



Diversity of culturable heterotrophic bacteria from the Mariana Trench and their ability to degrade macromolecules

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Abstract

The Mariana Trench is the deepest location on earth and harbors unique microbial communities as evidenced by 16S rRNA gene amplicon and metagenomic sequencing. Obtaining culturable microorganisms from the Mariana Trench will contribute to a further understanding of hadal biogeochemical processes and act as a unique microbial reservoir with potential applications. Here, 825 bacterial strains, identified by 16S rRNA gene sequencing, were isolated from 12 water depths (0–10,400 m) of the Mariana Trench with 2216E and R2A media at 4 °C or 28 °C on four cruises during 2015–2017. These bacteria belong to four phyla, nine classes, 27 orders, 45 families and 108 genera. *Alphaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria_c*, *Bacilli* and *Flavobacteriia* were the most abundant classes, accounting for 37.9%, 33.0%, 11.8%, 8.6% and 8.0% of the total bacterial isolates, respectively. 2216E and R2A media were found to have a better selectivity to *Bacilli* and *Flavobacteriia*, respectively. Fifty strains were potential novel bacterial species with a 16S rRNA gene similarity < 98.65%, and a higher percentage of novel strains were obtained from R2A than 2216E medium. Additionally, 301 (150 species) out of 354 strains (178 species) selected from each depth could degrade at least one of the ten kinds of macromolecules tested. These results indicate that there is a high diversity of culturable bacteria in the Mariana Trench and they can produce a variety of extracellular enzymes. Our study provides a valuable resource of microorganisms for investigating their biogeochemical roles in the Mariana Trench and for industrial applications.

Keywords Diversity · Heterotrophic bacteria · The Mariana Trench · Macromolecule · Water column

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Introduction

The Mariana Trench, which extends to 10,984 m as the greatest depth in the Challenger Deep, is the deepest location on earth (Gardner et al. 2014). Due to the unique "funnel" geomorphology, low temperature and extreme high hydrostatic pressure (Fujioka et al. 2002; Simonato et al. 2006), the microorganisms in the Mariana Trench, especially in the hadal zone, likely have a specific community composition and unique metabolic characteristics compared to those in the open ocean. Hadal microbial communities are of great scientific interest due to both their significant roles in global biogeochemical cycle and their potential applications.

Due to the rapid progress of deep-sea sampling techniques in recent years, the microbial community structures at different water depths of the Mariana Trench have been extensively studied by either 16S rRNA gene high-throughput sequencing (Nunoura et al. 2015; Peoples et al. 2018; Tarn et al. 2016; Tian et al. 2018) or metagenomic sequencing (Liu et al. 2019c; Wang et al. 2019). There have also

been a few reports on bacterial cultivation from the Mariana Trench (Kato et al. 1998). However, the isolation and purification of a wide range of cultivable bacteria from the whole water column of the Mariana Trench and analysis of their diversity remain rare. Although culturable bacteria only account for a small proportion of the total bacterial population (Kogure et al. 1979), a comprehensive understanding of the physiology, biochemistry and metabolic activities of these organisms and their roles in complex biogeochemical cycles will undoubtedly require their cultivation.

To understand the diversity of heterotrophic bacteria from the Mariana Trench and to investigate their potential roles in the organic carbon cycle, water samples were collected from different water depths (0–10,400 m; the trench water > 6000 m) of the Mariana Trench during four expeditions aboard the R/V *Dong Fang Hong 2* from 2015 to 2017. Heterotrophic bacteria were isolated using 2216E and R2A media and identified by 16S rRNA gene sequencing following our recent study on a culture-independent approach (Liu et al. 2019a, b, c). The capacity of selected bacterial strains to degrade a variety of macromolecules was also investigated. A large number (825 isolates) of diverse bacterial strains (including 50 potential novel bacterial strains) was obtained and many of them were able to produce active extracellular enzymes. These bacterial cultures provide a valuable resource of microorganisms for investigating their ecological roles as well as for industrial applications.

Results

Bacterial colony-forming unit (CFU) counts at different water depths

During the cruise of December 2015, bacterial CFU was counted from different water depths cultured on both marine agar 2216E (2216E) and marine R2A agar (R2A) agar plates. The bacterial CFU counts were $(1.0\text{--}2.4) \times 10^4$ CFU/ml at depths of 0–4000 m but increased significantly to $3.2 \times 10^4\text{--}1.0 \times 10^5$ CFU/ml at greater depths in the trench (> 6000 m; Fig. 1 and Supplementary Table S1). Nevertheless, there appeared to be a declining trend in the diversity of isolates with depth as bacterial colonies were more colorful at the surface than in the deeper water (Fig. 1). The CFU counts on the two types of media were comparable and of the same order of magnitude.

Diversity of heterotrophic bacteria isolated from the Mariana Trench

A total of 825 bacterial strains were isolated, 423 strains from 2216E medium and 402 from R2A medium. According to 16S RNA gene similarity analysis, these bacterial

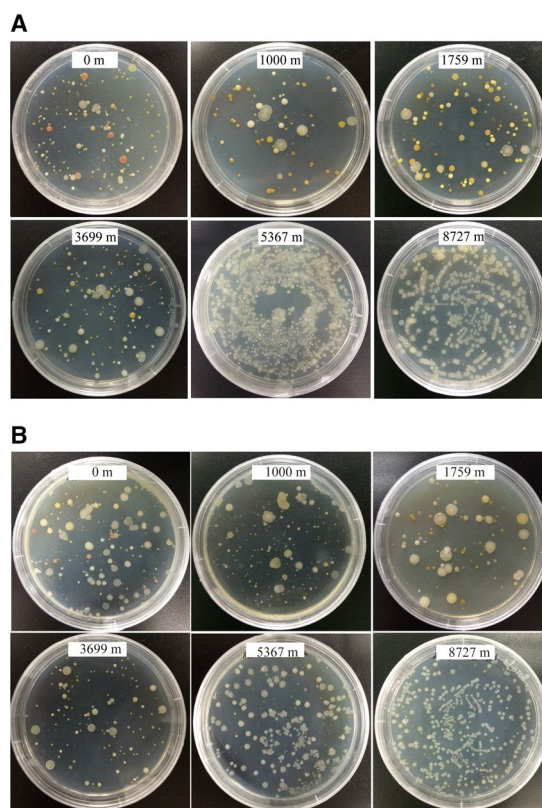


Fig. 1 The visible bacterial colonies on two kinds of culture media with water samples collected from different water depths of the Mariana Trench in cruise of December 2015. **a** On 2216E agar plates; **b** on R2A agar plates

strains belonged to four phyla, nine classes, 27 orders, 45 families, 108 genera and 190 species. The four phyla were *Proteobacteria* (586 strains, mainly composed of *Gammaproteobacteria* and *Alphaproteobacteria*), *Actinobacteria* (99 strains), *Firmicutes* (71 strains) and *Bacteroidetes* (69 strains) (Figs. 2, 3). At the class level, *Alphaproteobacteria* (313 strains), *Gammaproteobacteria* (272 strains), *Actinobacteria_c* (97 strains), *Bacilli* (71 strains) and *Flavobacteriia* (66 strains) were the most abundant five classes, accounting for 37.9%, 33.0%, 11.8%, 8.6% and 8.0% of the total isolates, respectively. The other four classes, i.e., *Cytophagia*, *Epsilonproteobacteria*, *Nitrospirillum* and *Acidimicrobiia*, contained only 1–3 strains (Figs. 2, 3). Consistently, *Alphaproteobacteria* (45 genera), *Gammaproteobacteria* (23 genera), *Actinobacteria_c* (13 genera), *Flavobacteriia* (12 genera) and *Bacilli* (nine genera) contained diverse genera, while the other four classes contained only one or two genera (Supplementary Table S2).

