Updated definitions on piezophily as suggested by hydrostatic pressure dependence on temperature

Alberto Scoma

Engineered Microbial Systems Laboratory (EMS-Lab), Biological and Chemical Engineering Section, Dept. of Engineering, Aarhus University, Høngøvej 2, 8200, Aarhus N, Denmark

Abstract

Microbial preference for elevated hydrostatic pressure (HP) is a recognized key feature of environmental and industrial processes. HP effects on macromolecules and, consequently, cell functionality has been accurately described in the last decades. While there is little debate about the importance of HP in shaping microbial life, a systematic definition of microbial preference for increased HP is missing. The lack of a consensus about 'true' piezophiles, and 'low' or 'high' HP levels, has deleterious repercussions on microbiology and biotechnology. As certain levels are considered 'low' they are not applied to assess microbial activity. Most microorganisms collected in deep waters or sediments have not been tested (nor isolated) using the corresponding HP at which they were captured. Microbial response to HP is notoriously dependent on other environmental parameters, most notably temperature, but also on availability of nutrients, growth substrate, pH and salinity. This implies that countless isolates retrieved from ambient pressure conditions may very well require increased HP to grow optimally, as already demonstrated in both Archaea and Bacteria.

In the present study, I collected the data from described piezophilic isolates and used the fundamental correlation existing between HP and temperature, as first suggested in seminal works by Yayanos, to update the definition of piezophiles. Thanks to the numerous new piezophilic isolates available since such seminal studies, the present analysis brings forward updated definitions which concern 1) the actual beginning of the piezosphere, the area in the deep sea where piezophiles thrive; 2) the HP thresholds which should be considered low, medium and high HP, and their implications for experimental design in Microbiology; and 3) the nature of obligate piezophiles and their location in the deep sea.

Introduction

Hydrostatic pressure (HP) is the force exerted on an area by a fluid at rest. Its influence on macromolecules of biological relevance contributed to the evolution of life. In the biosphere, HPs from 0.1 to 200 MPa can affect intermolecular distances and conformation of polynucleotides (DNA and RNA), lipid bilayers and multimeric proteins (Oger and Jebbar 2010). Increased HP features today planktonic crustaceans, and marine invertebrates, fish, and deep-diving mammals, with Bacteria and Archaea able to thrive at tens of MPa (Bartlett 2002). Elevated HPs characterize the largest microbial ecosystems on Earth (*i.e.* the deep sea and seafloor (Whitman et al 1998)), may feature extraterrestrial life (Bartlett 2002), and are key to the inactivation of pathogens and viruses in food and medicine production (Aertsen et al 2009). Yet, a systematic definition of microbial preference for increased HP is missing. Such preference is generally referred to as piezophily, descriptive of microorganisms growing better at increased HP, with obligate piezophiles (or hyperpiezophiles) unable to grow at ambient pressure, and piezosensitive strains growing best at ambient pressure. The lack of consensus about 'true' piezophiles extends to the formalization of 'low' or 'high' HP levels and to the actual beginning of the piezosphere, the area of the deep sea where piezophiles thrive.

HP linearly increases by 1 MPa every 100 m of seawater depth, and about three times as much in sediments (Oger and Jebbar 2010). Enhanced growth at elevated HP has been used to locate the beginning of the piezosphere at corresponding depths (*e.g.* 1'000 m below seawater level [bswl] if HP was equal to the corresponding ~10 MPa). However, different HP thresholds were proposed for both piezophiles and hyperpiezophiles: ≥ 10 MPa (Jannasch and Taylor 1984)); ≥ 10 MPa for piezophiles and ≥ 50 MPa for hyperpiezophiles (Fang et al 2010); > 0.1 MPa for piezophiles and ≥ 60 MPa for hyperpiezophiles (Yayanos 1995)), but indicating moderate piezophily if HP optimum [HP_{opt}] of an isolate under investigation was between 10

