ISSN 2616-5872 Vol.6, Issue 3: 20-29, DOI: 10.25236/AJEE.2024.060303

Comparison of Community Composition and Function of Particle-associated Microorganisms and Free-living Microorganisms in the Mariana Trench

Qianyi Hui[1,2,a,*]

[1]*College of Oceanography and Ecological Science, Shanghai Ocean University, Shanghai, 201306, China*
[2]*Shanghai Engineering Research Center of Hadal Science and Technology, Shanghai, 201306, China*
[a]*18956122962@163.com*
[*]*Corresponding author*

Abstract: *Deep-sea environments have unique habitat characteristics, including extremely high hydrostatic pressure, low temperatures, and minimal light. In addition, the deep sea also has a unique energy flow and material circulation system. In this special biological environment, the species, genetic composition and ecological function diversity of deep-sea microorganisms have been improved, and the abyss is the deepest part of the deep-sea environment, which has the most special biological environment in the deep-sea and is one of the least understood marine environments by human beings so far. The abyss contains abundant microbial biomass and exhibits active organic carbon turnover characteristics, which is a "hot spot" area for organic carbon degradation in the deep sea. In the marine environment, microorganisms exist in different ways, with Particle-associated microorganisms (PA) and free-living microorganisms (FL) representing two distinct lifestyles. However, little is known about the species composition and metabolic potential of abyssal microorganisms. In this paper, we used metagenomic technology and bioinformatics analysis to study the community composition and functional characteristics of these two microorganisms in the "Challenger Deep" (water depth of 10,500 meters) in the Mariana Trench, and to find the metabolic differences caused by their different lifestyles based on their functional characteristics. The results show that the nitrogen fixation ability, extreme environment adaptability and antioxidant capacity of free microorganisms are stronger than those of epiphytic microorganisms in the abyssal environment, which provides some help for the future research on abyssal microorganisms.*

Keywords: *Mariana Trench, Particle-associated Microorganisms, Free-living Microorganisms, Species Composition and Ecological Function*

1. Introduction

The abyss is the deepest part of the Earth's oceans, with seawater depths exceeding 6000 m. It covers 1-2% of the seafloor area and 45% of the vertical depth of the ocean [1-4], and is an important part of the marine ecosystem. Although the abyss is characterised by extreme environmental features such as high pressure, low temperature and complex geological activities [5], recent studies have found that abyssal trench sediments have an organic matter content and microbial carbon turnover rate far exceeding those of ordinary deep-sea environments, making them a "hotspot" for deep-sea organic carbon degradation [6]. Abyssal microbial-mediated degradation and transformation of organic matter is an important part of the chemical turnover process in the deep sea, and its contribution to the structural and functional diversity of marine prokaryotic communities should not be overlooked [6-7], so the study of functional and metabolic diversity of abyssal microorganisms is of extraordinary significance to human exploration and understanding of the deep-sea living environment.

Current studies of abyssal microbial communities have mostly used traditional isolation and culture methods [8-10] or high-throughput sequencing based on 16S rRNA genes to study the diversity of microbial communities [11-22], while less is known about the functional characteristics of trench microbes. Sporadic studies have been conducted to show that the microbial communities in the seawater of the Puerto Rico Trench have a rich and unique functional potential [23]. In Mariana Trench seawater, microbial communities with hydrocarbon degrading bacteria (e.g., Oceanospirillales) as the dominant taxa and a large number of genes encoding enzymes related to hydrocarbon degradation are

ISSN 2616-5872 Vol.6, Issue 3: 20-29, DOI: 10.25236/AJEE.2024.060303

present [24]. Another study of seawater from the Mariana Trench showed that abyssal microorganisms are primarily engaged in heterotrophic metabolism, but may also derive energy by oxidising CO [25]. Furthermore, studies in the Yap Trench have shown that the predominant metabolic mode in seawater (5000 m, 5700 m and 6000 m) microorganisms is heterotrophic metabolism (i.e., degradation of carbohydrates, hydrocarbon compounds and aromatic compounds) [26].