In terms of strain numbers within each genus, *Alteromonas* (*Gammaproteobacteria*) had the most strains with 77 belonging to six species, accounting for 9.3% of the total

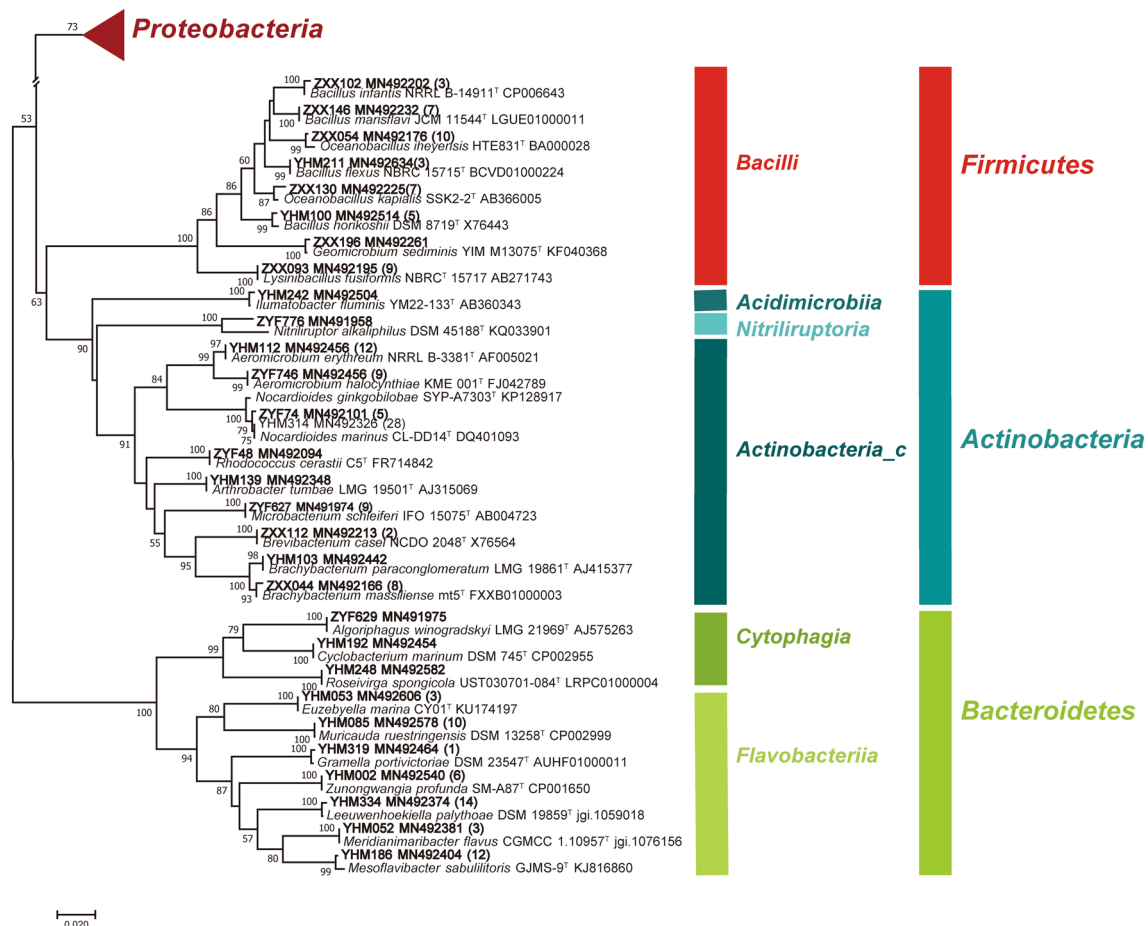


Fig. 2 Neighbor-joining phylogenetic tree of representative cultivated bacteria isolated from the Mariana Trench seawater and their closest relatives retrieved from the GenBank database based on 16S rRNA

bacterial isolates; *Erythrobacter* (*Alphaproteobacteria*) was the second largest genus with 61 strains belonging to 11 species, accounting for 7.4% of the total bacterial isolates. The numbers of *Marinobacter* (*Gammaproteobacteria*) and *Henriciella* (*Alphaproteobacteria*) strains were also relatively high, including 40 strains of seven species and 35 strains of three species, respectively (Supplementary Table S2). Some other genera, such as *Roseivirgaspongicola* and *Nitriliruptoria*, only contained a single isolate (Supplementary Table S2), but these genera can also reflect the diversity of microorganisms in the water column of the Mariana Trench.

Diversity of culturable bacteria at different depths

There were clear differences in the diversity of culturable bacteria at different depths (Figs. 1, 4). At class level, *Alphaproteobacteria*, the most abundant class in the Mariana Trench, had the highest relative abundance at ~4000 m and decreased slightly below 4000 m. The relative abundance of *Gammaproteobacteria*, the second most abundant class,

gene sequences. The numbers inside brackets represents the amount of strains within the same species. Bar, the number of substitutions per site

however, increased slightly at depths deeper than 7500 m and had the highest relative abundance (~90%) near the bottom water (10,400 m) of the trench. Like *Alphaproteobacteria*, *Actinobacteria_c* also decreased in relative abundance at depths greater than 4000 m, although its relative abundance was much lower than *Alphaproteobacteria*. *Bacilli* mainly existed within 2000–8000 m and had the highest relative abundances at ~7500 m and ~8000 m. In addition, *Flavobacteriia* was found at relatively low abundance throughout the water column; however, it had the highest relative abundance at ~9000 m (36%). *Cytophagia* (three strains) was only isolated at ~6000 m, ~9000 m and 9600 m, and *Epsilonproteobacteria* (one strain), *Nitriliruptoria* (one strain) and *Acidimicrobiia* (one strain) were each only isolated at a single depth (Fig. 4a).

At genus level (Fig. 4b; Supplementary Table S2), *Alteromonas*, the most abundant genus (77 strains), was distributed throughout the whole water column except for 200 m, and it had the highest relative abundance at a depth of 8289 m. *Erythrobacter*, the second largest genus (61 strains), was

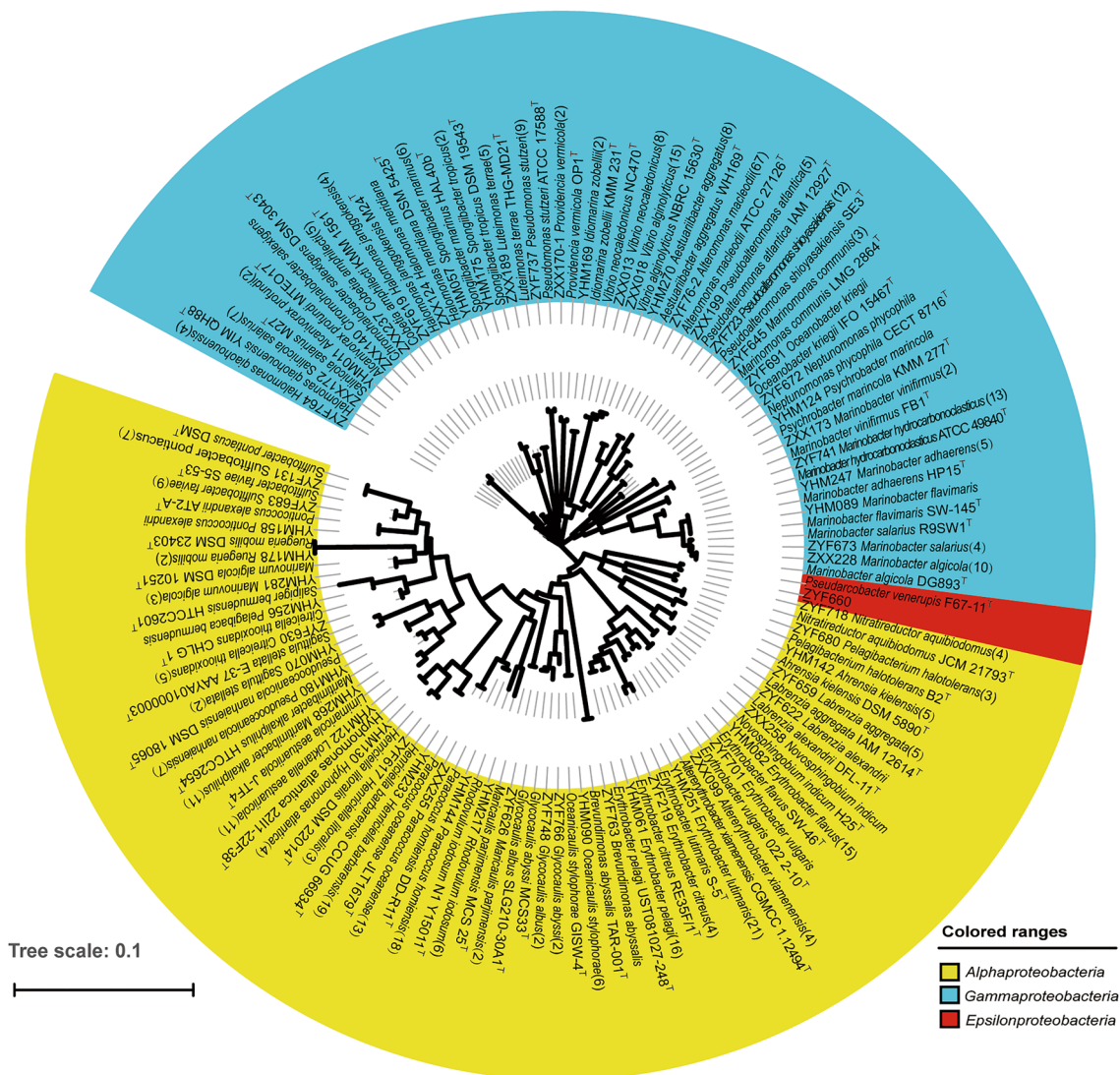


Fig. 3 Neighbor-joining phylogenetic tree of members of *Proteobacteria*. Bar, the number of substitutions per site

isolated from nine of 12 depths, and showed little variation in abundance with depth. *Marinobacter*, the third largest genus (40 strains), was most abundant at greater depths (> 4000 m). Inversely, the relative abundance of *Henriciella* (35 strains) and *Nocardioides* (33 strains) were higher in upper (< 4000 m) rather than deeper (> 6000 m) waters (Fig. 4b; Supplementary Table S2). *Bacillus* (33 strains) was mainly present in 2000–8000 m samples, while *Oceanobacillus* (20 strains) was mainly found in the samples of 4000–8000 m. At 10,400 m, the community of culturable bacteria was less diverse compared to other depths, with *Marinobacter* being the most abundant (Fig. 4b).