and 30 MPa (Eloe et al 2008); ≥ 40 MPa (as the average depth of the oceans is about 3750 m, (Horikoshi and Bull 2011)). Following deep-sea sampling, isolation and lab-scale testing, a seminal work by Yayanos in 1986 proposed that true piezophiles populated water depths of at least 2'000 m (≥ 20 MPa). A most significant concept introduced by Yayanos was that of PTK diagrams, where growth rates are plotted in three dimensional graphs versus HP and temperature (T). Such diagrams indicate that maximum growth rate [$\mu_{\max}$] at HP_{opt} is observed when concomitantly adjusting T to an optimal value [T_{opt}]. However, Yayanos work was almost exclusively based on psychrophiles (microorganisms with a $T_{\text{opt}} \leq 15^{\circ}\text{C}$). The isolation of several new piezophiles in recent years, particularly with $T_{\text{opt}} > 15^{\circ}\text{C}$, is an opportunity to revise the role of T on piezophiles. Deep-sea environments exposed to elevated HPs commonly experience low T (*i.e.* 2°C). Exceptions are the deep, warm Sulu ($\sim 10^{\circ}\text{C}$), Mediterranean ($\sim 13.5^{\circ}\text{C}$) and Red sea ($\sim 20^{\circ}\text{C}$); deep hydrothermal vents, where microorganisms may grow at $> 110^{\circ}\text{C}$; and deep sub-seafloors where T increases 25°C every km underground (Jebbar et al 2015, Yayanos 1995). Notwithstanding the many HP-T combinations in the environment, this correlation has not been systematically addressed.

The most commonly accepted definition for piezophily is physiological, and states that piezophiles show a $\mu_{\max}$ above the atmospheric pressure of 0.1 MPa. This straightforward definition, however, does not appropriately describe microbial preference for elevated HPs. Can a microorganism with a HP_{opt} of 5 MPa be regarded as piezophilic as one with a HP_{opt} of 50 MPa? If intermediate HP values distinguish 'moderate' and 'true' piezophiles, they should be appropriately assessed *e.g.* by cultivation.

Cultivation is however a complicated task. Maximum cell density and $\mu_{\max}$ may occur at different HPs. (Martini et al 2013) proposed a cross coefficient to describe piezophily including both such factors. Although this cross coefficient has not been adopted in later

reports, the study has merit in highlighting the need for proper classification of piezophiles. Furthermore, aside T (Yayanos 1986), microbial preference for increased HP may also depend on availability of nutrients (Jannasch and Wirsén 1984), growth substrate (Jannasch and Taylor 1984, Yayanos and Chastain 1999), pH (Matsumura et al 1974) and salinity (Harrison et al 2013). All such dependencies are seldom tested on new piezophilic isolates. The absence of an exhaustive and commonly accepted definition of microbial preference for HP has deep implications for the microbiology and biotechnology of piezophiles. As certain HP levels may be generally considered low, they are not applied to assess microbial activity. Most microorganisms collected underwater have not been tested (nor isolated) using the corresponding HP at which they were captured. The fact that several cultivation parameters can influence the microbial response to HP implies that several microorganisms originally collected from surface waters may have their HP_{opt} above atmospheric pressure. This was already demonstrated in both Archaea (*Methanococcus thermolithotrophicus* (Bernhardt et al 1988)) and Bacteria (*Clostridium paradoxum* (Scoma et al 2019)), where true μ_{max} at increased HP were determined by concomitantly varying T. At the time of writing the present note, there are only 86 documented microbial isolates with a $HP_{opt} > 0.1$ MPa, an incredibly small group for a condition featuring the largest reservoir of prokaryotes on the planet (Whitman et al 1998). This lack of information has the worst implications when assessing the activity of microorganisms collected at shallower depths in the oceans (*e.g.* from meso- and bathypelagic waters), where hydrological conditions favor the presence of both HP-adapted (autochthonous) and HP-sensitive communities (allochthonous, *e.g.* sinking from surface waters), as opposed to deep, stratified waters (Tamburini et al 2013).

In the present note, I have collected data describing known piezophiles whose preference for increased HP has been assessed in laboratory tests, and discussed such preference based on their T_{opt} . The objective was to improve the standing definition of piezophily relying on the

existing dataset of cultured isolates, and individuate whether intermediate HPs exist to distinguish 'moderate' from 'true' piezophiles, indicative of the beginning of the piezosphere. Even though cultivation-based definitions may be biased by microbial cultivability, with only a minority of the existing microorganisms in the cultivable fraction, characterization of available isolates is the only way to learn the true HP ranges for a particular species.

Results

The 86 described piezophiles ($HP_{opt} > 0.1$) were grouped according to their T preference in piezopsychro- ($T_{opt} \leq 15$ °C), piezomeso- ($16 < T_{opt} < 49$ °C) and piezothermophiles ($T_{opt} \geq 50$ °C). There are currently 48, 17 and 21 prokaryotic species of documented piezopsychro-, piezomeso- and piezothermophilic nature, 71 of which are Bacteria and 15 Archaea (Table 1).

