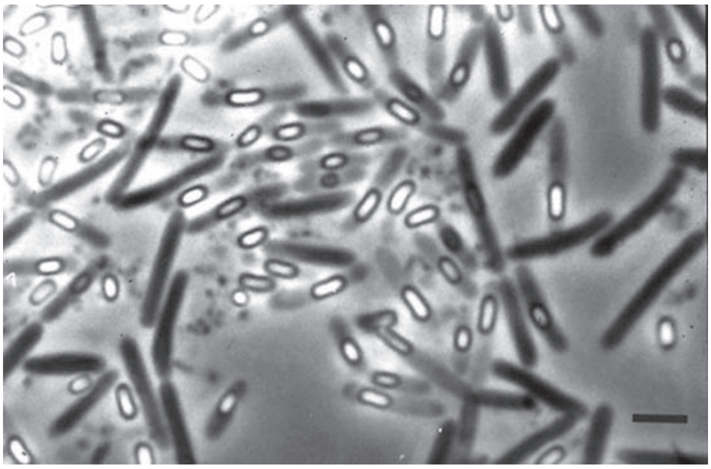

Fig. 8.5 Photomicrograph of sporangia and vegetative cells of *Bacillus fumarioli* viewed by phase contrast microscopy; ellipsoidal and cylindrical spores lie paracentrally and subterminally in unswollen sporangia. Bar represents 2 µm

despite repeated sampling; nor was it isolated from 25 cold soils local to Mt Melbourne. It was even more surprising, therefore, that using BFA De Clerck et al. (2004) were able to isolate *B. fumarioli* from gelatin production plants in Belgium, France, and the USA. Although both ecosystems have similarly low pH and moderately high temperatures, their geographical separations are huge, and the organic load in Antarctic soils is very low compared with that of gelatin. A polyphasic taxonomic comparison showed very close relationships between the Antarctic and gelatin isolates; the latter organisms did, however, produce an abundant protein with high similarity to a stress response protein, and subtractive DNA hybridization revealed genomic differences between the two sets of isolates that might indicate adaptive evolution to a specific environment (De Clerck et al. 2004).

It is of special interest that the NW ridge of Mt Melbourne failed to yield *B. fumarioli* from samples taken on two occasions. Why this particular geothermal site, lying a short distance from Cryptogam Ridge, should not yield the organism is not understood. It was noted that moss was absent from the NW ridge, yet *B. fumarioli* was isolated from both mossy and moss-free areas of Cryptogam Ridge and Mt Rittmann. Broady et al. (1987) remarked on the low diversity of Victoria Land warm ground bryophytes compared with Deception Island and the South Sandwich Islands in the maritime Antarctic, and suggested that as none of the local cold-ground bryophytes of Victoria Land had colonized the local volcanoes, it may be inferred that the soil chemistries of the fumarole environments might be unsuitable; indeed, as noted above, Bargagli et al. (1996) and Pepi et al. (2005) found appreciable differences in the mineral contents of these different soils. Broady et al. (1987) also noted that the geothermal areas of Mt Erebus and Mt Melbourne are, in comparison with maritime Antarctica, much farther from the rich propagule sources of more temperate lands to the north and west, and well south of the circumpolar westerly airstream that might carry and deposit such propagules; however, the discovery of *B. fumarioli* in Europe and America suggests that this organism is widely dispersed. Llarch et al. (1997) did not find *B. fumarioli* in their geothermal soils from Deception Island, but they did not cultivate at pH 5.5 from their samples.

