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Survival and Activity of Bacteria in a Deep, Aged Lake Sediment (Lake Constance)

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Abstract

Viable counts and potential activities of different bacteria were determined as a function of depth in the deep profundal sediment of Lake Constance, Germany. The sediment layer at the bottom of the lake had a total depth of about 7 m and was deposited in the time after the last ice age, i.e., over the past 13,000 years. The high clay content of the sediment prevents seepage. Below 25 cm all of the viable heterotrophic bacteria were present as heat-resistant spores. Numbers of viable spores of both aerobic and anaerobic heterotrophic bacteria decreased exponentially with sediment depth and were below the detection limit (5–55 cells ml^{-1}) at 4–6 m, i.e., in about 8,900-year-old sediment. Absence of viable heterotrophic bacteria in deeper sediment layers demonstrated that aseptic sampling conditions were achieved. The decrease of viable spores with depth may be interpreted as time-dependent death of spores resulting in a death rate of about 0.0013–0.0025 $year^{-1}$. Viable units of specific metabolic groups of bacteria were detected only in the upper sediment layers (0–50 cm). Nitrifying bacteria could not be detected below 30 cm. Methane-oxidizing bacteria were present in the sediment down to >30 cm, but were in a dormant state. Nitrate reduction activity decreased by a factor of 6 within the upper 25 cm of the sediment, but was still detected at 50 cm. Sulfate reduction, on the other hand, could not be detected at depths of 20 cm and below. By contrast, methanogenesis and methanogenic bacteria could be detected down to 50 cm. These observations indicate that bacteria eventually become nonviable in aged sediments.

Introduction

Spore-forming microorganisms are able to survival in a dormant state for long times. Sneath [43] found viable spores of *Bacillus subtilis* in 320-year-old soil samples from a herbarium. The data allowed only a rough estimation of the death rate of the spores (about 0.046 $year^{-1}$). Bacteria and fungi were also isolated from the body of a prehistoric man who was conserved in glacier ice for 5,300 years [20]. Viable bacterial spores of various species of *Thermoactinomyces* were recovered from an archeological excavation containing

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plant debris deposited between 85 and 125 AD [40]. Viable endospores of *Thermoactinomyces* were found in 9,000-year-old lake sediments [32]. Abyzov [1] reported viable microorganisms in Antarctic ice 10,000–13,000 years old. Recent observations on amber suggest that bacterial spores may stay viable for more than 25 million years [10].

In sediment cores that are obtained by drilling of deep aquifers, the numbers of bacteria often do not decrease with depth [14]. The absence of a decrease may be due to seepage of viable bacteria or growth substrates to deeper sediment layers. Low numbers of viable microorganisms were found in those sediment layers that were relatively rich in clay and silt. It was argued that the reason for the low numbers of bacteria was that the clay-rich sediment was less permeable for intrusion of water containing bacteria and substrates [6, 15, 34, 37].

Numbers and/or activity of bacteria in the top layers of seawater and freshwater sediments have been frequently studied [11, 21]. The presence of viable bacteria in deep, aged sediment layers has not received as much attention. A study on deep layers of marine sediments was reported by Parkes et al. [33, 34]. These sediments contained active bacteria at depths >500 m. However, the sediments were permeable and exhibited brine incursions so that bacteria may have been swept in from other sites or have been stimulated by influx of substrates. Similar data from a limnic environment are lacking, probably because of the logistic difficulties in taking long sediment cores in lakes as this requires the availability of special ships. The deep sediments of Lake Constance were recently investigated within a paleolimnological cooperative program between the ETH of Zürich and the University of Konstanz [48]. Thus, we were provided with a long sediment core. Since the sediment of Lake Constance is impermeable due to its high clay content [30] and, thus, is probably not affected by seepage, we studied the vertical distribution of culturable bacteria.

Materials and Methods

Sampling and Processing of Sediment

A sediment core of 7.33 m length and 6 cm diameter was taken with a piston corer [28] from the profundal sediment at a water depth of 115 m in the northern arm (47°41′22″N, 9°13′41″E) of Lake Constance, Germany in June 1988. The sediment core, which was contained in a polyvinyl chloride core liner [26], was retrieved from the piston corer and was cut into 1-m sections. The ends of the core liners were stoppered and the core sections were stored in upright position at 4°C for six months, before processing. The coring method disturbed the upper part of the core. Therefore, sediment cores from the upper 1 m were taken by the method described by Tessenow et al. [46] with the modifications of Frenzel et al. [17]. These sediment cores were taken at the deepest point (145 m water depth) of the northern arm of Lake Constance, the so-called Überlinger See. A total of 13 cores were sampled over the period of February 1988 to March 1989. Processing of these cores (8 cm diameter) started immediately after return to the laboratory, and was finished within 10 days. In between, the cores were stored in upright position with water on top at the in-situ temperature (4°C). Porewater samples were taken by centrifugation (6,000 rpm) of slices of the sediment core. The supernatant was stored at −25°C until analysis.