Growth rates

All μ_{max} values in described piezophiles were plotted versus either HP_{opt} (Fig. S1A) or T_{opt} (Fig. S1B) to explain growth based on HP or T independently of one another. High HP_{opt} is consistent with low μ_{max} while the opposite is true for T_{opt} . Aside these general observations, no strict correlation appears evident. Another approach was to divide piezophiles for T preference but neglect HP_{opt} . The average μ_{max} in piezopsychro-, piezomeso- and piezothermophiles increases with T, namely 0.22 ± 0.13 , 0.42 ± 0.38 and 1.25 ± 0.84 h⁻¹ (Table 1). However, only the average μ_{max} in piezothermophiles is significantly different from the other groups (t-test, $p < 0.0011$), and there is no difference between piezopsychro- and piezomesophiles ($p > 0.05$). The opposite approach (*i.e.* neglecting T classification) was

attempted using HP thresholds as suggested by (Yayanos 1995): piezophiles >0.1 MPa up to 60 MPa; and hyperpiezophiles >60 MPa. Average $\mu_{\max}$ was comparable ($p>0.05$, 0.55 ± 0.61 and 0.41 ± 0.69 h⁻¹, respectively). Overall, neither HP_{opt} nor T_{opt} alone appropriately describe the differences in piezophiles.

The combined effect of HP and T on growth was then assessed. The HP_{opt} and T_{opt} of every piezophile was 'normalized' dividing each by their corresponding $\mu_{\max}$ (Fig. 1), thus expressing the rate at which either HP_{opt} or T_{opt} increases with respect to their $\mu_{\max}$. These rates were exponentially correlated in piezopsychro-, piezomeso- and piezothermophiles (r^2 equal to 0.62 (n=37), 0.78 (n=12) and 0.58 (n=17), respectively). The regression line indicates that to attain $\mu_{\max}$ HP and T must be combined in a specific, optimal proportion. Suboptimal combinations of HP and T not resulting into $\mu_{\max}$ plot far from the regression line (*i.e.* have a lower μ). The relevance of HP-T correlations is only observed when separating piezophiles in the three T groups, as it can be inferred by the large variations in the axes of Fig. 1. Two data points were omitted from piezopsychrophiles, namely *Profundimonas piezophila* YC-1 and *Rhodobacterales bacterium* PRT1. These are the two slowest piezophiles isolated so far ($\mu_{\max}$ <0.02 h⁻¹), therefore normalized HP_{opt} and T_{opt} resulted out of scale. However, their inclusion would increase r^2 from 0.62 to 0.87. One data point was removed from piezothermophiles for the same reason (*Archaeoglobus fulgidus* VC-16^T), its inclusion slightly reducing r^2 from 0.58 to 0.54.

Capture depth and HP_{opt}

The relationship between piezophiles' capture depth and HP_{opt} is reported in Fig. 2A, while that between HP_{opt} and T_{opt} in Fig. 2B. There is an almost linear correlation between capture depth and HP_{opt} in piezopsychrophiles ($r^2=0.69$, n=48). This correlation is irrelevant in

piezomeso- ($r^2=0.04$, $n=15$) and piezothermophiles ($r^2=0.06$, $n=21$). Isolates with a markedly lower HP_{opt} than that experienced at capture depth may indicate they did not originally belong in that environment (*i.e.* they were allochthonous). As HP increase differs underground, two piezomesophiles collected at about 2 km in the deep seafloor (Fang et al 2017) were removed when assessing linear correlation, all others were included. It may be concluded that capture depth in the water column is seldom (if at all) relevant except for piezopsychrophiles. Removal of the six Bacteria from piezothermophiles to identify a correlation only in Archaea had just a slight effect on the goodness of fit (from 0.06 to 0.09).

The reason why HP_{opt} in piezothermophiles does not correlate with HP at capture depth is unclear. Microbial dispersion from the deep hot biosphere may partially account for this (Hoshino et al 2017), however, most piezothermophilic isolates derive from hydrothermal vents. Resilience of sinking allochthonous microorganisms from warmer surface waters may represent a relevant bias in piezomesophiles. Five isolates show HP_{opt} remarkably lower compared to HP at capture depth (*Salinimonas sediminis* N102^T, *Paraoceanicella profunda* D4M1, *Shewanella profunda* LT13a and *Pseudodesulfovibrio indicus* J2^T and *Muricauda hadalis* MT-229^T; Table 1B). Their removal increases the goodness of fit from $r^2=0.04$ to 0.53. However, such a selective data treatment may just confirm the lack of correlation between capture depth and HP_{opt} in piezomesophiles. A similar data refining for piezopsychrophiles (five strains) has no effect on the goodness of fit. The small discrepancy between linear HP increase with depth (dotted line, Fig. 2A) and linear regression of HP_{opt} with capture depth in piezopsychrophiles (straight line, Fig. 2A) indicates a slightly lower HP_{opt} in piezopsychrophiles as compared to HP at capture depth. This consolidates previous observations with single piezopsychrophiles (Deming et al 1984, Yayanos et al 1982).