Two other kinds of aerobic endospore formers were isolated from Mt. Melbourne during the expedition of 1998–1999 (Logan et al. 2000). They were scanty and difficult to cultivate, and so were not studied further at that time, but additional strains were isolated from the same soil samples back in Glasgow. Allan et al. (2005) subjected 13 such strains to polyphasic taxonomic study, and proposed seven isolates from the NW slope of Mt Melbourne as the new species *Brevibacillus levickii* (Fig. 8.6), and proposed six isolates from Cryptogam Ridge and the vents and summit of Mt Rittmann as another new species, *Aneurinibacillus terranovensis* (Fig. 8.7). *Brevibacillus* strains were not isolated from the sites at Mt Rittmann or Cryptogam Ridge and *Aneurinibacillus* strains were not isolated from the NW slope of Mt Melbourne. The distribution of *An. terranovensis* thus correlates with that of *B. fumarioli* on Mts Melbourne and Rittmann, whereas *Br. levickii* was only found in soils of the NW slope of Mt Melbourne, from which *An. terranovensis* and *B. fumarioli* could not be isolated; perhaps these observations owe something to the differences in soil chemistries that Bargagli et al. (1996) and Pepi et al. (2005) discovered at these sites.

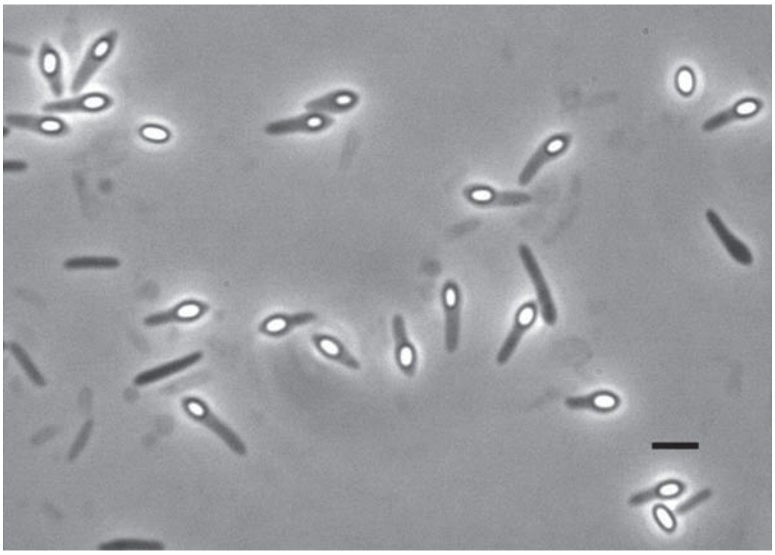

Fig. 8.6 Photomicrograph of sporangia and vegetative cells of *Brevibacillus levickii* viewed by phase-contrast microscopy; ellipsoidal spores lie subterminally and terminally in swollen sporangia. Bar represents 2 µm

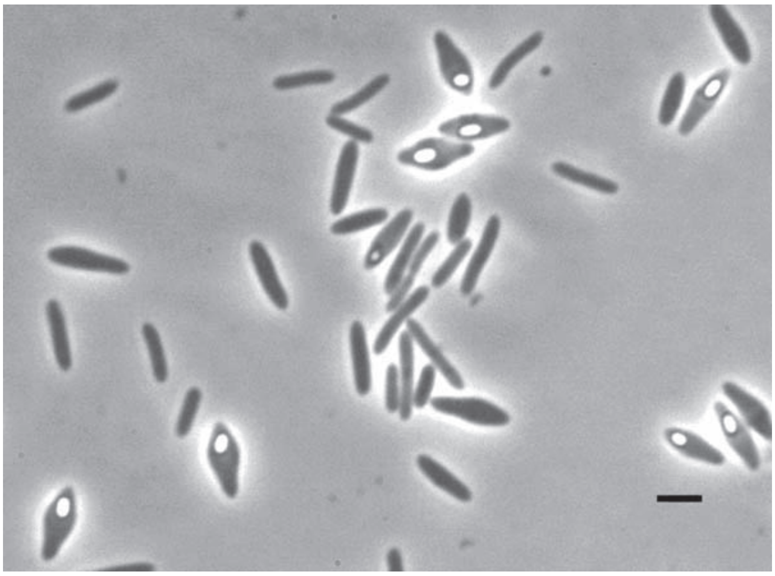

Fig. 8.7 Photomicrograph of sporangia and vegetative cells of *Aneurinibacillus terranovensis* viewed by phase-contrast microscopy; ellipsoidal spores lie centrally, paracentrally and subterminally in swollen sporangia. Bar represents 2 µm