Sediment samples used for most probable number (MPN) counting were taken aseptically by pushing the upper end of the core with a piston into a core liner. The top of the upper core liner was closed with a rubber stopper. The core was then cut between the two core liners to about one-third of its original diameter and broken in the direction of the cut. This procedure resulted in a smooth, freshly-exposed surface for one-third of the core diameter which was contaminated by the knife, and in an uncontaminated rough surface for the broken part of the core. A subcore was taken from the broken area by using a sterile syringe with a cut-off tip. Samples from the upper 25 cm of the sediment were directly taken with a syringe, since these sediment layers were too soft for breaking. For sampling from layers below 1 m depth the whole core was cut longitudinally through the middle. Then a syringe of 2 cm diameter with a cut-off tip was pushed 1 cm deep into the sediment at a distinct depth. An aluminum plate (0.5 mm thick) was pushed from the side under the tip of the syringe to cut the subcore in the syringe for one-third of its diameter. Then the subcore was broken, and the sediment was aseptically sampled from the broken area.

Sediment samples for counting of methanotrophic bacteria and their activities were obtained by taking subcores with a syringe with a cut-off tip through predilled holes on the side of the sediment corer [17].

Counting of Bacteria

All media were adapted to the low salt concentrations in Lake Constance [4]. All cultures (MPN and plate counts) were incubated at 21°C. The agar plates were kept in a closed box containing 2% CO_2 in air. CO_2 was included to adjust the growth conditions to the relatively high carbonate content that is typical for Lake Constance sediment [30]. Pasteurization of samples was achieved by exposure of the serial dilutions to 80°C for 10 min.

Aerobic heterotrophic bacteria were enumerated in tenfold serial dilutions using the plate count technique. Aliquots (100 µl) from each serial dilution were plated onto agar plates using a Drigalsky spatula. The agar had the following composition per liter: agar, 15 g; caseinpeptone, 0.5 g; bactopeptone, 0.5 g; soluble starch, 0.5 g; K_2HPO_4, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; trace element solution [49], 1 ml and glycerin, 1 ml brought to pH 7 with HCl.

Anaerobic bacteria were counted by the MPN method using tenfold dilutions with three tubes [2]. This MPN method gives a

95% confidence limit of typically 1 order of magnitude. The mineral medium was prepared as described elsewhere [50] with the following composition per liter: NaCl, 0.4 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; KCl, 0.15 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; NH_4Cl, 0.1 g; KH_2PO_4, 0.08 g. After autoclaving, $NaHCO_3$, 10 mM; Na_2S, 1.25 mM; trace-element solution [49], 2 ml; vitamin solution [51], 1 ml; Na_2SeO_3, 10 nM; and Na_2WO_4, 10 nM were added per liter.

Heterotrophic anaerobic bacteria were counted in the mineral medium supplemented with 5 mM fumarate, 5 mM pyruvate, and 0.1% w/w yeast extract. The MPN tubes were incubated under an atmosphere of 10% CO_2 and 90% N_2 at 21°C for 60 days.

Nitrate-reducing bacteria were counted in the mineral medium supplemented with 2 mM $NaNO_3$, 1 mM glucose, 2 mM Na-acetate, 2 mM Na-butyrate, and 0.05% w/w yeast extract. The MPN tubes were incubated under an atmosphere of 10% CO_2 and 90% N_2 at 21°C for 120 days. Tubes were counted positive when >1 mM nitrate was reduced.

Sulfate-reducing bacteria were counted in the mineral medium supplemented with 10 mM Na_2SO_4 and either 6 mM Na-lactate plus 3 mM Na-acetate for lactate-utilizing sulfate reducers, or 10 mM Na-acetate for acetate-utilizing sulfate reducers. Acetate was included in the lactate medium to serve as an additional carbon source. The MPN tubes were completely filled with medium and incubated at 21°C for 170–189 days. Tubes were scored as positive when >0.1 mM sulfide was produced.