Minimum HP_{opt}

HP_{opt} is the HP at which μ_{max} is observed in a piezophile. In particular, the lowest HP_{opt} values indicate that no piezophile was found growing optimally with less HP. Examination of the lowest HP_{opt} may thus be used to distinguish 'low' from 'high' HP.

There is presently a slight difference in the lowest HP_{opt} according to T preference: in piezopsychrophiles, 8 isolates have HP_{opt} around 10 to 20 MPa (with the sole exception of *Shewanella* sp. SC2A at 7 MPa, although its growth rate is almost identical up until 14 MPa, 0.076 vs. 0.072 h⁻¹, respectively (Yayanos et al 1982)); in piezomesophiles, the lowest HP_{opt} is always 10 MPa; for piezothermophiles, the minimum is at 20 MPa with the sole exception of *Thioprofundum lithotrophica* 106 at 15 MPa. When piezophiles are considered altogether, the minimum HP_{opt} ranges between 10 and 20 MPa. In other words: i) at least 10 MPa are generally required to observe piezophily, and ii) ≥ 20 MPa piezophilic traits can be found in any T group.

Using minimum HP_{opt} in described piezophiles as a threshold to define true piezophily has several advantages: it is independent of sampling procedures and environment (*i.e.* issues with sample preservation in challenging environments; sampling of allochthonous microorganisms); given its cultivation-based nature, it can be reproduced in any laboratory, meaning that the scientific debate is open to a broader research community; optimization of culture conditions allows to update these thresholds in time. Nonetheless, the current trends may just reflect the lack of laboratory testing at very low HPs (*e.g.* 5 MPa) although some of the isolates showing such minimum HP_{opt} were also grown at lower HPs (12.5, 25 and 50% in piezopsychro-, piezomeso- and piezothermophiles, respectively). Besides, countless microorganisms isolated from surface water have never been tested for HP tolerance. This is particularly relevant for psychrophilic *Colwellia* and *Shewanella* collected from surface

waters, as these genera have several members among the most extreme piezopsychrophiles (Table 1A); or for *Shewanella* in piezomesophiles (Table 1B).

T_{opt} and HP_{opt}

Microbial preference for different T_{opt} and HP_{opt} defines three areas where described piezophiles grow optimally (Fig. 2B). Piezopsychrophiles, by definition growing in a narrow range of T , form a rectangle that stretches up to 120 MPa. Piezomesophiles grow in a quadrilateral area that currently lacks representatives at extreme T_{opt} - HP_{opt} combinations. Piezothermophiles, typically growing within large T_{opt} variations, have an upper HP_{opt} not higher than 55 MPa, with one single exception (*Methanocaldococcus jannaschii* JAL-1).

Each of these areas is consistent with existing ecological niches. Cold, deep-oceans do experience the T - HP combinations which are optimal for the isolated piezopsychrophiles, a consideration which extends to piezothermophiles as hydrothermal vents are found at depths of ~5'000 m (Jebbar et al 2015). However, piezothermophiles are mostly Archaea (15/21, 71%), the most extreme bacterium represented by *Marinitoga piezophila* KA3^T (T_{opt} 65°C, HP_{opt} 40 MPa). Seafloor HP and T for the warmest deep seas on our planet (the Mediterranean, Sulu and Red sea) are indicated (Fig. 2B). These define an imaginary triangle which concerns piezopsychro- and piezomesophiles. The physiological properties of described piezomesophiles extend to T_{opt} - HP_{opt} combinations well beyond this triangular area, particularly to higher T_{opt} which often involve anaerobic isolates collected from subseafloors (Table 1B). Nonetheless, no piezomesophile thus far has been isolated which requires the most extreme T_{opt} - HP_{opt} combinations e.g. 45 °C and 50 MPa. Recently *Virgibacillus pantothenicus* 19R1-5 and *Bacillus subtilis* 29R7-12 were isolated from deep-subseafloor samples (between 1.9 and 2.5 km below seafloor level) characterized by such

high T and HP (Fang et al 2017). These have T_{opt} close to T at capture depth (42-43 °C), but considerably lower HP_{opt} (20 and 10 MPa; while *in situ* levels were 53 and 65 MPa, respectively) (Fang et al 2017)). The difficulty in collection, preservation and cultivation of these samples represents a critical limit for isolation of extreme piezomesophiles.