Our emphasis thus far has been on thermophilic or thermotolerant organisms, but the same geothermal soils have also yielded mesophilic species. Bargagli et al. (2004) isolated strains related to *Paenibacillus validus* (a species that we have found repeatedly in unheated Antarctic soils) and a strain they identified as *P. apiarius* on Mt Melbourne, and found that these organisms, from iron-rich soils, often benefited from iron supplements in their growth media (Pepi et al. 2005). The novel species *B. luciferensis*, *B. shackletonii* (Logan et al. 2002, 2004a), *Paenibacillus cineris*, and *P. cookii* were isolated from Candlemas Island; *P. cookii*, like *B. fumarioli,* has also been isolated from a gelatin production plant (Logan et al. 2004b). The presence of *Paenibacillus* species in these soils is of particular interest, as these organisms often fix nitrogen; Rodríguez-Díaz et al. (2005) demonstrated the presence of the *nifH* gene in *Paenibacillus wynnii*, from unheated soil on Alexander Island, and capacity for acetylene reduction in *P. cineris* and *P. cookii*. Logan et al. (2000) also found strains of the mesophiles *B. sphaericus* (also found on Alexander Island by Rodríguez-Díaz et al. (2005)) and *B. cereus,* and the thermophile *B.* (now *Geobacillus*) *thermodenitrificans* on Candlemas Island.

8.5 Adaptations for Growth at High Temperatures

Organisms growing at high temperatures need enzyme adaptations to give molecular stability as well as structural flexibility, heat-stable protein-synthesizing machinery, and adaptations of membrane phospholipid composition. They differ from their mesophilic counterparts in the fatty acid and polar headgroup compositions of their phospholipids. The effect of temperature on the membrane composition of *G. stearothermophilus* has been intensively studied. Phosphatidyl glycerol (PG) and cardiolipin (CL) comprise about 90% of the phospholipids, but as the growth temperature rises the PG content increases at the expense of the CL content. The acyl-chain composition of all the membrane lipids also changes; the longer, saturated-linear and iso fatty acids with relatively high melting points increase in abundance, and anteiso fatty acids and unsaturated components with lower melting points decrease. As a result, the organism is able to maintain nearly constant membrane fluidity across its whole growth temperature range; this has been termed homeoviscous adaptation. An alternative theory, homeophasic adaptation, considers that maintenance of the liquid-crystalline phase is more important than an absolute value of membrane fluidity in bacteria (Tolner et al. 1997).

The major cellular fatty acid components of *Geobacillus* species following incubation at 55°C are (with ranges as percent of total given in parentheses) iso-$C_{15:0}$ (20–40%; mean 29%), iso-$C_{16:0}$ (6–39%; mean 25%) and iso-$C_{17:0}$ (7–37%; mean 19.5%), that account for 60–80% of the total (Nazina et al. 2001). The figures given by Fortina et al. (2001) for *G. caldoxylosilyticus* and Sung et al. (2002) for *G. toebii* generally lie within these ranges, with the exception that strains of the former species showed 45–57% of iso-$C_{15:0}$. Such higher levels of iso-$C_{15:0}$ are also found in *Anoxybacillus* species (Dulger et al. 2004). *Geobacillus thermoleovorans* subsp. *stromboliensis* (Romano et al. 2005), isolated from Italian geothermal soil, showed fatty acid patterns within the ranges seen for other *Geobacillus* species. *Thermobacillus xylanilyticus* Touzel et al. (2000) shows a fatty acid profile dominated by iso $C_{16:0}$, whereas the profile of *Vulcanibacillus modesticaldus* is dominated by iso $C_{15:0}$ (L'Haridon et al. 2006). Direct comparison of profiles between the obligately thermophilic species and mesophilic aerobic endospore formers is not normally possible, as the assays of members of the two groups have not usually been done at the same temperature.