Generally, there were clear differences in the communities of culturable heterotrophic bacteria with depth. The majority of culturable bacteria from 0 to 6000 m in the upper Trench were *Proteobacteria*

(*Gammaproteobacteria* and *Alphaproteobacteria*) and *Actinobacteria* (*Actinobacteria_c*), which were mainly composed of *Vibrio*, *Henriciella* and *Nocardioides*; the majority of culturable bacteria from 6000 to 8000 m were *Proteobacteria* (*Gammaproteobacteria* and *Alphaproteobacteria*) and *Firmicutes* (mainly composed of *Bacilli*); *Proteobacteria* were most abundant from 8000 to 10,400 m, especially at 10,400 m, near the bottom, which was mainly composed of *Marinobacter*.

Comparison of bacterial diversity isolated from different cultivation media

Two culture media, i.e., 2216E and R2A, were used to isolate bacteria in this study. While 423 isolates (seven classes) were obtained from 2216E medium, 402 isolates

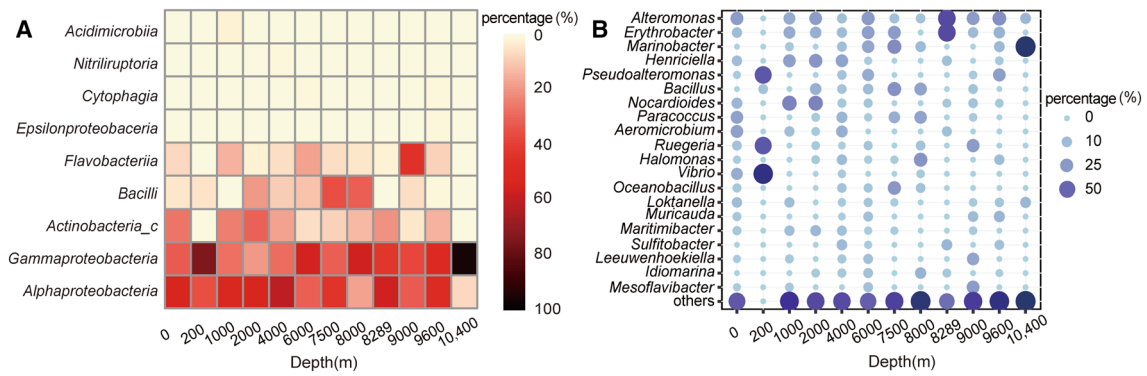


Fig. 4 The bacterial diversity of cultivable bacteria isolated from different water layers. **a** At class level; **b** at genus level (top 20 dominant genera)

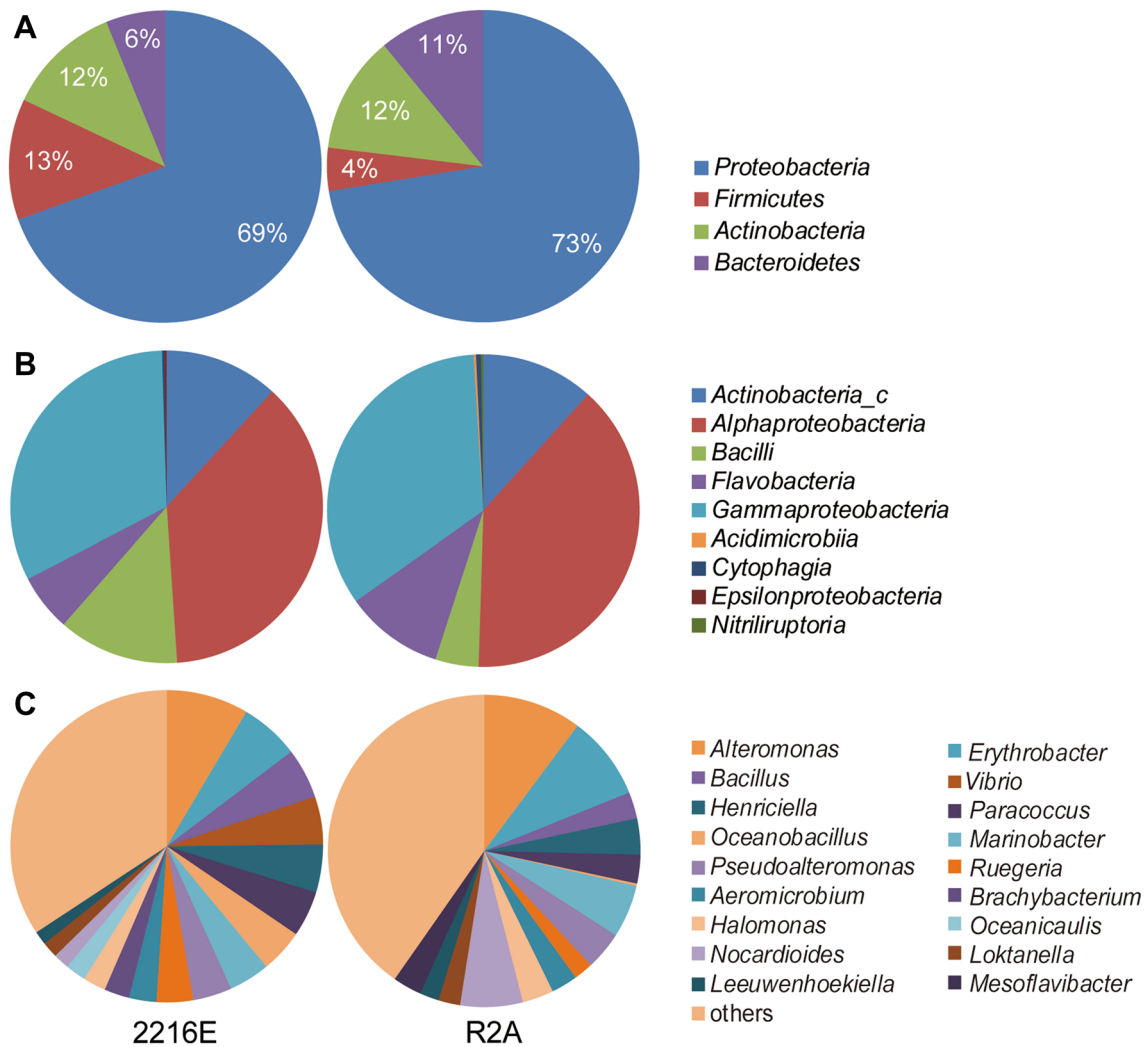


Fig. 5 The community composition of cultivable bacteria on different culture media. **a** At phylum level; **b** at class level; **c** at genus level (top 18 dominant genera)