Hyperpiezophiles

While there are several obligate piezopsychrophiles (9/48), there is none for piezomesophiles, and just one for piezothermophiles (*Pyrococcus yayanosii* CH1) (Table 1). The term hyperpiezophiles thus currently describes a group of psychrophiles. Their lowest HP_{opt} is generally ~70 MPa (4/9 strains), with the exception of *P. piezophila* YC-1 at 50 MPa. The shallowest capture depth ranges between 6'000-6'500 m bswl.

Discussion

The most widely shared definition of microbial preference for increased HP states that a microorganism is piezophilic when its μ_{max} is obtained at HPs >0.1 MPa. By setting the threshold to such a low reference value, this definition neglects the large variation in HP preference that exists among described piezophiles and the differential effects enhanced HP may impose on the vast diversity of microbial processes in nature. An additional cultivation-based approach is required to identify the threshold level above which HP-adapted microorganisms clearly separate from those thriving at ambient pressure.

In the present meta-analysis, the relevance of HP-T combinations first described by Yayanos (1986) was updated to include all currently described piezophiles, which were classified according to their T preference in piezopsychro-, piezomeso- and piezothermophiles. T

preference appears to define piezophiles in several regards, namely: 1) $\mu_{\max}$ is attained at specific HP-T combinations; the rates of both HP_{opt} and T_{opt} increase with respect to $\mu_{\max}$ are exponentially correlated, and such correlation differs in the three T groups; 2) the concept of capture depth and its relation to HP_{opt} is only relevant in piezopsychrophiles; 3) piezopsychrophiles have a HP_{opt} slightly lower than the HP at capture depth; 4) there are no extreme piezomesophiles, perhaps an indication of the technical limitations in their collection and cultivation; 5) similarly, there are no obligate piezomesophiles; 6) obligate piezophily features piezopsychrophiles almost exclusively, with a minimum HP_{opt} of 70 MPa and capture depth of at least 6'000-6'500 m bswl; 7) $HP_{\text{opt}} > 75$ MPa exclusively belong to piezopsychrophiles.

Updated definitions

Based on the present meta-analysis, the following updated definitions are proposed:

1) As capture depth is only linearly correlated with HP_{opt} in piezopsychrophiles, and it complicates the comparison of isolates that present-day technology allows to collect from deep subseafloors, the definition of piezophily and piezosphere should refer to HP at capture depth (in MPa) rather than meters below sea or seafloor level.

2) Minimum HP_{opt} may be considered to define low, medium and high HP levels, *e.g.* when assessing microbial activity with environmental samples in laboratories or ship boards using samples carrying an unknown mixture of autochthonous and allochthonous microorganisms. Low HPs may be considered as those below which piezophiles across all T levels seldom have their HP_{opt} ; high HPs those above which piezophiles typically have their HP_{opt} irrespective of their T preference; and mild HP levels those in between these two extremes.

The present data across all available prokaryotic isolates (n=86) suggests these thresholds to be: low <10 MPa; mild ≥ 10 and <20 MPa; and high ≥ 20 MPa.

3) As a consequence of point 2, moderate piezophiles may be defined as those with $HP_{opt} < 20$ MPa, and true piezophiles those with $HP_{opt} \geq 20$ MPa. The piezosphere would therefore begin at 20 MPa.

4) True hyperpiezophiles are so far almost exclusively psychrophilic in nature. Until isolation of a consistent number of piezomeso- and piezothermophiles indicates otherwise, true hyperpiezophiles are captured at least at 6'000-6'500 m bswl and have $HP_{opt} \geq 70$ MPa.

Acknowledgements

I am grateful to Prof. Kasper Urup Kjeldsen and Dr. Grégoire Michoud for the scientific discussions.

Conflict of interest

I declare no conflict of interest

Figure legends

Figure 1. Correlation between the rate increase in HP_{opt} (HP_{opt}/μ_{max}) and rate increase in T_{opt} (T_{opt}/μ_{max}) with respect to μ_{max} in the piezopsychro-, piezomeso- and piezothermophiles.

Statistical correlation was obtained with GraphPad Prism 5, nonlinear regression, exponential growth equation, least square (ordinary) fit.