Nicolaus et al. (1995) studied the effects of growth temperature on polar lipid patterns of aerobic endospore formers from geothermal and unheated soils from Antarctica; at 60°C, the strain subsequently proposed as *Bacillus thermantarcticus* (and which presently awaits reassignment to *Geobacillus*) showed a level (27%) of iso-$C_{17:0}$ fatty acid which is similar to that of *Bacillus thermoglucosidasius,* but a high level (36%) of anteiso $C_{17:0}$ fatty acid in comparison with *Bacillus* and *Geobacillus* species. Nicolaus et al. (2002) reviewed their lipid studies on Antarctic isolates. Strains tentatively identified as *Bacillus* showed increased phosphoglycolipid contents with increased growth temperature, at the expense of phosphoaminolipid and phospholipids; higher-melting point acyl chains such as iso-$C_{17:0}$ were favoured at maximum growth temperatures, whereas iso-$C_{15:0}$ was synthesized at minimum growth temperature.

Llarch et al. (1997) compared the fatty acid profiles of aerobic endospore formers isolated from Antarctic geothermal environments; their six isolates had temperature ranges with minima between 17 and 45°C and maxima between 62 and 73°C, with optima of 60 to 70°C. Two strains (temperature ranges 37–70 and 45–73°C) were found to lie nearest to *G. stearothermophilus* in a phenotypic analysis, and two other isolates could be identified as strains of *B. licheniformis* (temperature range 17–68°C) and *B. megaterium* (temperature range 17–63°C) whose maximum growth temperatures were extended beyond those seen in strains from temperate environments. The fatty acid profiles for all of these strains were compared following incubation at 45°C, and the results suggested that any potential distinctions between the rather variable fatty acid profiles of *Geobacillus* species and *Bacillus* species are largely lost when strains of each group are incubated at the same temperature.

Members of *Alicyclobacillus* possess an apparently unique phenotype, as the main membranous lipid components of most species are ω-alicyclic (ω-cyclohexane or ω-cycloheptane) fatty acids; it has been shown that ω-alicyclohexyl fatty acids pack densely, resulting in low diffusion at high temperatures (Kannenberg et al. 1984). Polar lipids based upon hopanoids are also important chemotaxonomic markers for this genus, and hopanoid content is increased in response to elevated temperatures at low pH. Together with lipids containing ω-cyclohexane fatty acids, hopanoids are important for forming a biological membrane stable enough to withstand extreme temperature and pH conditions.

Nicolaus et al. (2002) found that the percentage of cyclohexyl fatty acids increased as the growth temperature was raised for their Antarctic *Alicyclobacillus* isolates. However, an *Alicyclobacillus* isolate from fruit juice, that did not possess ω-alicyclic fatty acids, showed a fatty acid profile similar to that of *Bacillus tusciae* (Goto et al. 2003).

8.6 Nutrition and Growth Conditions of Thermophiles

Most aerobic endospore formers are chemo-organotrophs and, despite the very wide diversity of the genus, will grow well on routine media such as nutrient agar or trypticase soy agar. However, some isolates, particularly those from nutritionally poor environments, may grow poorly if at all on these standard media because of their neutral pH, and/or insufficient salinity, or because they are nutritionally too rich. Most species will use glucose and/or other fermentable carbohydrates as sole sources of carbon and energy. Inorganic and organic sources of nitrogen are used. Many species will utilise an ammonium salt as their sole nitrogen source; amino acids are also widely utilized, and strains of some species can use urea. Most thermophilic species conform to this general nutritional pattern for aerobic endospore formers.