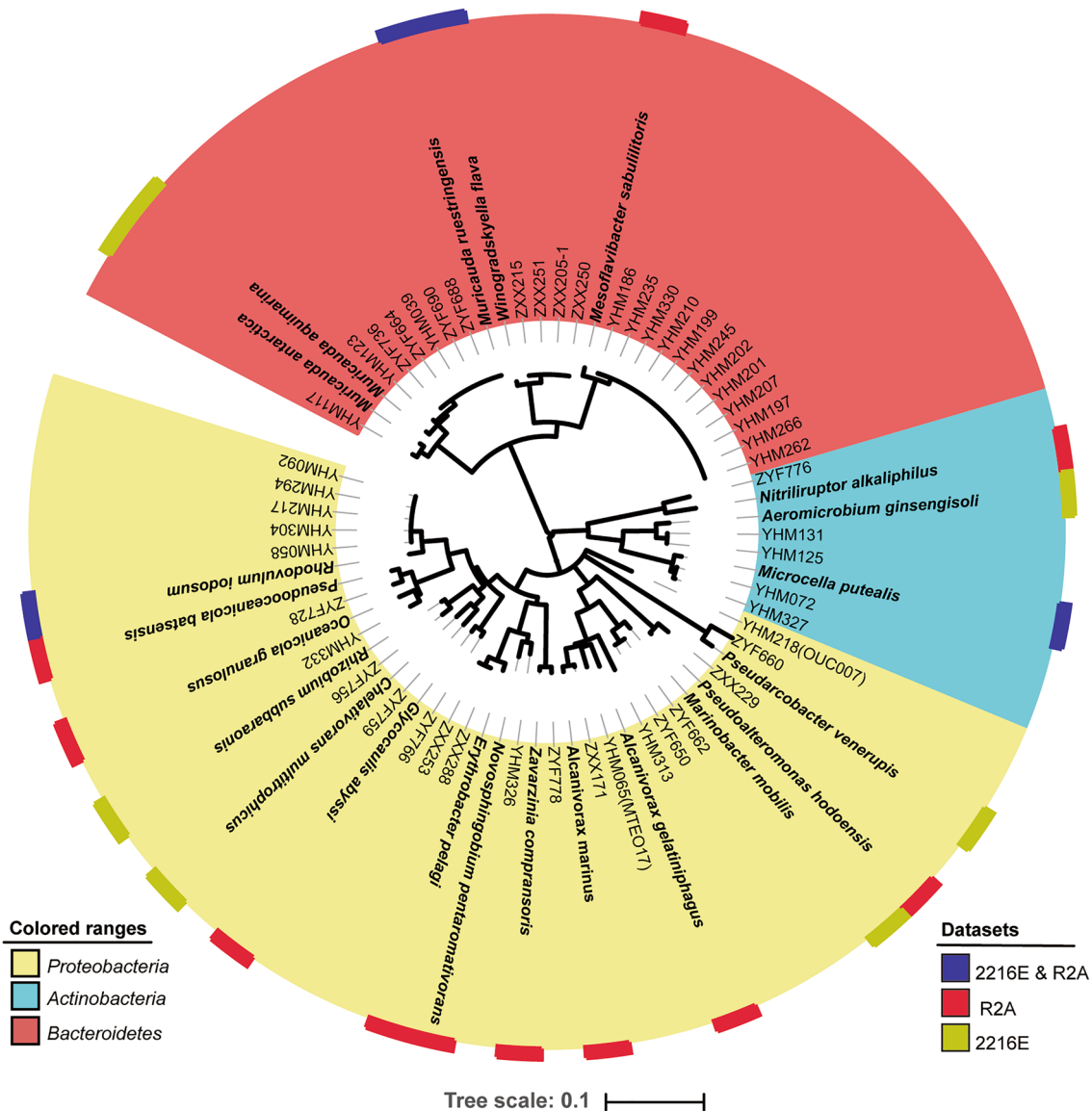


Fig. 6 The diversity of potential novel bacterial strains under different culture conditions. Top-hit taxon of potential novel strains marked with bold font. Bar, the number of substitutions per site

(eight classes) were obtained from R2A medium. 2216E medium had better selectivity to *Firmicutes* at phylum level and *Bacilli* at class level, while R2A medium had better selectivity to *Bacteroidetes* at phylum level and *Flavobacteriia* at class level (Fig. 5a). Additionally, *Acidimicrobiia* and *Nitriiliruptoria* were only obtained from R2A medium, while *Epsilonproteobacteria* was only isolated from 2216E medium (Fig. 5b).

Of the total 108 genera, 85 were obtained from 2216E medium, while 69 were obtained from R2A medium. Forty-eight genera (45.7%) were found in both 2216E and R2A media. While *Vibrio* (22 strains), *Brachybacterium* (11 strains) and *Oceanicaulis* (nine strains) were isolated

only from 2216E medium, *Mesoflavibacter* (12 strains) was isolated only from R2A medium. In addition, 2216E medium had a better selectivity to *Bacillus*, *Henriciella*, *Paracoccus*, *Oceanobacillus* and *Ruegeria*, while R2A medium was more selective to *Alteromonas*, *Erythrobracter* and *Nocardioidea* (Fig. 5c).

Comparison of bacterial diversity isolated at different temperatures

All the bacterial strains were isolated at temperatures of 4 °C and 28 °C. While 176 isolates (belonging to five classes) were obtained at 4 °C, 649 isolates (belonging to nine

classes) were obtained at 28 °C. All the four phyla were found at both 4 °C and 28 °C. However, the low temperature had a better selectivity to *Proteobacteria*, while the higher one was more selective to *Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Supplementary Fig. S1a). At class level, *Acidimicrobiia*, *Cytophagia*, *Epsilonproteobacteria* and *Nitiliruptoria* were only isolated at 28 °C. In addition, 28 °C had better selectivity to *Actinobacteria_c*, *Bacilli* and *Flavobacteriia*, while 4 °C had better selectivity to *Alphaproteobacteria* and *Gammaproteobacteria* (Supplementary Fig. S1b). At genus level, *Ruegeria*, *Vibrio*, *Oceanobacillus*, *Muricauda*, *Leeuwenhoekiella*, *Mesoflavibacter*, *Brachybacterium* and *Microbacterium* were only isolated at 28 °C, while *Cobetia*, *Winogradskyella* and *Croceicoccus* were only isolated at 4 °C. Moreover, 28 °C had better selectivity to *Henriciella*, *Nocardioides*, *Bacillus* and *Aeromicrobium*, while 4 °C was more selective to *Erythrobacter*, *Pseudoalteromonas*, *Marinobacter*, *Sulfitobacter*, *Pseudomonas* and *Idiomarina* (Supplementary Fig. S1c).

Potential novel bacterial species from the Mariana Trench

Fifty bacterial strains (7.0% of the total isolates) showed 16S rRNA gene similarities of less than 98.65% to the type strains of their closest known bacterial species, and represented 24 potential novel species, details of three of these have recently been published (Liu et al. 2019a; Sun et al. 2019; Zhou et al. 2018). These strains were isolated from different depths, including the surface (nine strains), 1000 m (five strains), ~2000 m (three strains), ~4000 m

strains), ~8000 m (one strain), ~9000 m (nine strains) and 9600 m (eight strains); no strains of potential novel species were obtained from 200 m, 8289 m and 10,400 m (Supplementary Table S3). The proportions of potential novel bacteria were highest at 0 m (18.0%) and 9000 m (18.0%) (Supplementary Table S3). Moreover, 33 (16 species) of the 50 potential novel strains were obtained from R2A medium, while the remaining 17 strains (10 species) were obtained from 2216E medium (Fig. 6). These potential novel strains belonged to *Flavobacteriia* (23 strains), *Alphaproteobacteria* (14 strains), *Gammaproteobacteria* (seven strains), *Actinobacteria_c* (four strains), *Nitiliruptoria* (one strain) and *Epsilonproteobacteria* (one strain).

The abilities of bacterial isolates to degrade various macromolecules

To investigate the degradation activity of bacterial isolates from the Mariana Trench to various macromolecules, at least one strain from each bacterial species at different water depths were selected. In total, 354 isolates belonging to 178 species were screened for their degradation activity against ten kinds of macromolecules (Supplementary Table S4). More than 50% (194 out of 354 isolates) of the isolates showed an ability to degrade Tween 40 (59.9%), gelatin (57.9%) and Tween 20 (56.5%), 29%–35% of the isolates were positive for hydrolysis of Tween 80 (34.2%), starch (30.2%) and DNA (29.9%) but less than 10% of the isolates were positive for hydrolysis of casein (8.2%), CM-cellulose (7.1%), chitin (3.4%) and alginate (1.7%). Furthermore, no strains had a broad range of biodegradability to all