Figure 2. Correlation between optimal HP (HP_{opt}) and capture depth (A) and between HP_{opt} and optimal temperature (T_{opt}) (B) in described piezophilic isolates. In Fig (A): the straight

line indicates the linear regression for piezopsychrophiles ($r^2=0.69$, $n=48$); the dotted line indicates the linear increase of HP with increasing depth in seawater. In Fig (B): approximate maximum T at the deepest point (expressed as equivalent HP) in the warmest seas in the world; dotted quadrilaterals define areas of $HP_{opt}-T_{opt}$ for known piezophiles (all data in Table 1). Keys reported in the graph. In Fig. (A) statistical correlation was obtained with GraphPad Prism 5, linear regression.

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Table 1A. Described piezopsychrophilic isolates and their main features. Temperature boundaries were defined based on Morita RY (1975). Psychrophilic bacteria. Bacteriol Rev 39: 144-167.

Keys: n.d., not determined; T, temperature; HP, hydrostatic pressure; (T), type strain. Asterisks (*) refer to their corresponding Note.

N.	Strain	T optimum (°C)	HP optimum (MPa)	HP range (MPa)	Capture depth (m)	Maximum growth rate (h ⁻¹)	References	Lowest HP tested >0.1 MPa	Notes
1	Shewanella sp. SC2A	2	7	0.1-42	1957	0.08	Yayanos et al., 1982	7	
2	Photobacterium profundum DSJ4	10	10	0.1-70	5110	0.45	Nogi et al., 1998	10	
3	Strain PE31	2*	14	0.1-69	3584	0.14	Yayanos et al., 1982	14	Unclear if optimal temperature
4	Strain PE35	2*	14	0.1-83	3584	0.12	Yayanos et al., 1982	14	Unclear if optimal temperature
5	Carnobacterium sp. strain AT12	2	20	0.1-60	2550	n.d.	Lauro et al., 2007*	not reported	data from Jebbar et al., 2015
6	Psychromonas sp. #503	4	20	0.1-70	3064	0.27	Arakawa et al., 2006	20	
7	Moritella sp. #304	4	20	0.1-70	3064	0.31	Arakawa et al., 2006	20	
8	Shewanella psychrophila WP2	10-15	20	0.1-50	1914	0.41	Xiao et al., 2007	10	
9	Psychromonas profunda 2825 (T)	10	25	0.1-50	2770	0.15	Xu et al., 2003		
10	Vibrio sp. WM145	2	27	0.1-82	4575	0.21	Deming et al., 1984		

11	Photobacterium profundum SS9	15	28	0.1-70	2551	0.5	De Long et al., 1997	
12	Shewanella sp. CT01	8	30	0.1-70	4819	n.d.	Sekiguchi et al., 2010a,b	
13	Shewanella sp. CT03	8	30	0.1-70	4819	n.d.	Sekiguchi et al., 2010a,b	
14	Moritella sp. CT08	8	30	0.1-70	4819	n.d.	Sekiguchi et al., 2010a,b	
15	Moritella sp. JT01	5-8	30	0.1-70	5000-7000	n.d.	Sekiguchi et al., 2010a,b	
16	Shewanella benthica #302	4	30	0.1-70	3064	0.35	Arakawa et al., 2006	
17	Moritella profunda 2674 (T)	6	30	0.1-50	2815	0.17	Xu et al., 2003	
18	Shewanella benthica F1A	8	30	0.1-70	4900	0.15	Wirsén et al., 1987	
19	Moritella abyssi 2693 (T)	10	30	0.1-50	2815	0.2	Xu et al., 2003	
20	Shewanella violacea DSS12	10	30	0.1-70	5110	0.28	Kato et al., 1995	
21	Vibrio sp. UM40	2	41	0.1-82	5920	0.22	Deming et al., 1984	
22	Moritella sp. strain PE36	10	41	0.1-70	3584	0.28	Yayanos et al., 1986	
23	Strain MT52	2*	42	0.1-83	5672	0.08	Yayanos et al., 1982	Unclear if optimal temperature
24	Strain MT62	2*	42	0.1-83	5672	0.09	Yayanos et al., 1982	Unclear if optimal temperature
25	Moritella sp. CT12	8	50	0.1-70	4819	n.d.	Sekiguchi et al., 2010a,b	

