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Heuer, Verena B.; Inagaki, Fumio; Morono, Yuki; Kubo, Yusuke; Spivack, Arthur J.; Viehweger, Bernhard; Treude, Tina; Beulig, Felix; Schubotz, Florence; Tonai, Satoshi; Bowden, Stephen A.; Cramm, Margaret; Henkel, Susann; Hirose, Takehiro; Homola, Kira; Hoshino, Tatsuhiko; Ijiri, Akira; Imachi, Hiroyuki; Kamiya, Nana; Kaneko, Masanori; Lagostina, Lorenzo; Manners, Hayley; McClelland, Harry Luke; Metcalfe, Kyle; Okutsu, Natsumi; Pan, Donald; Raudsepp, Maija J.; Sauvage, Justine; Tsang, Man Yin; Wang, David T.; Whitaker, Emily; Yamamoto, Yuzuru; Yang, Kiho; Maeda, Lena; Adhikari, Rishi R.; Glombitza, Clemens; Hamada, Yohei; Kallmeyer, Jens; Wendt, Jenny; Wörmer, Lars; Yamada, Yasuhiro; Kinoshita, Masataka; Hinrichs, Kai Uwe

Published in:
Science

DOI:
10.1126/science.abd7934

Publication date:
2020

Link:
Link to publication in PEARL

Citation for published version (APA):
Heuer, V. B., Inagaki, F., Morono, Y., Kubo, Y., Spivack, A. J., Viehweger, B., Treude, T., Beulig, F., Schubotz, F., Tonai, S., Bowden, S. A., Cramm, M., Henkel, S., Hirose, T., Homola, K., Hoshino, T., Ijiri, A., Imachi, H., Kamiya, N., ... Hinrichs, K. U. (2020). Temperature limits to deep subseafloor life in the Nankai Trough subduction zone. *Science*, *370*(6521), 1230-1234. https://doi.org/10.1126/science.abd7934

Temperature limits to deep subseafloor life in the Nankai Trough subduction zone

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One sentence summary: In deep subseafloor sediments above 45°C, microbial cells are rare, endospores prevail, and life still persists at 120°C.

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Abstract: Microorganisms in marine subsurface sediments substantially contribute to global biomass. Sediments warmer than 40°C account for roughly half the marine sediment volume, but the processes mediated by microbial populations in these hard-to-access environments are poorly understood. We investigated microbial life in up to 1.2 km deep and up to 120°C hot sediments in the Nankai Trough subduction zone. Above 45°C, concentrations of vegetative cells drop two orders of magnitude and endospores become more than 6,000 times more abundant than vegetative cells. Methane is biologically produced and oxidized until sediments reach 80-85°C. In 100°C to 120°C sediments, isotopic evidence and elevated cell concentrations demonstrate the activity of acetate-degrading hyperthermophiles. Above 45°C, populated zones alternate with zones up to 192 m thick where microbes were undetectable.

Scientific ocean drilling has demonstrated the ubiquity of microbial life in deep subseafloor environments down to 2.5 km below seafloor (*1-3*). As sediment temperature increases with burial depth, more than 50% of the global marine sediment volume is situated above 40°C (*4*). So far, the vast majority of subseafloor-life studies have targeted environments with in-situ temperatures <30°C; the habitability of hotter sediments is largely unexplored. Microbes with growth temperatures up to 122°C have been isolated at hydrothermal vents (*5*), where the metabolism of these hyperthermophiles is fueled by high fluxes of oxidants and reductants (*6*). However, in deeply buried sediments, energy is limited and with increasing depth and temperature the slow-growing microbial communities struggle to meet the cellular maintenance energy requirement (*3, 7, 8*). Even in organic-matter rich petroleum reservoirs, microbial activity appears to cease at temperatures of ~80°C (*9, 10*).