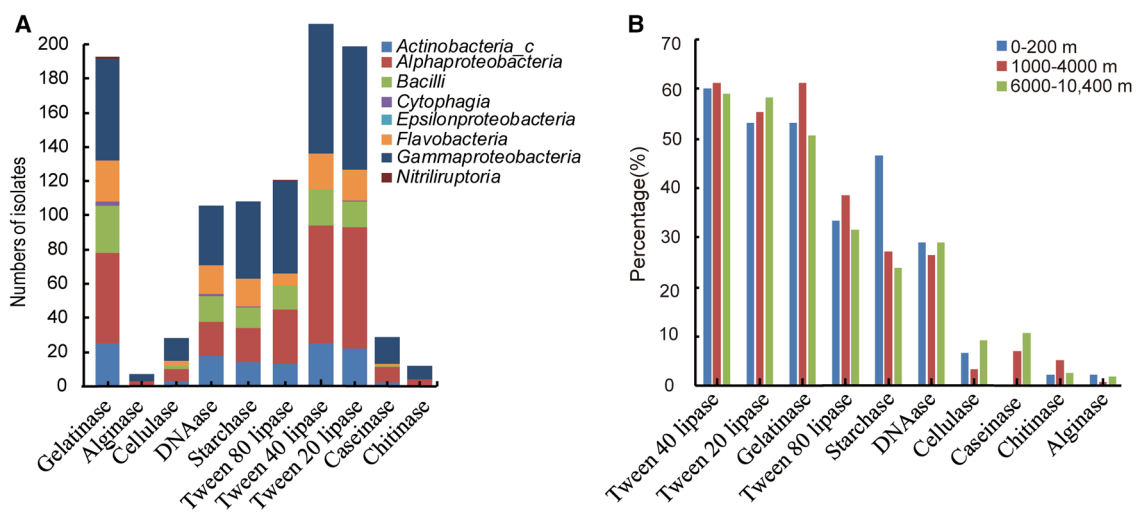


Fig. 7 Macromolecule degradation activities of bacterial isolates. **a** Number of bacterial isolates that could degrade different macromolecules. **b** Macromolecule degradation activities of bacterial isolates at different water layers

(five strains), ~6000 m (four strains), ~7500 m (six

Table 1 Proportion of bacterial strains that can degrade various macromolecules at different water depths

Water depths (no. of bacterial strains)	Alginate (%)	Cellulose (%)	Gelatin (%)	DNA (%)	Starch (%)	Tween 80 (%)	Tween 40 (%)	Tween 20 (%)	Casein (%)	Chitin (%)
0–200 m (45)	2.22	6.66	53.33	28.88	46.67	33.33	60	53.33	0	2.22
1000–4000 m (113)	0.87	3.51	61.40	26.31	27.20	38.60	61.40	55.26	7.02	5.26
6000–10,400 m (196)	2.04	9.18	50.50	29.08	23.98	31.63	59.18	58.16	10.71	2.55

macromolecules and all were only able to degrade a small number of compounds.

Among the isolates, *Gammaproteobacteria* and *Alphaproteobacteria* were positive for hydrolysis of all the ten macromolecules, while *Actinobacteria_c*, *Bacilli* and *Flavobacteriia* were negative for hydrolysis of alginate and chitin. In addition, *Cytophagia* was positive for hydrolysis of gelatin, DNA, starch and Tween 20 (Fig. 7a).

There were fewer isolates that could degrade alginate, chitin and CM-cellulose compared with other macromolecules. Only seven strains from seven genera, *Cobetia*, *Paracoccus*, *Pseudoalteromonas*, *Aestuariibacter*, *Marinovum*, *Pseudoceanicola* and *Salinicola*, were positive for hydrolysis of alginate, and these belonged to *Alphaproteobacteria* and *Gammaproteobacteria*. Twelve strains belonging to seven genera were positive for hydrolysis of chitin, including eight strains from *Gammaproteobacteria* and four strains from *Alphaproteobacteria* (Fig. 7a). Twenty-eight strains belonging to 23 genera were positive for hydrolysis of CM-cellulose, mostly from *Gammaproteobacteria* (13 strains) and *Alphaproteobacteria* (seven strains) and a few from *Actinobacteria_c* (three strains), *Flavobacteriia* (three strains) and *Bacilli* (two strains). In most cases, the proportion of strains that could degrade the same macromolecule was comparable at different depths (Table 1). However, it was noticed that the proportion of strains with starch degradation activity decreased with increasing water depth, while the proportion of strains with casein degradation activity exhibited an opposite trend (Fig. 7b).

Discussion

Although culture-independent methods (e.g., 16S rRNA gene high-throughput sequencing and metagenomics) have been extensively used to explore the diversity and potential metabolic pathways of microorganisms in marine environments, cultivation of microorganisms is still required to fully understand the physiology and biogeochemical functions of these organisms. As a complement to previous molecular studies, a large number of bacterial cultures were isolated from the seawater of the Mariana Trench in this study and their ability to degrade various macromolecules was investigated.

Highly diverse heterotrophic bacteria were obtained from the Mariana Trench

In this study, 825 bacterial strains (belonging to 108 genera and including 50 potential novel strains) were isolated from different water depths of the Mariana Trench. This culturable bacterial community was mainly composed of the phyla

Proteobacteria, *Actinobacteria*, *Firmicutes* and *Bacteroidetes*, which was similar to previous culture-independent studies by 16S rRNA genes high-throughput sequencing and metagenomics in the Mariana Trench (Liu et al. 2019c; Nunoura et al. 2015; Peoples et al. 2018; Tarn et al. 2016). At class level, *Alphaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria_c*, *Bacilli* and *Flavobacteriia*, which accounted for the majority of the culturable bacteria, were also the dominant classes (Liu et al. 2019c; Nunoura et al. 2015; Peoples et al. 2018; Tarn et al. 2016). In addition, the distribution and abundance patterns were found to be distinct from those in abyssal waters. For example, *Gammaproteobacteria* was abundant in hadal waters. This difference between hadal and abyssal waters was also noted in previous studies with culture-independent data (Nunoura et al. 2015; Peoples et al. 2018). The abundance of *Gammaproteobacteria*, and thus heterotrophic activity, at the bottom of the trench was considered to be caused by organic matter sinking downward and funneling into the trench topography (Danovaro et al. 2003; Glud et al. 2013; Ichino et al. 2015; Peoples et al. 2018; Wenzhöfer et al. 2016). In fact, *Gammaproteobacteria* is the most commonly reported bacteria in many different oceanic areas worldwide, such as the South Atlantic Ocean (Wang et al. 2017), East China Sea and South China Sea (Lu et al. 2012), the Pacific nodule province (Xu et al. 2005) and the Mid-Atlantic Ridge (Nercessian et al. 2005).

Many abundant genera from the cultures, such as *Alteromonas*, *Marinobacter*, *Pseudoalteromonas*, *Erythrobacter*, *Henriciella*, *Bacillus*, *Nocardiodides*, *Aeromicrobium*, *Halomonas*, *Vibrio*, *Oceanobacillus* and *Pseudomonas*, were also present in many of the culture-independent studies (Liu et al. 2019c; Peoples et al. 2018; Tarn et al. 2016). These results indicate that the culturable bacteria studied herein reliably reflect the total microbial communities of the Mariana Trench. The culturing also recovered some rare genera from the waters deeper than 6000 m, including *Lysinibacillus*, *Citricella*, *Altererythrobacter*, *Croceicoccus*, *Luteimonas* and *Winogradskyella*. These genera have been previously found in deep-sea environments (Fan et al. 2014; Lai et al. 2009, 2011; Xu et al. 2009) and some have been shown to have the ability to degrade (e.g., *Lysinibacillus* can degrade xylan, fomesafen and ethanethiol) (Lee et al. 2010; Liang et al. 2009; Wan et al. 2010), indicating that they may have evolved specific strategies to survive in deep-sea environments such as the Mariana Trench. Some of the abundant groups in other culture-independent study, e.g., *Epsilonproteobacteria* and *Cytophagia* (Liu et al. 2019c; Tarn et al. 2016), were rare in the cultivation study reported here, indicating that the culture conditions still have many limitations. Different culture media and culture conditions will be necessary to obtain a more diverse bacterial community.

In summary, the heterotrophic bacterial diversity changed significantly with water depth, which was probably caused by changes in physicochemical properties such as pressure, salinity, temperature, oxygen, light penetration and primary productivity. In addition, other biotic and abiotic characteristics such as dissolved organic carbon (DOC), particulate organic carbon (POC) and dimethylsulfoniopropionate (DMSP) may also help explain changes in the community structure (Phoma et al. 2018).

Fifty potential novel strains (belonging to 24 potential novel species) were isolated from the Mariana Trench, including *Abyssibacter profundus* gen. nov., sp. nov. (Zhou et al. 2018), *Alcanivorax profundus* sp. nov. (Liu et al. 2019a) and *Glycoaulis profundus* sp. nov. (Sun et al. 2019); descriptions of these have recently been published. Nine other novel species have also been identified from the Mariana Trench (Hu et al. 2015; Kusube et al. 2017; Meng et al. 2019; Pathom-aree et al. 2006a, b, c, 2007; Takai et al. 1999; Wei et al. 2018). This shows that the Mariana Trench contains abundant novel microbial resources and further study is bound to identify many more.