26	<i>Profundimonas piezophila</i> YC-1	8	50	20-70	6000	0.017	Cao et al., 2014
27	<i>Psychromonas kaikoe</i> JT7304 (T)	10	50	0.1-70	7437	0.15	Nogi et al., 2002
28	<i>Psychromonas kaikoe</i> JT7301 (T)	10	50	0.1-70	7437	0.15	Nogi et al., 2002
29	<i>Shewanella benthica</i> DB6101	10	50	0.1-70	5110	0.35	Kato et al., 1995
30	<i>Moritella japonica</i> DSK1	15	50	0.1-70	6356	0.4	Kato et al., 1995
31	<i>Psychromonas</i> sp. strain CNPT3	12	52	0.1-85	5782	0.19	Yayanos et al., 1979
32	<i>Psychromonas hadalis</i> K41G (T)	6	60	0.1-90	7542	0.14	Nogi et al., 2007
33	<i>Colwellia piezophila</i> Y223G (T)	10	60	0.1-80	6278	0.14	Nogi et al., 2004
34	<i>Colwellia piezophila</i> Y251E (T)	10	60	0.1-80	6278	n.d.	Nogi et al., 2004
35	<i>Shewanella benthica</i> DB172R	10	60	0.1-70	6499	0.45	Kato et al., 1996
36	<i>Shewanella benthica</i> DB5501	15	60	0.1-70	2485	0.35	Kato et al., 1995
37	<i>Shewanella benthica</i> DB6705	15	60	0.1-70	6356	0.4	Kato et al., 1995
38	<i>Shewanella benthica</i> DB6906	15	60	0.1-70	6269	0.35	Kato et al., 1995
39	<i>Colwellia</i> sp. strain MT41	8	69	38-103	10476	0.07	Yayanos et al., 1981
40	<i>Shewanella benthica</i> DB172F	10	70	50-100	6499	0.41	Kato et al., 1996

41	Shewanella benthica DB21MT-2	10	70	60-100	10898	0.17	Kato et al., 1998
42	Rhodobacterales bacterium PRT1	10	80	20-100	8350	0.019	Eloe et al., 2011
43	Moritella yayanosii DB21MT-5	10	80	50-100	10898	0.2	Nogi and Kato, 1999
44	Shewanella sp. PT64	9	90	0.1-120	7100	0.06	Yayanos et al., 1986
45	Colwellia hadaliensis BNL-1	10	90	37-102	7410	0.12	Deming et al., 1988
46	Shewanella benthica KT99	2	98	40-140	9856	n.d.	Lauro et al., 2007
47	Colwellia sp. KT27	2	98	n.d.	9856	n.d.	Lauro et al., 2007
48	Colwellia marinimaniae MTCD1 (T)	6	120	80-140	10918	0.08	Kusube et al., 2017
		Min	2	7	0.1	Mean	0.22
		Max	15	120	140	s.d.	0.13

Table 1B. Described piezomesophilic isolates and their main features. Temperature boundaries were defined based on the definition of psychrophiles and thermophiles.

Keys: n.d., not determined; T, temperature; HP, hydrostatic pressure; (T), type strain. Asterisks (*) refer to their corresponding Note.

N.	Strain	T optimum (°C)	HP optimum (MPa)	HP range (MPa)	Capture depth (m)	Maximum growth rate (h ⁻¹)	References	Lowest HP tested >0.1 MPa	Notes
1	Salinimonas sediminis N102 (T)	28	10	0.1-70	4700	n.d.	Cao et al., 2018	not reported	
2	Pseudodesulfovibrio profundus 80-55 (T)	25	10	0.1-40	900	n.d.	Bale et al., 1997	5	Growth rates based on H ₂ S production
3	Pseudodesulfovibrio piezophilus C1TLV30 (T)	30	10	0.1-30	1693	0.07	Khelaifia et al., 2011	5	
4	Paraoceanicella profunda D4M1	37	10	0.1-70	10890	0.13	Liu, Ding et al., 2019	10	
5	Shewanella profunda LT13a	25-30	10	0.1-50	4790	1.33	Toffin et al., 2004	10	
6	Pseudodesulfovibrio indicus J2 (T)	30-35	10	0.1-30	3173	0.38	Cao et al., 2016	10	
7	Bacillus subtilis 29R7- 12	43	10	0.1-60	1180+24 06*	0.58	Fang et al., 2017	10	Depth refers to m below sea surface water level plus m below seafloor level. <i>In situ</i> HP was 53 MPa
8	Petrocella atlantisensis 70B-A	25	14	n.d.	1150	0.45	Quéméneur et al., 2019		
9	Pseudodesulfovibrio profundus 500-1 (T)	25	15	0.1-40	900	n.d.	Bale et al., 1997		
10	Carnobacterium sp. strain AT7	20	20	0.1-60	2415–246 0	0.75	Lauro et al., 2007*		data from Jebbar et al., 2015
11	Shewanella piezotolerans WP3	15-20	20	0.1-50	1914	0.5	Xiao et al., 2007		