Aiming to fill the vast knowledge gaps regarding the response of microbial life to increasing temperature, we investigated up to 1.2 km deep and up to 120°C hot sediments in the Nankai Trough off Cape Muroto, Japan (fig. S1). In this area, an up to 16 million year (My) old, ~600 m thick succession of hemipelagic mudstones and tuffs has been rapidly buried by an equally thick layer of trench deposits over the past ~0.4 My (*11;* fig. S2). Sediments concurrently heated by approximately 50°C, and the onset of subduction formed a décollement separating the accreting and underthrusted domains (*11, 12*). First indications for the presence of microbial life in ~800 m deep, ~80-90°C warm sediments at a nearby drill site date back two decades (*11, 12*). However, insufficient sensitivity in cell detection at that time compromised the habitability assessment of this environment (*12*). We designed Expedition 370 of the International Ocean Discovery Program (IODP) to achieve maximal sensitivity in life detection together with accurate determination of in-situ temperatures, and established Site C0023 (32°22.0018'N, 134°57.9844'E, 4776 m water depth; fig. S1) in the vicinity of the previous drill site (*13,* see Supplementary Materials). Rigorous precautions during sampling and improvements in cell enumeration techniques increased the sensitivity in cell detection by five orders of magnitude compared to the previous study (*12*). For the quantification of cells that can be stained by a fluorescent dye (hereafter termed vegetative cells), the procedural blank was 4.2 ± 4.0 cells cm^{-3} of sediment (N = 20), thereby yielding a minimum quantification limit (MQL) of 16 cells cm^{-3}. Temperature measurements in the borehole constrained a steady-state temperature profile with a gradient of 110°C km^{-1} and a temperature of 120 ± 3°C in the deepest core retrieved from the basement at 1177 m below seafloor (mbsf) (figs. S3, S4). The combination of authigenic minerals and thermally altered biomarkers reveals a history of episodic, short-term ingression of ~140-220°C hot hydrothermal fluids along permeable strata in the underthrust domain (*14,* fig. S2).

At Site C0023, the depth profile of cell concentrations deviates notably from the global trend of gradually decreasing cell concentrations observed in similarly deep but substantially colder (<30°C)

sediments (*1*, *2*). At ~300-400 mbsf, concentrations of vegetative cells drop abruptly by two orders of magnitude and approach the MQL as temperature rises from 40°C to 50°C (Fig. 1A). Concurrently, concentrations of endospores, i.e., dormant, resistant structures affiliated with the bacterial phylum Firmicutes (fig. S5), which are widely found in marine sediments and soils (*15, 16*), increase to 2×10^5 cm^{-3} (Fig. 1B). Nevertheless, a small microbial population persists at >50°C in the form of both vegetative cells and endospores (Fig. 1). Down to the 120°C hot basement, sediments harboring microbial communities with up to 400 vegetative cells cm^{-3} are interspersed with intervals of up to 192 m thickness, in which no cells were detected (Fig. 1A; fig. S6). We rule out the possibility that the detection of cells resulted from contamination because cell concentration is neither related to the abundance of fractures in sediment cores nor to the concentration of the perfluorocarbon-based contamination tracer supplied during drilling operation (fig. S7); such relationships would be expected if contaminant cells were introduced via drilling fluids. Consistent with the extremely low concentrations of vegetative cells and the difficulty of extracting DNA from endospores (*17*), DNA yields were insufficient for producing reliable DNA-based community data for samples buried more deeply than 320 mbsf (*13*). In samples shallower than 320 mbsf, the community resembled those found in shallow subsurface sediments (*13*).

In contrast to the scattered distribution of vegetative cells in sediments >50°C, endospores show a clear zonation (Fig. 1B), as quantified by measurement of the diagnostic biomarker dipicolinic acid (DPA) (*18*). We rule out that substantial levels of DPA could have accumulated after the decay of endospores, given the propensity of 2-carboxylated pyridines to decarboxylate upon moderate short-term heating (*19*). Endospore concentrations rise prominently in a ~200-m interval of 75-90°C hot sediments, with a maximum of 1.2×10^6 endospores cm^{-3} at 85°C. The average endospore-to-vegetative cell ratio exceeds 6,000 in sediments below 350 mbsf (table S1) and is thus 2-3 orders of magnitude higher than in cold subseafloor sediments (*18*). Plausible scenarios for the accumulation of endospores in sediments that are nearly barren of vegetative cells relate to the thermal history of the site since the onset of trench conditions ~0.4 My ago (*12, 14*) and involve the transitory growth of a thermophilic population of endospore formers (cf. ref. *16*) after temperature rose to ~50°C and its subsequent sporulation (fig. S8). Interestingly, in two expanded horizons, at 570-633 mbsf and 829-1021 mbsf, neither vegetative cells nor endospores were detected (Fig. 1, fig. S6).