Geobacillus species utilize a wide range of substrates, including carbohydrates, organic acids, peptone, tryptone, and yeast extract; the ability to utilize hydrocarbons as carbon and energy sources is a widely distributed property in the genus (Nazina et al. 2001; Marchant et al. 2006). A strain of *G. thermoleovorans* has been found to have extracellular lipase activity and high growth rates on lipid substrates such as olive oil, soybean oil, mineral oil, tributyrin, triolein, and Tweens 20 and 40 (Lee et al. 1999).

Alicyclobacillus species are obligately acidophilic, and have been found particularly in fruit juices and solfataric environments; isolates from the latter show different patterns of resistance to metal salts (Simbahan et al. 2004).

Bacillus schlegelii grows chemolithoautotrophically, using H_2 as electron donor and CO_2 as carbon source (CO will satisfy both requirements), or chemo-organoheterotrophically; it can also grow autotrophically on thiosulfate (Hudson et al. 1988). Hydrogenase is constitutive and has a temperature optimum between 70 and 75°C. Carbohydrates are not used. Organic acids and a small number of amino acids are utilized as sole carbon sources, whereas ammonium ions, asparagine, and urea can be utilized as sole nitrogen sources. *Bacillus tusciae* also grows chemolithoautotrophically, using H_2 as electron donor and CO_2 as carbon source, or chemoorganoheterotrophically. Carbon and nitrogen sources are similar to *B. schlegelii*, and some alcohols can also be used as carbon sources.

Members of the genus *Sulfobacillus* are facultative autotrophs that can obtain their energy by oxidizing ferrous iron, as well as elemental sulfur or its reduced compounds. None of the three established species from this genus have been reported from natural geothermal environments, but unidentified *Sulfobacillus* strains have been reported from geothermal waters (see Table 8.1).

Amino acid transport in *G. stearothermophilus* is Na^+-dependent, which is unusual for neutrophilic terrestrial organisms, but common among marine bacteria and alkalophiles; however, the possession of primary and secondary Na^+-transport systems may be advantageous to the organism by allowing energy conversion via Na^+-cycling. Although the phospholipid adaptations needed to give optimal membrane fluidity at the organism's growth temperature also result in increased proton permeability, this may be counteracted by increased proton pumping activity using the less permeable sodium ions as coupling ions (de Vrij et al. 1990; Tolner et al. 1997).

Allan (2006) used a [^{14}C] l-glutamic acid tracing technique to study the type strains of *Br. levickii* and *An. terranovensis* with a view to understanding why they could not be isolated from the same habitats on Mt Melbourne and Mt Rittmann (see Section 8.4 above and Fig. 8.1). Results for both species showed distinct differences in the mechanisms used for l-glutamic acid uptake; *Br. levickii* possesses a secondary uptake system specific for l-glutamic acid which is dependent on K^+ and possibly H^+, and *An. terranovensis* possesses multiple uptake systems which are capable of transporting other amino acids as well as l-glutamic acid, a process which appears to be dependent on multiple factors including Na^+, K^+, H^+, the electrical gradient across the cell membrane, and osmotic conditions. These studies showed that both strains utilize glutamate, which is probably available from cyanobacteria and microalgae in their natural habitats (Siebert and Hirsch 1988). Glutamate is nonessential, however, as the organisms grew on a defined medium from which it had been omitted. For comparative purposes, l-glutamic acid uptake by strains of *B. fumarioli* and *B. cereus* were also investigated. *B. fumarioli* was found to possess an uptake system similar to that of *An. terranovensis* (consistent, perhaps, with their similar habitats), whereas the *B. cereus* strain possessed multiple uptake systems capable of transporting both the d and l isomers of glutamic acid.

8.7 Conclusions

It is clear that our understanding of the bacteriology of Antarctic geothermal soils remains in its infancy. Although several new species of aerobic endospore formers have been discovered in these niches in the last 15 years, almost nothing is known about the relationships of the prokaryotes and eukaryotes within these environments, whereas knowledge of the ecologies of deep-sea hydrothermal vents is ever increasing (Reysenbach et al. 2006).

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