Additionally, the near bottom water of the Mariana Trench had more abundant culturable bacteria than the upper waters, according to our CFU count results, consistent with previous culture study results using the dilution counting method (Nunoura et al. 2015). Indeed, at the near bottom water of the Trench, the abundance of total bacteria identified by the 16S rRNA gene qPCR method was also found to increase (Liu et al. 2019c; Nunoura et al. 2015), and the near bottom water was supposed to be dominated by heterotrophic bacteria (Nunoura et al. 2015).

Different culture conditions are important for obtaining a highly diverse heterotrophic bacteria community

This study identified 108 genera of culturable heterotrophic bacteria from the Mariana Trench, well exceeding the 33 genera obtained in a previously (Peoples et al. 2018). Sixteen genera were shared by both studies, with *Pseudoalteromonas*, *Halomonas* and *Vibrio* being the dominant genera in both studies. While 90 genera were only obtained in this study, 17 genera were only found in the study by Peoples et al. (2018). This difference in bacterial diversity may be caused by the culture conditions used. Peoples et al. (2018) cultured the bacteria on 2216E plates and in bulbs at 4 °C, whereas this study used two media (2216E and R2A) and cultured at two temperatures (4 °C and 28 °C). Therefore, the more diverse media and temperatures allowed more bacterial genera to be isolated. In addition, the different sampling methods may also have contributed to the difference in the diversity of the bacterial cultures; while a CTD was used

to collect all the samples in this study, Peoples et al. (2018) used several different kinds of collection methods.

Regarding culture media, both 2216E and R2A are commonly used heterotrophic culture media. In this study, 423 bacterial isolates (belonging to seven classes and 85 genera) were obtained using 2216E medium, while 402 isolates (belonging to eight classes and 73 genera) were obtained using R2A medium. Fifty genera (47.0%) were isolated from both 2216E and R2A media. Results of this study showed that 2216E medium had better selectivity to *Firmicutes*, while R2A medium had better selectivity to *Bacteroidetes*. In addition, R2A medium was able to isolate some less common bacteria species. For example, one rare isolate of *Bacteroidetes*, *Euzebryella marina* RN62, which possessed a considerable capacity for carbohydrate metabolism, was isolated from hadal water by R2A medium (Liu et al. 2019b). Moreover, most (33 out of 50) of the potential novel strains (belonging to 16 potential novel species) obtained in this study were isolated by R2A medium. The reason for the differences in bacterial selectivity might be because these two media have different carbon sources and nutrient concentrations; R2A medium is a relatively oligotrophic medium compared with 2216E medium and is more similar to natural marine waters.

Previous studies by us have isolated some bacterial taxa with selective media that were rare or absent in 2216E and R2A media in this study (Liu et al. 2019c). For example, strains of *Alcanivorax venustensis* and *Alcanivorax dieselolei*, which can efficiently degrade alkanes, were only obtained from medium supplemented with alkane as a sole carbon source; strains of *Vibrio campbellii* (unpublished data) were only obtained from TCBS (thiosulfate citrate bile salts sucrose) medium, which is a selective medium for *Vibrio*. These results indicated that the combination of general media (e.g., 2216E and R2A) and selective media (e.g., TCBS and medium supplemented with alkane) can be used to obtain a more diverse range of culturable bacteria.

Cultivated bacteria from the Mariana Trench can degrade a diverse range of macromolecules

To understand the potential roles of heterotrophic bacteria in organic carbon cycling, strains of a number of genera from each depth were tested for their ability to degrade ten kinds of macromolecules, including Tween 20, Tween 40, Tween 80, gelatin, starch, DNA, casein, CM-cellulose, chitin and alginate. Tween 20, Tween 40 and Tween 80 were used as substrate to examine the lipase activities, following previous studies (Li et al. 2001; Plou et al. 1998; Tindall et al. 2007). Tween 20 (lauric acid ester), Tween 40 (palmitic acid ester) and Tween 80 (oleic acid ester) represent three different esters, so they can reflect different kinds of lipase activity in

bacteria. It was interesting to find that these bacterial strains had a variety of extracellular enzyme activities. While many macromolecules could be degraded by most bacterial strains, some refractory macromolecules, such as alginate and chitin, could be degraded by only a few strains, belonging to *Gammaproteobacteria* and *Alphaproteobacteria*. Some strains of *Actinobacteria_c*, *Bacilli* and *Flavobacteriia* were positive for hydrolysis of casein and cellulose. In addition, bacterial strains isolated from the Mariana Trench found it easier to use Tween 40 and Tween 20 than Tween 80 (Fig. 7).

Some of the representative strains have the potential to be used as model microorganisms to study the organic carbon cycling in the Mariana Trench. For example, one flavobacterial strain isolated from hadal water in this study was found to have a capacity for carbohydrate utilization and possessed a potential ability for intracellular cycling of the glycogen/starch pathway (Liu et al. 2019b). In addition, some of the strains with high activity to degrade macromolecules have potential value for industrial applications. For example, *Alteromonas* has been shown to produce extra- and intracellular alginate lyases (Sawabe et al. 1997), and *Bacillus* can be used as a probiotic in aquaculture (Ziaei-Nejad et al. 2006). Additionally, five strains of *V. campbellii* and four strains of *Labrenzia aggregate* isolated from different depths of the Mariana Trench were investigated for their capacity to utilize carbohydrate and adapt to the deep-sea environment (unpublished data). Meanwhile, some strains from our study were found to have DMSP synthesis and degradation capabilities (unpublished data).

Conclusions

In summary, 825 heterotrophic bacterial strains (belonging to 108 genera and including 50 potential novel strains) from 12 water depths (0–10,400 m) of the Mariana Trench were isolated with 2216E and R2A media at 4 °C or 28 °C on four cruises during 2015–2017. The diversity of these bacterial strains was much higher than found in previous studies. To obtain a more abundant culturable bacterial diversity, it was essential to employ a variety of culture conditions, i.e., different culture media, temperatures, etc. Although culturable bacteria account for only a small part of all bacteria, compared with culture-independent studies, bacterial cultures can be used for a variety of purposes. Many of the strains obtained here are novel taxa. In addition, some bacterial strains can be used as model organisms to study their biogeochemical functions or their deep-sea adaptation mechanisms in deep waters such as the Mariana Trench. Moreover, some of the strains may have potential values for industrial application.

Materials and methods

Sample collection

Seawater samples were collected from the Mariana Trench (Supplementary Table S5) aboard the R/V *Dong Fang Hong 2*, on four cruises; December 2015, September 2016, March 2017 and October 2017. For the convenience of statistics, samples from adjacent depths were divided into 12 depths, namely 0, 200, 1000, 2000, 4000, 6000, 7500, 8000, 8289, 9000, 9600 and 10,400 m. The samples were collected using a 12 L Niskin bottles (with either 8 or 24 bottles). 10 ml subsamples were transferred into 15 ml sterile conical tubes, and stored at 4 °C (within 1 h) prior to bacterial isolation. The basic environmental parameters, such as temperature, salinity and pH were measured by CTD (Supplementary Table S2).

Bacterial isolation

The seawater samples were diluted to 10^{-3} in a 1-to-10 dilution series. Aliquots (0.1 ml) of each dilution were spread on duplicated marine agar 2216E (1 g yeast extract, 5 g peptone, 0.01 g ferric phosphate, 20 g agar, 1,000 ml sea water, pH 7.6) or marine R2A agar (0.5 g yeast extract, 0.5 g peptone, 0.5 g casein, 0.5 g glucose, 0.5 g soluble starch, 0.5 g sodium pyruvate, 20 g agar, 750 ml sea water, 250 ml distilled water) plates, respectively. The inoculated plates were incubated at either 4 °C or 28 °C in onboard incubators. The colony-forming units (CFU) were counted at 4 weeks (at 4 °C) or 7 days (at 28 °C) when most morphologically different colonies appeared on the plates. Individual colonies were picked randomly, purified by streaking three times on fresh media, and incubated at the same temperature. Bacterial stocks were preserved at –80 °C freezer in sterile 0.85% (w/v) NaCl supplemented with 15% (v/v) glycerol after identification by 16S rRNA gene sequencing.

Genomic DNA extraction

For Gram-negative bacteria, the genomic DNA of the bacterial isolates was extracted by the simplified “colony boiling method” (Margassery et al. 2012), as the cell wall of Gram-negative bacteria is susceptible to cell disruption. Briefly, the bacterial isolate was subcultured on an 2216E/R2A agar plate, and the bacterial colonies were picked into Eppendorf tubes containing 200 μ l 1 \times TE buffer (1 mol/L Tris–HCl, 0.5 mol/L EDTA, pH 8.0). The Eppendorf tube was oscillated on a micro-vortex mixer, heated on water bath of boiling water (100 °C) for 10 min, and immediately ice bathed for 30 min. Subsequently, the mixture was centrifuged at

14,000 g for 5 min, and the supernatant was used as the DNA template.