Most of the studies that have been carried out in China and abroad on the functional metabolism of microorganisms in abyssal trenches have focused on seawater microorganisms [27-30], and there has been limited research on the metabolic potential of microorganisms in the abyss below 6,000 m. In this study, we will analyse the differences in community composition and functional metabolism between epiphytic and free-living microorganisms in the Challenger Deep of the Mariana Trench (up to 10,500 m depth) using a combination of macro-genomics and bioinformatics. The results of this study will help to unravel the microbial community structure and metabolic processes in the abyssal biosphere of the Mariana Trench and fill the research gap of differences in metabolic pathways among abyssal microorganisms of different lifestyles in the abyssal microbial communities. Metabolic pathway differences, enhance the understanding of biogeochemical cycles and potential microbial driving mechanisms in the abyssal trenches, and further the understanding of species diversity and metabolic differences among abyssal microbial communities.

2. Methods of Data Acquisition and Analysis

2.1 Data Acquisition

The data used in this study were four data sets (SRR6144757; SRR6144761; SRR6144760; SRR6144758) downloaded from ncbi (https://www.ncbi.nlm.nih.gov) based on SSR numbering which contained 10400 and 10500 m FL and PA macrogenomic information.

2.2 Macrogenomic Data Analysis

In this study, the raw data obtained after sequencing were subjected to quality control (Table 1), and the sequences obtained from sequencing were de-jointed and low quality bases (bases) were removed using trimmomatic (v. 0.38) [31] (parameters: LEADING:30, TRAILING:30CROP:90, HEADCROP:10. SLIDINGWINDOW:4:25, MINLEN:50). Clean reads were assembled and spliced into contigs (parameter: kmer range 50-80, step 15) by IDBA-UD (v. 1.1.3) [32], and then the overlap clusters with lengths ≥500 bp were filtered in the splicing results as the final assembly results. Gene prediction was performed using prodigal (V2.6.3) [33] for Open Reading Frame (ORF) prediction (parameter: -p meta) of the contigs in the splicing results to obtain the coding sequences. Species classification (parameter: default) based on KAIJU (http://kaiju.binf.ku.dk/) for macrogenomic sequences [34], obtaining species annotations at different classification levels and combining intergroup samples into one whole sample based on the number of reads of the species. The coding sequences were then compared to the Kyoto Encyclopedia of Genes and Genomes (KEGG) [35] using the BlastKOALA [35] (https://www.kegg.jp/ghostkoala/) tool (parameters: default); Obtain the results of functional annotation of macro genome sequences, obtain the results of functional annotation of macro genome sequences, and calculate the abundance information of genes based on the results of functional annotation. The TPM (Tags Per Million) method was chosen for the abundance calculation. (TPM=(Ni/Li)*1000000/sum(Ni/Li+..... + Nm/Lm), Ni: number of reads mapped to gene i; Li: sum of exon lengths of gene i), the data were divided into two groups (FL and PA), and T-tests were applied to analyse the differentially expressed genes between the two groups, and these differentially expressed genes were enriched to find their metabolic pathways.

3. Results

3.1 Species Composition of Free and Attached Microorganisms in the Mariana Trench

The total number of bases obtained in this study was 137.36 Gb, and the optimised number of bases was 110.26 Gb after quality control processing, i.e., the clean sequences accounted for more than 80% of the original sequences (raw reads). The total number of phyla, families and genera obtained after the species classification process was 903, 4641 and 18337, respectively (see Table 1).

ISSN 2616-5872 Vol.6, Issue 3: 20-29, DOI: 10.25236/AJEE.2024.060303

Table 1: Metagene grouping and species annotation information

Sample	Group	Filtered Bases/Gb	Class	Genus
SRR6144757/PA-10400	PA	27.88	229	4616
SRR6144758/FL-10500	FL	26.16	224	4466
SRR6144760/FL-10400	FL	27.93	224	4568
SRR6144761/PA-10500	PA	28.29	226	4687