Pore-water profiles of microbial substrates and products provide evidence for microbial activity down to the ~16 My old oceanic crust (Fig. 2). High concentrations of methane with a mean carbon isotopic composition (δ^{13}C-CH_4) of -61.3 ± 3.0 per mil (‰) (Fig. 2A-B) indicate biogenic methanogenesis at least down to the 80-85°C hot sulfate methane transition zone (SMTZ) at ~730 mbsf. The positive excursion in δ^{13}C-CH_4 in the SMTZ (Fig. 2B) points to a biogenic methane sink and is consistent with previous observations from cultivation-based approaches that demonstrated the activity of thermophilic anaerobic methane-oxidizing communities at these temperatures (*20-21*). Below the SMTZ, methane is only present in micromolar concentrations, with rising δ^{13}C-CH_4 values and decreasing methane/ethane ratios indicating a relative increase of thermogenic hydrocarbons (Fig. 2B). Remarkably, a reversal of this trend at >1000 mbsf hints at a biogenic methane source above 100°C.

Diffusive profiles of pore-water constituents do not allow the distinction between current and recent in-situ biogeochemical processes, while radiotracer experiments specifically target on-going microbial activity, albeit with some unavoidable deviation from in-situ conditions. At Site C0023, radiotracer experiments reveal present-day methanogenic activity in 65% of the investigated samples (Fig. 2D). Potential rates of methanogenesis via CO_2 reduction in sediments below 300 mbsf are

generally below 4 pmol cm^{-3} d^{-1} and thus within the range of previous observations made in the deep subseafloor (*22*). Their depth distribution is consistent with cellular concentrations (Fig. 1) and activities deduced from the pore-water profiles of methane (Fig. 2A-B). Rates are highest in the methanic zone, decrease distinctly to <0.6 pmol cm^{-3} d^{-1} below the SMTZ, and drop to undetectable levels in 63% of the samples taken from the deep expanded horizon with no detectable cells and endospores (Fig. 2D). Strikingly, potential methanogenesis rates rise again to values observed in the methanic zone in the three deepest samples (Fig. 2D), thus confirming the existence of active methanogenic communities in 110-120°C hot sediments and pillow basalts above basement.

Acetate is a key microbial substrate, and its generation from sedimentary organic matter upon heating has been suggested to fuel microbial life in deeply buried sediments (*23*). Throughout the sediment column of Site C0023, reactions degrading acetate via sulfate reduction and methanogenesis are exergonic, with Gibbs free energy yields becoming increasingly negative with depth (fig. S9). The concentrations of acetate and its carbon isotopic compositions (δ^{13}C-acetate) (Fig. 2C) indicate distinct changes in acetate utilization with temperature and depth. In the up to 60°C hot upper 600 mbsf, low concentrations of acetate around 26 ± 22 µM (N=19) are consistent with a steady state governed by tightly coupled microbial production and consumption, as observed in other sedimentary environments. The fluctuation of δ^{13}C-acetate around its average of -25.5 ± 3.4‰ implies ongoing metabolic activity (*24*). In sharp contrast, acetate utilization is minimal at 60°C to 100°C. At 60-75°C, acetate concentrations rise steeply with the simultaneous decline of methane concentrations and accumulation of endospores, suggesting that microbial consumption is no longer balancing the release of acetate from sedimentary organic matter. Nevertheless, a local minimum in acetate concentration at the SMTZ (Fig. 2C) is consistent with some microbial utilization at this geochemical interface. Below the SMTZ, acetate concentrations level at 9.2 ± 2.4 mM with an invariable δ^{13}C-acetate around -18.8 ± 0.5‰. The combination of high concentration and low isotopic variability implies an acetate pool without significant turnover within the endospore-dominated zone as well as in the underlying 200 m thick zone, where neither cells nor endospores were detected.