Methanogenic bacteria were counted in the mineral medium supplemented with either 5 mM methanol or 10 mM acetate. The MPN tubes were incubated under an atmosphere of 90% N_2 plus 10% CO_2 at 21°C for 137–150 days. Tubes were counted positive when >10 μmol CH_4 ml^{-1} medium was produced.

Nitrifying bacteria were counted by the same MPN method using the following mineral medium per liter: KCl, 2.7 g; Na_2SO_4, 0.7 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; $MgCl_2 \cdot 6H_2O$, 0.05 g; $AlCl_3$, 0.1 g; $NaH_2PO_4 \cdot H_2O$, 0.07 g; $Na_2HPO_4 \cdot 2H_2O$, 0.09 g; and trace element solution [49], 2 ml. The mineral medium was supplemented with 10 mM NH_4Cl, 0.2% w/w $CaCO_3$, and 0.2 mM Na-acetate. The MPN tubes were incubated under air at 21°C for 56 days. Tubes were scored as positive when >0.5 mM nitrate was produced (nitrite was not detected).

Methanotrophic bacteria were counted by an MPN method using twofold serial dilutions in eight parallels. The serial dilutions and incubations were done in microtiter plates. The procedure and media were described previously [7]. This MPN method gives a standard error that is typically <60%.

Rate Measurements

Sulfate reduction potentials were measured in slurries which were prepared by cutting the sediment core and transferring the slices into Erlenmeyer flasks (2 l) flushed with nitrogen. After addition of the same volume of oxygen-free water, the flasks were closed with rubber stoppers and stirred at 4°C for 1 h. Then 50-ml portions of the slurries were filled under nitrogen gas into 100-ml Erlenmeyer flasks. The rate measurements were started by addition of 0.5–1 ml sulfate solution to the slurries, resulting in a final concentration of 200 to 500 μM. The slurries were incubated at 15°C and stirred slowly. Samples (5 ml) were taken from the flasks under anoxic conditions [5] and centrifuged at 6,000 rpm. The supernatant was stored at −25°C until analysis by ion chromatography. Nitrate reduction potentials were measured analogously in slurries prepared from subcores (20 ml volume) of the sediment core. The subcores were transferred directly into the 100-ml flasks containing 20 ml oxygen-free water. Methane production rates were measured in subcores transferred directly into pressure tubes as described previously [17]. Methane oxidation rates were measured in serum bottles containing sediment slurry and an initial CH_4 concentration of 370 ppmv in air as described elsewhere [8]. Potential rates of CH_4 oxidation were measured after induction of the dormant methanotrophs by adding 10% CH_4 into the air headspace of the serum bottle and following the oxidation of CH_4 until the rate had reached a maximum [9]. The different metabolic rates were calculated from the linear slope of four to five concentration measurements. All the rate measurements were made in duplicate. Controls with autoclaved sediment showed no activity.

Sulfate, nitrite, and nitrate concentrations were analysed by ion chromatography [5]. Methane was measured by gas chromatography with a flame ionization detector [12]. Sulfide was measured by a photometric test [13].

Results

Characteristics of the Sediment

The sediment of mesotrophic Lake Constance showed alternating thick gray and thin black layers which are typical for alpine lakes [31, 52]. With increasing depth of sediment the layers became less conspicuous. The laminar structure was not disturbed by the coring procedure. The dominating sediment color was gray in the upper layers, and turned to yellowish grey below about 5 m. At about 6 m, a 5-mm thick layer of tuff was observed which may have originated from the volcanic eruption 11,000 years ago that is known to have formed the basin of the Lake of Maria Laach [47]. At 7.33 m the sediment material abruptly changed to glacier gravel. Since the last ice age ended 13,000 years ago, the sediment layer just above the glacier gravel should have this age. The median particle diameter in the sediment is only 1 to 5 μm [30]. This small particle size results in such dense packing that the sediment material is watertight in thick layers [30].

We measured the bulk density of the sediment for only the upper 50 cm. The density (gram dry weight per volume) increased linearly from 0.11 g cm^{-3} at the surface to 0.54 g cm^{-3} at 42 cm. Using this density and the sedimentation rate of solid material of 0.08 g cm^{-2} that was observed in recent times [44] we estimated an age-depth correlation of 675 year m^{-1}. Since the age of the sediment at 7.3 m was about 13,000

years, the average age-depth correlation of the entire sediment was about 1,780 year m^{-1}. These data indicate that the sediment becomes more compact with depth and/or the sedimentation rate was slower in the past. These results are in agreement with radiocarbon data [48].