For Gram-positive bacteria, genomic DNA was extracted by the traditional “phenol/chloroform method”, according to the previous published procedures (Moore et al. 1999, 2004), as Gram-positive bacteria have much thicker cell walls containing more peptidoglycans than Gram-negative bacteria which are difficult to break.

16S rRNA gene PCR amplification and sequencing analysis

The 27F (5'-AGAGTTTGTGATCCTGGCTCAG-3')/1492R (5'-GGTTACCTTGTACGACTT-3') primer pairs (DeLong 1992) were used as bacterial universal primers for 16S rRNA gene amplification. The 50 μ l PCR reactions contained 5 μ l of TaKaRa PCR 10 \times buffer, 5 μ l of Super pure dNTPs (2 mol/L), 0.25 μ l of TaKaRa Taq (5U/ μ l), 0.5 μ l of the primer pairs (20 μ mol/L) and 2.0 μ l of genomic DNA. The PCR program was set as follows: 95 °C for 5 min, and 30 cycles of 95 °C for 60 s, 55 °C for 60 s and 72 °C for 90 s, and a final extension of 72 °C for 10 min. The PCR amplification products were purified, digested by the restriction endonuclease *Hae*III and checked with agarose gel electrophoresis. Two or three bacterial isolates from each of the restriction endonuclease digestion profiles on 16S rRNA gene PCR products were selected and their 16S rRNA genes were sequenced by Beijing Genomics Institute (BGI, Shenzhen, China). The effective fragments (~650 bp) of 16S rRNA gene sequence were blasted by EzBioCloud server (<https://www.ezbiocloud.net/>) for comparison, and the single-track similarity values between bacterial isolates and their closely related type strains were calculated.

Screening for the abilities of bacterial strains to degrade various macromolecules

In total, 354 (out of 825) bacterial strains isolated from the waters of the Mariana Trench were chosen to perform macromolecules hydrolysis tests. Generally, at least one bacterial strain belonging to each species was selected from each depth. A total of ten macromolecules, including Tween 20, Tween 40, Tween 80, gelatin, starch, DNA, casein, CM-cellulose, chitin and alginate, were tested. Hydrolysis of casein, gelatin, starch, cellulose, and Tween 20, Tween 40 and Tween 80 were carried out according to the standard methods of Tindall et al. (2007). Hydrolysis of DNA was determined by DNase test agar (Qingdao Hope Bio-technology Co. Ltd, China) prepared with sterile seawater. HCl (mol/L) solution was used to flood the plates to detect DNase producers, and a clear transparent zone appearing around the colony indicating positive result. Alginate hydrolysis activity

was determined by the formation of depression zones around colonies on alginase test agar [2 g (NH₄)₂SO₄, 3 g KH₂PO₄, 7 g K₂HPO₄·3H₂O, 0.1 g MgSO₄·7H₂O, 0.05 g FeSO₄·7H₂O, 30 g NaCl, 5 g sodium alginate, 15 g agar, 1000 ml distilled water, pH 7.5] (Zhang 2016). The activity could be further confirmed by adding a few drops of 10% CaCl₂ around colonies and a transparent or opalescent zone will appear for positive strain. Chitinase activity was determined by the method of previous used (Zhang 2016). Briefly, bacterial strains were inoculated onto chitin agar [2216E agar supplemented with 10% (w/v) chitin colloid (Ocean University of China)]. Typically, the plates were required to be incubated for ~2 weeks to observe the results, as chitin is a refractory macromolecule. The appearance of a transparent zone around a colony indicated that the strain was positive for chitinolytic activity.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences of 706 cultivated bacterial strains have been deposited in the GenBank database under the accession numbers MN491931–MN492266, MN492268–MN492635, MK660097 and MN515145.

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Author contributions X-HZ designed the experiments, analyzed the data and wrote the manuscript. XZ isolated the bacteria, screened the bacterial strains for macromolecules degradation abilities, analyzed the data and wrote the manuscript. SZ, JL and YZ collected the water samples and isolated the bacteria. YW isolated the bacteria. KK analyzed the data. All authors edited and approved the final manuscript.

Compliance with ethical standards

Conflict of interest All the authors declare that there are no conflicts of interest.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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References

- Danovaro R, Della CN, Dell'Anno A, Pusceddu A (2003) A depocenter of organic matter at 7800 m depth in the SE Pacific Ocean. *Deep Sea Res Part I* 50:1411–1420
- DeLong EF (1992) Archaea in coastal marine environments. *Proc Natl Acad Sci USA* 89:5685–5689
- Fan X, Yu T, Li Z, Zhang X-H (2014) *Luteimonas abyssi* sp. nov., isolated from deep-sea sediment. *Int J Syst Evol Microbiol* 64:668–674
- Fujioka K, Okino K, Kanamatsu T, Ohara Y (2002) Morphology and origin of the Challenger Deep in the Southern Mariana Trench. *Geophys Res Lett* 29:1011–1014
- Gardner JV, Armstrong AA, Calder BR, Beaudoin J (2014) So, how deep is the Mariana Trench? *Mar Geod* 37:1–13
- Glud RN, Wenzhöfer F, Middelboe M, Oguri K, Turnewitsch R, Canfield DE, Kitazato H (2013) High rates of microbial carbon turnover in sediments in the deepest oceanic trench on Earth. *Nat Geosci* 6:284
- Hu J, Zhang W-Y, Zhang X-Q, Cheng-H Z-F, Wu M (2015) *Muriicola marianensis* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* 65:407–411
- Ichino MC, Clark MR, Drazen JC, Jamieson A, Jones DOM, Martin AP, Rowden AA, Shank TM, Yancey PH, Ruhl HA (2015) The distribution of benthic biomass in hadal trenches: a modelling approach to investigate the effect of vertical and lateral organic matter transport to the seafloor. *Deep Sea Res Part I* 100:21–33
- Kato C, Li L, Nogi Y, Nakamura Y, Tamaoka J, Horikoshi K (1998) Extremely barophilic bacteria isolated from the Mariana Trench, Challenger Deep, at a depth of 11,000 meters. *Appl Environ Microbiol* 64:1510–1513
- Kogure K, Simidu U, Taga N (1979) A tentative direct microscopic method for counting living marine bacteria. *Can J Microbiol* 25:415–420
- Kusube M, Kyaw TS, Tanikawa K, Chastain RA, Hardy KM, Cameron J, Bartlett DH (2017) *Colwellia marinimaniae* sp. nov., a hyperpiezophilic species isolated from an amphipod within the Challenger Deep, Mariana Trench. *Int J Syst Evol Microbiol* 67:824–831
- Lai Q, Yuan J, Shao Z (2009) *Altererythrobacter marinus* sp. nov., isolated from deep seawater. *Int J Syst Evol Microbiol* 59:2973–2976
- Lai Q, Fu Y, Wang J, Chen S, Zhong H, Sun F, Shao Z (2011) *Citri-cella marina* sp. nov., isolated from deep-sea sediment. *Int J Syst Evol Microbiol* 61:728–731
- Lee C-S, Jung Y-T, Park S, Oh TK, Yoon JH (2010) *Lysinibacillus xylanilyticus* sp. nov., a xylan-degrading bacterium isolated from forest humus. *Int J Syst Evol Microbiol* 60:281–286
- Li CY, Cheng CY, Chen TL (2001) Production of *Acinetobacter radi-oresistens* lipase using Tween 80 as the carbon source. *Enzyme Microb Technol* 29:258–263
- Liang B, Lu P, Li H, Li R, Li S, Huang X (2009) Biodegradation of fomesafen by strain *Lysinibacillus* sp. ZB-1 isolated from soil. *Chemosphere* 77:1614–1619
- Liu J, Ren Q, Zhang Y, Li Y, Tian X, Wu Y, Tian J, Zhang X-H (2019a) *Alcanivorax profundus* sp. nov., isolated from deep seawater of the Mariana Trench. *Int J Syst Evol Microbiol* 69:371–376
- Liu J, Xue C-X, Sun H, Zheng Y, Meng Z, Zhang X-H (2019b) Carbohydrate catabolic capability of a *Flavobacteriia* bacterium isolated from hadal water. *Syst Appl Microbiol* 42:263–274