At >1030 mbsf, however, acetate concentrations decline and δ^{13}C-acetate monotonically increases with depth, reaching a maximum of -7.9‰ in the deepest pore-water sample recovered from 1101 mbsf. This trend is consistent with active hyperthermophiles degrading preferentially ^{13}C-depleted acetate, leaving the residual acetate isotopically enriched. Without continued consumption, diffusion would homogenize δ^{13}C-acetate variations, as observed in the overlying sediments. The drawdown of the acetate pool requires isotopic fractionation factors of -7.7 to -15.4‰ (fig. S10), which are consistent with those observed in lab cultures (*25*). The size of the sink would have to be on the order of 5 x 10^{-12} mol cm^{-3} $year^{-1}$. Given cellular concentrations of 10 to 100 cm^{-3} in sediments corresponding to this acetate sink, the required cellular metabolic rates are 2-3 orders of magnitude lower than observed in lab cultures of the hyperthermophilic archaea *Pyrococcus furiosus* (*26*) and *Archaeoglobus fulgidus* (*27*), but 2-3 orders higher than in-situ rates in deep sediments with temperatures <30°C (*28*). Thus, acetate profiles are consistent with the existence of a small acetate-utilizing microbial community at >100°C and suggest that the microbes at this high temperature require more energy and therefore turn over substrates faster than at lower temperature (*8*). Syntrophic acetate oxidation coupled to consumption of the resulting CO_2 and electrons by methanogens is a known acetate sink in deep sediments (*29*) and considered to be particularly important at elevated temperatures (*30*). This process is exergonic under in-situ conditions (fig. S9) and could account for the elevated methanogenesis rates (Fig. 2D) and the isotopic signature of methane (Fig. 2B) in the deepest portion of the borehole.

Our findings reveal the impact of increasing temperature with depth on microbial life. This is exemplified in the massive collapse of the population of vegetative cells in <0.4 My old sediments at 300-400 mbsf. In this interval, temperatures of 40-50°C are within the upper growth range of mesophiles. The coincident accumulation of endospores as a result of a putative sporulation of mesophilic endospore-forming Firmicutes (Fig. 1) supports the conclusion that the abundance of microbial populations is primarily controlled by temperature-dependent physiological factors down to 600 mbsf. In the deeper portion of Site C0023 geological processes exert additional control. A sharp decline in biogenic methanogenesis and acetate utilization at 70°C to 75°C coincides with the upper growth range of thermophiles, but notably, this depth interval concurrently spans the lithological boundary between Upper and Lower Shikoku Basin (cf. Fig. 1). At this boundary, tuffs (indurated volcanic ash) cease to be present. Tuff alteration forms smectite, and microbial reduction of Fe(III) in smectite serves as an energy yielding process and has in fact been found to promote smectite-to-illite conversion at 500-600 mbsf at Site C0023 (*31*). Thus, a modulation of some types of microbial activity by microbe-mineral interactions is conceivable. Peak endospore concentrations at 85°C coincide with both the SMTZ and the plate boundary décollement. While brief periods of frictional heating to temperatures of potentially up to ~1000°C during differential plate motion (*32*) likely cause additional challenges for microorganisms in this zone, endospores and high acetate concentrations may provide a seed bank and energy, respectively, for an ecosystem recovery from episodic perturbations.

In the upper 200 m of the underthrust domain, at ~90-100°C, an expanded zone without detectable cells and with no geochemical signs of microbial activity traverses the sparsely populated sediments (Figs. 1, 2). In this zone, under-compacted and mechanically weak sediments are overpressurized and affected by ~145-220°C hot fluids for short durations (*14*, *33*). The short heating events may have locally sterilized sediment (*14*), but microbial cells, acetate consumption and methanogenic activity prevail again in >100°C sediments, where mechanical strength and salinity increase towards the sediment/basement interface (Figs. 1, 2, fig. S2). Hydraulic communication between basalts and overlying sediment is evidenced by shared styles of epigenetic mineralization in the form of calcite veins and ferruginous metal oxides. Mass transfer between basal sediment and a basalt-hosted aquifer would increase the habitability of the basal sediment by reducing formation fluid pressure, and by replenishing otherwise depleted substrates such as reduced iron and sulfate (*34*).

Our study reveals the dependence of microbial abundance and activity to critical temperatures around 40-50°C and 70°C; it moreover shows that life in the deep subseafloor is not constrained by an upper temperature limit below 120°C. Our findings highlight the interplay of geological processes, temperature and microbial life in the deep, hot sediments of the Nankai Trough, and suggest a critical influence of subduction-related geological processes on habitability.

Acknowledgments. This research used samples and data provided by the International Ocean Discovery Program (IODP). The authors are grateful to IODP and the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) for providing an opportunity to explore the deep biosphere during Expedition 370. The expedition comprised simultaneous offshore and onshore work, conducted on the drilling vessel *DV Chikyu* and in the Kochi Core Center (KCC), respectively. We thank all operational and technical staff members who have put our plans into action onboard *Chikyu* and at KCC. In particular, we would like to thank N. Eguchi, M. Kyo, and I. Sawada for their knowledgeable support during project design, and T. Saruhashi and K. Aoike for care- and successful drilling operations. This is a contribution to the Deep Carbon Observatory (DCO).