The upper layers of the sediment (0–24 cm) show a distinct profile of radioactive ^{137}Cs. The different peaks can be attributed to events such as nuclear weapon testing or to the fallout of the Chernobyl nuclear reactor accident. The conservative behavior of the tracer indicates a negligible perturbation of the sediment by biotic or abiotic processes [29].

Vertical Profiles of Heterotrophic Bacteria

The numbers of viable heterotrophic bacteria were determined in the sediment to a depth of 7.3 m (Fig. 1). Only in the top 5-cm section were the numbers of bacteria counted in pasteurized samples significantly lower ($P < 0.05$) than those in unpasteurized samples. In sediment layers below 25 cm, bacterial numbers in fresh and pasteurized samples were very similar, indicating that most of the bacteria in the deeper sediment layers were present as heat-resistant spores. Therefore, the data from the pasteurized and unpasteurized samples were pooled and analyzed together (Fig. 1). Only sections below 1 m were analyzed from the long sediment core. The age of the sediment below 1 m depth (>675 years) was much greater than the storage time (0.5 year) of the long sediment core, which was kept at in-situ temperature. Therefore, we are confident that the bacterial populations were not significantly affected by storage of the core. Haldeman et al. [18] reported only a small increase of viable bacterial counts from 4×10^4 to 1×10^5 when intact cores of tuff were stored for 1 week at 4°C. Perturbation by chipping the rock material, however, resulted in larger increases of bacterial counts and in shifts of the microbial community after storage [19]. The upper 1 m of the Lake Constance sediment was analyzed in fresh cores. The data were pooled and analyzed together with those from the long core (Fig. 1).

For analysis we assumed that the vertical profile of bacterial counts (N) was exclusively a function of the age (t) of the bacteria. The death rate (d) is then given by the following equations:

$$N = N_0 e^{-d t} \quad (1)$$

$$d = -t^{-1}(\ln N - \ln N_0) \quad (2)$$

If we assume that the age-depth relation ($r = tx^{-1}$) is constant with depth (x) of the sediment, we obtain

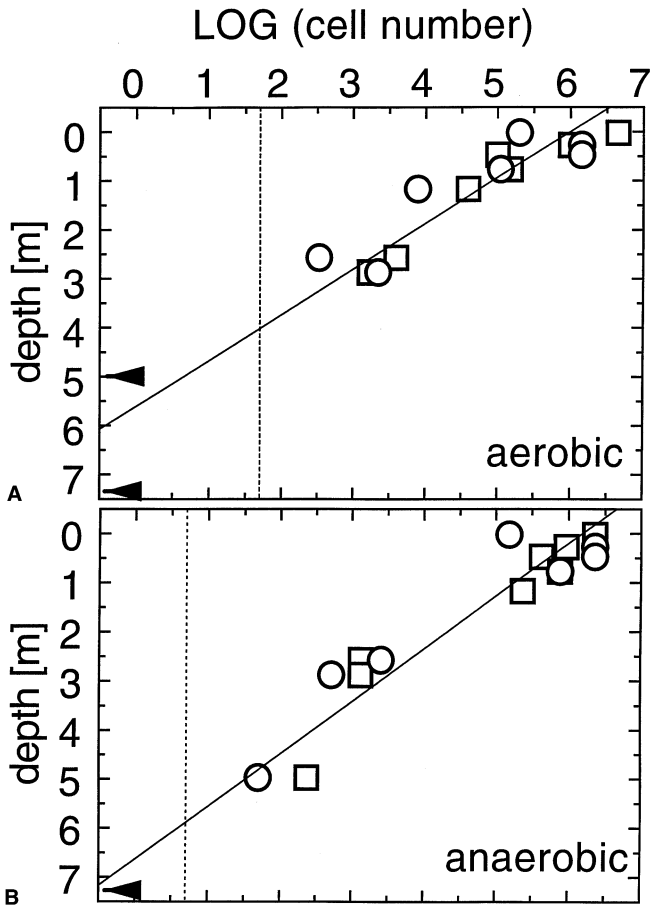

Fig. 1. Numbers (N; per ml sediment as decadic logarithms) of aerobic (**A**) and anaerobic (**B**) heterotrophic bacteria as function of sediment depth (x). The data points were fitted by linear regression and resulted in the following equations: **A:** logN = −1.021 x +5.872; **B:** logN = −0.932 x +6.190. The *vertical dotted line* indicates the detection limit of the counting procedure, i.e., the cell number where the probability of growth was >95%. Samples from the depths indicated by *arrows* were assayed, but did not result in bacterial growth. □, unpasteurized; ○, pasteurized.