12	Muricauda hadalis MT-229 (T)	35	20	0.1-70	10918	n.d.	Zhang et al., 2020	Depth refers to m below sea surface water level plus approximate m below seafloor level. <i>In situ</i> HP was 65 MPa
13	Virgibacillus pantothenticus 19R1-5	42	20	0.1-60	1180+(19 51- 1999)*	0.61	Fang et al., 2017	
14	Desulfovibrio hydrothermalis AM13 (T)	35	26	n.d.	2600	0.05	Alazard et al. 2003	
15	Desulfovibrio indonesiensis P23	45	30	n.d.	2656	0.07	Fichtel et al., 2015	
16	Profundibacter amoris BAR1	30	30-35	0.1-50	2216	0.07	Le Moine Bauer et al., 2019	
17	Dermacoccus abyssi MT1.1 (T)	28	40	n.d.	10898	n.d.	Pathom-aree et al., 2006	
Min		20	10	0.1	Mean	0.42		
Max		45	40	70	s.d.	0.38		

Table 1C. Described piezothermophilic isolates and their main features. Temperature boundaries were defined based on Zeikus JG (1979). Thermophilic Bacteria - Ecology, Physiology and Technology. Enzyme and Microbial Technology 1: 243-252.

Keys: n.d., not determined; T, temperature; HP, hydrostatic pressure; (T), type strain; Y, yes; N, No. Asterisks (*) refer to their corresponding Note.

N.	Strain	T optimum (°C)	HP optimum (MPa)	HP range (MPa)	Capture depth (m)	Maximum growth rate (h ⁻¹)	References	Lowest HP tested >0.1 MPa	Notes	Ar ch ae a
1	Thiopfundum lithotrophica 106	50	15	0.1-50	3626	0.30	Takai et al., 2009	5		N
2	Thermosiphon japonicus IHB1 (T)	72	20	0.1-60	972	1.33	Takai and Horikoshi, 2000	10		N
3	Thermococcus aggregans TY (T)	75	20	0.1-30	2000	n.d.	Canganella et al., 2000	20		Y
4	Pyrococcus abyssi GE5	96	20	0.1-50	2000	0.98	Erauso et al., 1993	20		Y
5	Anoxybacter fermentans DY22613	60-62	20	0.1-60	2891	2.20	Zeng et al., 2015	not reported		N
6	Archaeoglobus fulgidus VC-16 (T)	83	20	0.1-60	1 to 10	0.15	Stetter et al., 1987	10		Y
7	Clostridium paradoxum	60	22	0.1-30*	0	3.50	Scoma et al., 2019		not tested >30 MPa	N
8	Palaeococcus pacificus DY20341 (T)	80	30	0.1-80	2737	0.91	Zeng et al., 2013			Y
9	Palaeococcus ferrophilus DMJ (T)	84	30	0.1-60	1338	1.32	Takai et al., 2000			Y
10	Thermococcus eurythermalis A501 (T)	85	0.1-30	0.1-70	2000	1.25	Zhao et al., 2015			Y
11	Thermococcus guaymasensis TYS (T)	85	20-35	0.1-50	2000	n.d.	Canganella et al., 1998			Y

12	Piezobacter thermophilus 108	50	35	0.1-65	3626	0.46	Takai et al., 2009	N
13	Methanopyrus kandleri 116	105	40	0.1-50	2500	0.73	Takai et al., 2008	Y
14	Marinitoga piezophila KA3 (T)	65	40	0.1-60	2630	1.90	Alain et al., 2002a	N
15	Thermococcus barophilus MP	85	40	0.1-80	3550	1.50	Marteinsson et al., 1999	Y
16	Thermococcus peptonophilus OG-1 (T)	90	45	0.1-60	1380	1.38	Canganella et al., 1997	Y
17	Methanococcus thermolithotrophicus DSM2095	65	50	0.1-100	0.5	0.58	Bernhardt et al. 1988	Y
18	Thermococcus piezophilus CDGS (T)	75	50	0.1-120	4964	0.45	Dalmaso et al., 2016	Y
19	Pyrococcus kukulkanii NCB100 (T)	105	40-50	0.1-80	1997	n.d.	Callac et al., 2016	Y
20	Pyrococcus yayanosii CH1	98	52	20-120	4100	1.20	Zeng et al., 2009	Y
21	Methanocaldococcus jannaschii JAL-1	86	75	0.1-75	2610	2.36	Jones et al., 1983	Y
Min		50	15	0.1	Mean	1.25		
Max		105	75	120	s.d.	0.84		