Funding. This work was supported in part by the Japan Society for the Promotion of Science (JSPS) Strategic Fund for Strengthening Leading-Edge Research and Development (to JAMSTEC and F.I.), the JSPS Funding Program for Next Generation World-Leading Researchers (GR102 to F.I.), JSPS Grants-in-Aid for Scientific Research 26251041, 19H05503 and 20K20429 (to F.I. and T.H.), JP19H00730 (to Y.M., A.I., and T.H.), the Deutsche Forschungsgemeinschaft through projects 387745511 (to V.B.H.), 408178672 (to F.S.), 408249062 (to J.K.), 279667358 and Hi 616-14-1 (to K.-U.H.) and through the Cluster of Excellence "The Ocean Floor – Earth's Uncharted Interface" (project 390741603), the IODP U.S. Science Support Program (National Science Foundation prime award OCE-1450528 to T.T.), and the Natural Environment Research Council awards NE/P015182/1 and NE/R003408/1 (to S.A.B. and H.M., respectively). Additional support enabling this project was provided by the Deep Carbon Observatory.

Author contributions: K.-U.H., F.I., V.B.H., and Y.M. designed research; V.B.H., F.I., and Y.M. lead IODP Expedition 370 as co-chief scientists; Y.K. and L.M. coordinated research operations during IODP Expedition 370; V.B.H., F.I., Y.M., A.J.S., B.V., T.T., F.B., F.S., S.T., S.B., M.C., S.H., T.H., K.H., T.H., A.I., H.I., N.K., M.K., L.L., H.M., H.-L.M., K.M., N.O., D.P., M.J.R., J.S., M.-Y.T., D.T.W., E.W., Y.Y., K.Y., R.R.A., C.G., Y.H., J.K., J.W., and L.W. performed research; Y.Y. and M.K. provided crucial methods; V.B.H., F.I., Y.M., A.J.S., B.V., T.T., F.B., F.S., S.T., L.W., and K.-U.H. analyzed data; and V.B.H. and K.-U.H. wrote the paper with input from all authors.

Competing interests. None.

Data availability. All shipboard data are documented and are openly accessible in the IODP Expedition 370 Proceedings (*13*); with references to the respective tables and figures given in the Supplementary Materials. All shore-based data are accessible in the PANGAEA database (*35*).

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Fig. 1. Depth profiles of vegetative cells and endospores in relation to environmental factors at IODP Site C0023. (A) Concentrations of vegetative cells determined by counting of microbial cells fluorescently stained with SYBR Green I. (B) Concentrations of bacterial endospores derived from analysis of the diagnostic biomarker DPA; analytical sensitivity corresponds to a detection limit (DL) of 2.2×10^4 endospores cm^{-3}. (C) A schematic summary of temperature, tectonic units, and salinity showing the geochemical influence of basalt alteration in the basement; red symbols on the temperature axis designate the depth horizons where in-situ temperature measurements were made. Gray shading indicates zones where concentrations of both vegetative cells and endospores were undetectable in all samples; SMTZ indicates the location of the sulfate-methane transition zone (cf. Fig. 2).

Fig. 2. Geochemical signals of microbial metabolism at Site C0023. (A) Dissolved methane (*13*) and sulfate (*13*), (B) Methane/ethane ratios (*13*) and $\delta^{13}C\text{-}CH_4$, (C) dissolved acetate and $\delta^{13}C$-acetate, and (D) potential rates of methanogenesis (MG) based on conversion of $^{14}C\text{-}CO_2$ to $^{14}C\text{-}CH_4$; note that the value at 180 mbsf is off-scale. Potential MG (PMG) rates were determined at 40°C for ≤360 mbsf, 60°C for 405-585 mbsf, 80°C for 604-775 mbsf, and 95 °C for ≥816 mbsf. The minimum quantification limit (MQL) was 0.094 pmol $CH_4\,cm^{-3}\,d^{-1}$. Gray shading, SMTZ and temperature axis are as in Fig. 1. VPDB in panels B and D is the Vienna Pee Dee Belemnite standard.

Supplementary Materials:

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Materials and Methods and Supporting Text

Figs. S1 to S10

Tables S1 to S2

References (*36*–*83*)