$$d = -(rx)^{-1} (\ln N - \ln N_0) \quad (3)$$

Indeed, the bacterial counts decreased exponentially with depth and could be fitted to Eq. 3. Both aerobic (Fig. 1A) and anaerobic (Fig. 1B) bacteria showed almost the same depth profile and resulted with r = 1780 year m^{-1} in death rates of 0.00127 and 0.00139 $year^{-1}$, respectively. Extrapolation of the exponential decrease of the bacterial counts with depth resulted in 1 bacterium ml^{-1} at about 5.5 m and 6.5 m for culturable aerobic and anaerobic bacteria, respectively. However, the bacterial populations were already at the detection limit (5–55 bacteria ml^{-1}) at 4–6 m. Our bacterial enumeration probably underestimates actual population size,

since no attempt was made to separate the bacterial cells that were attached to particles or to each other, or to detect bacteria that were viable but not culturable [16].

Since the age-depth relation of the sediment is probably smaller in the surface layers than in the deep sediment (see above), the populations of aerobic bacteria (Fig. 1A) at the 0–1 m depth were fitted to Eq. 3 separately from those at 1–7 m. The data from the 0–1 m depth resulted with r = 675 year m^{-1} in a death rate of d = 0.00251 $year^{-1}$, those from the 1–7 m depth resulted with r = 1,780 year m^{-1} in a death rate of d = 0.00198 $year^{-1}$. The counts of the anaerobic bacteria (Fig. 1B) could not be used for such an analysis, since the data from the 0–1 m depth gave no significant ($P > 0.1$) fit to Eq. 3.

Aerobic bacteria isolated from deep sediment layers (>2 m) were typically thick rods with a centrally-located endospore, typical of *Bacillus* and related genera [35, 42]. Smaller cells, as compared with the aerobic cultures, were dominant in the anaerobic bacterial enrichment cultures from deep sediment layers (>2 m depth). Microscopic observation showed that the endospores were terminally located in a swollen host cell, typical for *Clostridium* and related genera [23]. However, cells with central spores were also found in anaerobic enrichment cultures. A small number of enrichment cultures from unpasteurized samples also showed no evidence of spore-forming organisms.

Vertical Profiles of Different Metabolic Types of Bacteria

Nitrate was detectable (about 40 μM) only in the overlaying water. Within the sediment, however, nitrate was generally <1 μM (detection limit). The potential nitrate reduction activity was relatively high in the upper sediment layers (0–5 cm), and decreased with depth (Table 1). The numbers of nitrate-reducing bacteria decreased at depths >30 cm (Table 1).

Sulfate concentrations decreased from >80 μM at the sediment surface to about 10–30 μM at 20 cm and below. Potential sulfate-reduction activity was found only in the upper sediment layers (0–5 cm), but was at the detection limit in deeper layers (Table 1). The numbers of lactate-utilizing sulfate-reducing bacteria decreased in parallel with the sulfate reduction potentials (Table 1). The numbers of the acetate-utilizing populations of sulfate reducers were low, 300–600 bacteria ml^{-1}, regardless of depth (Table 1).

The rates of CH_4 production at 25–30 cm were almost as high as at 0–5 cm (Table 1). A low activity was still detectable down to 50 cm. However, the numbers of methanogenic bacteria (acetate or methanol-utilizing) decreased in the same sediment core significantly between the surface (0–5 cm) and deeper (25–30 cm) layers (Table 1).