- Liu J, Zheng Y, Lin H, Wang X, Li M, Liu Y, Yu M, Zhao M, Pedentchouk N, Lea-Smith DJ, Todd JD, Magill CR, Zhang WJ, Zhou S, Song D, Zhong H, Xin Y, Yu M, Tian J, Zhang X-H (2019c) Proliferation of hydrocarbon-degrading microbes at the bottom of the Mariana Trench. *Microbiome* 7:47
- Lu J, Zhang X, Du L, Yang Z, Wu M, Lu L (2012) Bacterial isolation and diversity analysis of four seawater sampling sites of the East China Sea and the South China Sea. *J Zhejiang Univ Sci* 39:443–449
- Margassery LM, Kennedy J, O’Gara F, Dobson AD, Morrissey JP (2012) Diversity and antibacterial activity of bacteria isolated from the coastal marine sponges *Amphilectus fucorum* and *Eurypon* major. *Lett Appl Microbiol* 55:2–8
- Meng F-X, Li G, Fang C, Wu Y-H, Cheng H, Chen Y, Wang C-S, Shao Z, Xu X-W (2019) *Altererythrobacter aerophilus* sp. nov., isolated from deep-sea water of the north-west Pacific. *Int J Syst Evol Microbiol* 69:1689–1695
- Moore E, Arnscheidt A, Krüger A, Strömpl C, Mau M (1999) Simplified protocols for the preparation of genomic DNA from bacterial cultures. *Mol Microb Ecol Man* 1:1–15
- Moore E, Arnscheidt A, Krüger A, Strömpl C, Mau M (2004) Section 1 update: simplified protocols for the preparation of genomic DNA from bacterial cultures. *Mol Microb Ecol Man* 1:3–18
- Nercessian O, Fouquet Y, Pierre C, Prieur D, Jeanthou C (2005) Diversity of Bacteria and Archaea associated with a carbonate-rich metalliferous sediment sample from the Rainbow vent field on the Mid-Atlantic Ridge. *Environ Microbiol* 7:698–714
- Nunoura T, Takaki Y, Hirai M, Shimamura S, Makabe A, Koide O, Kikuchi T, Miyazaki J, Koba K, Yoshida N (2015) Hadal biosphere: insight into the microbial ecosystem in the deepest ocean on Earth. *Proc Natl Acad Sci USA* 112:1230–1236
- Pathom-aree W, Nogi Y, Ward AC, Horikoshi K, Bull AT, Goodfellow M (2006a) *Dermacoccus barathri* sp. nov. and *Dermacoccus profundus* sp. nov., novel actinomycetes isolated from deep-sea mud of the Mariana Trench. *Int J Syst Evol Microbiol* 56:2303–2307
- Pathom-aree W, Nogi Y, Sutcliffe IC, Ward AC, Horikoshi K, Bull AT, Goodfellow M (2006b) *Dermacoccus abyssi* sp. nov., a piezotolerant actinomycete isolated from the Mariana Trench. *Int J Syst Evol Microbiol* 56:1233–1237
- Pathom-aree W, Nogi Y, Sutcliffe IC, Ward AC, Horikoshi K, Bull AT, Goodfellow M (2006c) *Williamsia marianensis* sp. nov., a novel actinomycete isolated from the Mariana Trench. *Int J Syst Evol Microbiol* 56:1123–1126
- Pathom-aree W, Nogi Y, Ward AC, Horikoshi K, Bull AT, Goodfellow M (2007) *Dermacoccus barathri* sp. nov. and *Dermacoccus profundus* sp. nov., novel actinomycetes isolated from deep-sea mud of the Mariana Trench. *Int J Syst Evol Microbiol* 57:197–197
- Peoples LM, Donaldson S, Osuntokun O, Xia Q, Nelson A, Blanton J, Allen EE, Church MJ, Bartlett DH (2018) Vertically distinct microbial communities in the Mariana and Kermadec trenches. *PLoS ONE* 13:e0195102
- Phoma S, Vikram S, Jansson JK, Ansoorge IJ, Cowan DA, Van de Peer Y, Makhalyane TP (2018) Agulhas Current properties shape microbial community diversity and potential functionality. *Sci Rep* 8:10542
- Plou FJ, Ferrer M, Nuero OM, Calvo MV, Alcalde M, Reyes F, Balasteros A (1998) Analysis of Tween 80 as an esterase/lipase substrate for lipolytic activity assay. *Biotechnol Tech* 12:183–186
- Sawabe T, Ohtsuka M, Ezura Y (1997) Novel alginate lyases from marine bacterium *Alteromonas* sp. strain H-4. *Carbohydr Res* 304:69–76
- Simonato F, Campanaro S, Lauro FM, Vezzi A, D’Angelo M, Vitulo N, Bartlett DH (2006) Piezophilic adaptation: a genomic point of view. *J Biotechnol* 126:11–25
- Sun H, Hu Y, Zhou S, Zheng Y, Zhang X-H (2019) *Glycocaulis profundus* sp. nov., a marine bacterium isolated from seawater of the Mariana Trench. *Int J Syst Evol Microbiol*. <https://doi.org/10.1099/ijsem.0.003830>
- Takai K, Inoue A, Horikoshi K (1999) *Thermaerobacter marianensis* gen. nov., sp. nov., an aerobic extremely thermophilic marine bacterium from the 11000 m deep Mariana Trench. *Int J Syst Bacteriol* 49:619–628
- Tarn J, Peoples LM, Hardy K, Cameron J, Bartlett DH (2016) Identification of free-living and particle-associated microbial communities present in hadal regions of the Mariana Trench. *Front Microbiol* 7:665
- Tian J, Fan L, Liu H, Liu J, Li Y, Qin Q, Gong Z, Chen H, Sun Z, Zhou L, Wang X, Xu H, Bartlett D, Wang M, Zhang Y-Z, Zhang X-H, Zhang C (2018) A nearly uniform distributional pattern of heterotrophic bacteria in the Mariana Trench interior. *Deep Sea Res Part I* 142:116–126
- Tindall BJ, Sikorski J, Smibert RA, Krieg NR (2007) Phenotypic characterization and the principles of comparative systematics. In: Reddy C, Beveridge T, Breznak J, Marzluf G, Schmidt T, Snyder L (eds) *Methods for general and molecular microbiology*, 3rd edn. ASM Press, Washington, pp 330–393
- Wan S, Li G, An T, Guo B, Sun L, Zu L, Ren A (2010) Biodegradation of ethanethiol in aqueous medium by a new *Lysinibacillus sphaericus* strain RG-1 isolated from activated sludge. *Biodegradation* 21:1057–1066
- Wang K, Yan P, Ma R, Jia W, Shao Z (2017) Diversity of culturable bacteria in deep-sea water from the South Atlantic Ocean. *Bio-engineered* 8:572–584
- Wang Y, Huang J-M, Cui G-J, Nunoura T, Takaki Y, Li W-J, Li J, Gao Z-M, Takai K, Zhang A-Q, Stepanauskas R (2019) Genomics insights into ecotype formation of ammonia-oxidizing archaea in the deep ocean. *Environ Microbiol* 21:716–729
- Wei Y, Mao H, Xu Y, Zou W, Fang J, Blom J (2018) *Pseudomonas abyssi* sp. nov., isolated from the abyssopelagic water of the Mariana Trench. *Int J Syst Evol Microbiol* 68:2462–2467
- Wenzhöfer F, Oguri K, Middelboe M, Turnewitsch R, Toyofuku T, Kitazato H, Glud RN (2016) Benthic carbon mineralization in hadal trenches: Assessment by in situ O₂ microprofile measurements. *Deep Sea Res Part I* 116:276–286
- Xu M, Wang P, Wang F, Xiao X (2005) Microbial diversity at a deep-sea station of the Pacific nodule province. *Biodivers Conserv* 14:3363–3380
- Xu X-W, Wu Y-H, Wang CS, Wang X-G, Oren A, Wu M (2009) *Croceicoccus marinus* gen. nov., sp. nov., a yellow-pigmented bacterium from deep-sea sediment, and emended description of the family Erythrobacteraceae. *Int J Syst Evol Microbiol* 59:2247–2253
- Zhang X-H (2016) *Marine microbiology* (2nd edition) (in Chinese). Science Press, Beijing
- Zhou S, Ren Q, Li Y, Liu J, Wang X, Wu Y, Zhang Y, Zhang X-H (2018) *Abyssibacter profundus* gen. nov., sp. nov., a marine bacterium isolated from seawater of the Mariana Trench. *Int J Syst Evol Microbiol* 68:3424–3429
- Ziaei-Nejad S, Rezaei MH, Takami GA, Lovett DL, Mirvaghefi AR, Shakouri M (2006) The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. *Aquaculture* 252:516–524