Both subgroups of this study annotated to three orders with species relative abundance greater than 1% (Fig. 1a, Fig. 1b), with γ (Gammaproteobacteria), α (Alphaproteobacteria), and Actinomycetia making up respectively 58.6%, 16.97%, and 1.93%, respectively (Fig. 1a); accounting for 55.6%, 13.4% and 3.56% of all taxonomic annotations in the PA sample, respectively (Fig. 1b), the present study focused on genera with relative abundance in the top 12, with Oleibacter being the most abundant at the genus level in both groups, with relative abundance exceeding 23.5% in both cases; Alteromonas and Pseudooceanicola were also dominant genera in both sets of samples, with the relative abundance of Alteromonas exceeding 5.4% in both sets, and the relative abundance of Pseudooceanicola exceeding 2.4% in both sets (Fig. 1c).

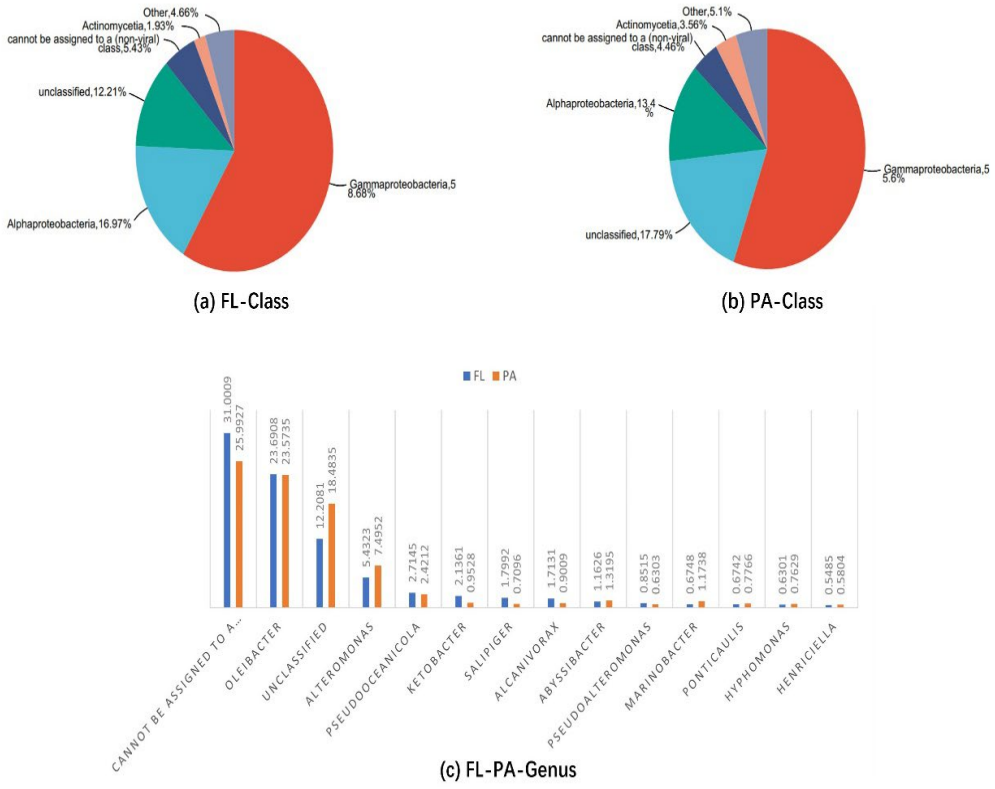
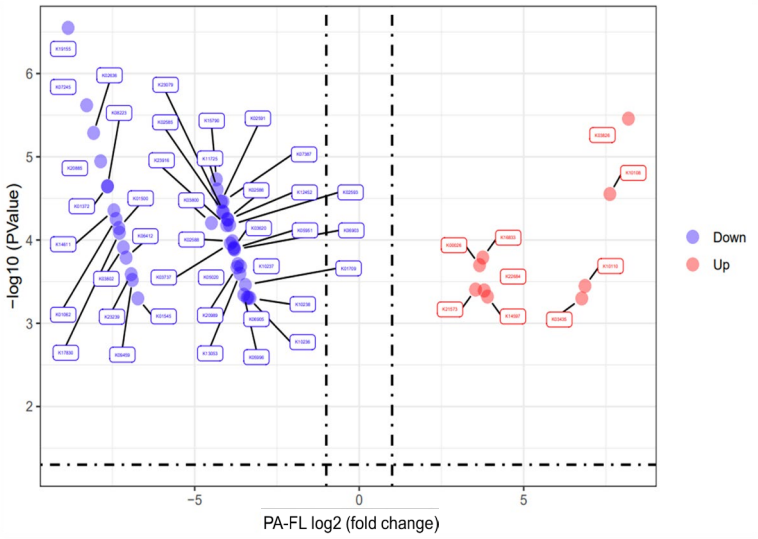