Table 1. Numbers and potential activities of anaerobic bacteria as function of depth of sediment[a]

Depth (cm)	Activity (nmol ml^{-1} day^{-1})	MPN (MPN ml^{-1})	
Nitrate reduction			
0–10	1710 ± 17	1.1×10^4	
20–30	268 ± 61	2.0×10^4	
40–50	87 ± 67	43	
Sulfate reduction		On lactate	On acetate
0–10	190 ± 17	2.4×10^6	296
20–30	ND[b]	93	296
40–50	ND	43	553
Methane production		On methanol	On acetate
0–5	4.89 ± 0.29	10.5×10^3	1.7×10^3
25–30	3.83 ± 0.04	169	10
45–50	0.11 ± 0.09	26	48
70–75	ND	ND	ND

[a] The activities are given as mean ± SD of the linear regression of data pooled from duplicate incubations. The 95% confidence limit of the MPN values was typically one order of magnitude
[b] ND, not detectable

Published data concerning Lake Constance indicate that O_2 is present only at depths above 3 mm [17], indicating that aerobic metabolism must be confined to these top sediment layers. Indeed, the numbers of nitrifying bacteria decreased significantly with depth and were no longer detectable below 45 cm (Table 2). Nitrite was not detected in the MPN tubes that contained ammonium as substrate, indicating that numbers of nitrite-oxidizing bacteria were at least as high as those of ammonium-oxidizing bacteria.

Numbers of methanotrophic bacteria also decreased with depth, but were still detectable at 30 cm (lower depths were not sampled) (Table 2). Methane oxidation was observed only in the upper 7.5 cm layer of the sediment (Table 2). However, if the sediment samples were incubated for 200–300 h under a headspace of 10% CH_4 in air to induce CH_4 oxidation, samples from deeper sediment also started to oxidize CH_4 (Table 2) indicating that the methanotrophic bacteria present in this layer were dormant.

Discussion

Our study showed that viable spores of heterotrophic bacteria were detectable down to 5–6 m of the impermeable

Table 2. Numbers and potential activities of aerobic bacteria as function of depth of sediment

Depth (cm)	Activity: Spontaneous (nmol ml^{-1} day^{-1})	Activity: After induction (μmol ml^{-1} day^{-1})	MPN (MPN ml^{-1})
Methane oxidation[a]			
0.5	39.6 ± 0.8	31.2 ± 0.3	22×10^4
1.0	46.2 ± 1.0	24.0 ± 0.3	
3.0	19.8 ± 0.5	23.5 ± 0.4	3×10^4
7.5	8.8 ± 0.7	5.9 ± 1.4	2×10^4
20	ND[b]	10.1 ± 1.2	8×10^4
32	ND	15.8 ± 1.4	5×10^4
Nitrification			
0–5			460
25–30			18
45–50			ND

[a] The activities are given as mean ± SD of the linear regression of data pooled from duplicate incubations. The standard error of the MPN values of CH_4-oxidizing bacteria was typically <±60%
[b] ND, not detectable

clay-rich profundal sediment of Lake Constance. By multiplying these depths with the age-depth correlation of 1,780 year m^{-1}, the bacterial spores are estimated to have survived for more than 8,900 years. Abyzov [1] found viable bacteria in 13,000-year-old Antarctic ice. However, almost no culturable bacteria were detected in lacustrine sediments from a deep aquifer with a groundwater age of at least 13,000 year [27]. Deep basalt aquifers, on the other hand, in which the age of the rock ranges from 6 to 17 million years, contained autotrophic bacteria that were able to survive on chemically-produced H_2 as substrate [45]. Hence, the presence of viable microorganisms in buried sediment layers seems to depend on the availability of substrates. Phelps et al. [36] estimated doubling times for the microbial community in deep aquifers to be in the range of centuries.

The fluctuation of the bacterial numbers that we observed was not unusual for MPN counts which, with three parallels, have 95% confidence limits of about 1 order of magnitude [2]. There may be other reasons for fluctuations in deep layers, such as compaction of sediments and sedimentation rates that are usually not constant over time [48], resulting in changing cell numbers per volume over depth. Also, the input of nutrients [52] has probably changed between geological epochs resulting in changes of the numbers of bacteria and spores formed. Finally, the death rate of spores is not necessarily constant, for example, due to secular changes in: natural radioactivity [43], temperature, and the embedding medium [10]. The demonstration of the absence of bacterial growth in samples from sediment layers below 5–6 m is evidence that our sampling procedure was aseptic. Otherwise the high cell numbers in the upper layers would have contaminated the samples of deeper layers.

The potential for transport of bacteria into deeper sediment layers was probably negligible in Lake Constance sediment because of the high clay content [30] which makes the sediment impermeable [30, 41]. Furthermore, spores cannot move actively, and, vegetative cells have no reason to move into deeper sediment layers, since fresh substrates are supplied only by sedimentation from above. Therefore, it is reasonable to assume that the bacteria detected as spores in deeper sediment layers were at least as old as the sediment layer itself. The decrease in the number of spores with depth may be interpreted in two ways: as the death rate of spores, or as an effect of increasing bacterial numbers in the top layer due to eutrophication of the lake since the ice age [52]. However, since bacteria can even be found in Antarctic ice [1], we assume that the sediment of Lake Constance was not completely sterile at the end of the ice age. Therefore, at least part of the depth-dependent decrease in the numbers of spores must be due to death. The similarity of the depth-dependent decrease of aerobic versus anaerobic spores, in combination with the different cell shapes and sizes observed in enrichment cultures, may be interpreted as similar death rates of the heat-resistant endospores of aerobic and anaerobic bacteria. The observed exponential decrease of viable spores with sediment depth resulted in death rates of 0.0013–0.0025 year^{-1} depending on the age-depth correlation of the sediment section used.

Activities and viable counts of different physiological groups of bacteria were determined only in the freshly exposed sediment core segments. Potential nitrate reduction rates and nitrate-reducing bacteria were found down to 50 cm. However, actual nitrate reduction would have been restricted to the upper sediment layers where nitrate was available. Nitrate reduction is found among spore-forming bacterial species such as *Bacillus* sp. Thus, the small nitrate-reducing bacterial population observed at the 40–50 cm depth may have been due to spore formers. Alternatively, nitrate reducers may survive by fermentation of organic substrates [24].

Rates of potential sulfate reduction and numbers of sulfate-reducing bacteria decreased dramatically below 10 cm and reached the detection limit at 20–30 cm. Obviously, the metabolism of this bacterial group was severely hampered in the sediment layers where sulfate was below detection [3].

The small number of lactate-utilizing sulfate reducers found at 40–50 cm may be the same population as that counted on acetate as electron donors, since the lactate medium included acetate. However, the number of acetate utilizers was low regardless of depth. The acetate utilizers may have belonged to the genus *Desulfotomaculum* which is the only genus of sulfate-reducing bacteria that form endospores. Since no sulfate reduction was detectable below 30 cm, the sulfate reducers found at 40–50 cm were probably present as spores, similarly as described for the littoral of Lake Constance [4].

The methanogenic activity decreased drastically below 25–30 cm. The rates of CH_4 production were measured in samples taken in the winter and, thus, were relatively low compared to earlier reports that are based on rates measured during summer [17, 39]. The numbers of acetate or methanol-utilizing methanogenic bacteria decreased by more than an order of magnitude when compared to sediment layers from 0–5 cm versus 25–30 cm. No methanogenic activity was detected below 50 cm. Since spore-forming methanogens are unknown, it is plausible that the methanogens in these sediments survive only as long as they are supplied with sufficient substrates by fermenting bacteria, which in this case is only above 50 cm.

The numbers of nitrifying bacteria decreased steadily from the surface layers down to 45 cm where they were no longer detectable. In Lake Constance sediment, the activity of nitrifiers is restricted to the uppermost surface layers of about 3 mm, since O_2 is absent below this depth [17]. The nitrifiers that were detected at 25 cm must either have been transported to this depth or have resided there since the sediment was deposited. Transport is unlikely because of the physical properties of the sediment (see above). Nitrifying bacteria do not form known spores or resting stages, but nevertheless are able to survive for up to 24 weeks under energy source deprivation when exposed to air [25]. However, with an age-depth correlation of 675 year m^{-1} for the upper sediment layers, the layers at 25 cm would have an age of about 170 years. Nitrifiers are apparently able to survive for prolonged periods under anoxic conditions.

Similarly to ammonium oxidizers, methanotrophic bacteria can be active only in the upper 3 mm sediment layer where O_2 is available [17]. Although methanotrophs were detected to a depth of 32 cm, methane oxidation in these layers was detected only if the sediment samples were preincubated under high CH_4 concentrations in presence of O_2. Thus, the methanotrophic bacteria are probably dormant. Methanotrophs form cysts as resting stages and thus are probably able to survive adverse conditions for quite some time [22]. It has been shown that they are able to survive under starvation conditions for up to 10 weeks and that survival is enhanced under anaerobic compared to aerobic conditions [38]. Our results suggest that some methanotrophs survived for about 170 years under anaerobic conditions.

In summary, our results demonstrate that buried clay-rich lake sediments may serve as a source of aged bacterial spores and as a model system to study the conditions in nature for the extended survival of different metabolic groups of microorganisms.

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