Figure 1: Species composition of microorganisms at the taxonomic level of classes and genus.

3.2 Comparison of Differences in Functional Annotations Based on KEGG Database

After KEGG annotation of the four samples, based on the grouping information, the component single samples were integrated by the number of reads for each gene, and finally the integration yielded 5,382 gene abundance information annotated through the KEGG official website, which were T-tested and yielded 49 differential KOs, of which 40 were FL-expressed significant genes and the other 9 were PA-expressed significant genes (Fig. 2).

ISSN 2616-5872 Vol.6, Issue 3: 20-29, DOI: 10.25236/AJEE.2024.060303

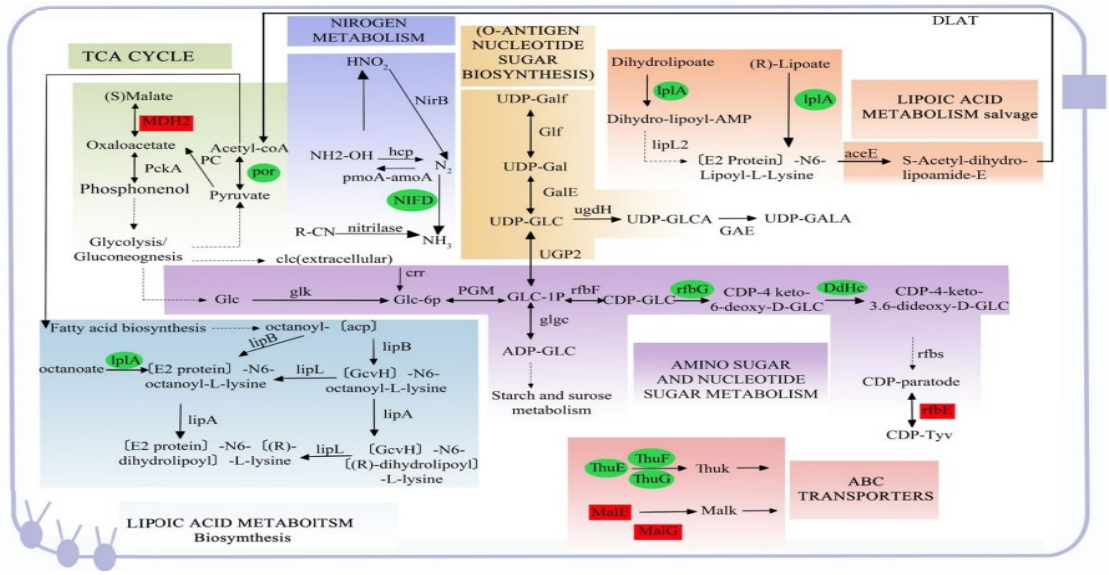


Comparison was made using samples from the PA group and the FL group, and results were obtained for declining genes indicating a significant decrease in the expression of these genes in the FL group compared to the PA group, and for increasing results indicating a significant increase in the expression of these genes in the FL group compared to the PA group.

Figure 2: Genes that differ from FL and PA

3.3 Comparison of Differences Based on KEGG Annotation Results of Two Microorganisms

Pathway enrichment analysis of differential KO numbers yielded six major differential pathways (Fig. 3) mainly including TCA CYCLE, AMINO SUGARAND Nucleotidesugar Metabolism, O-Antigennucleotide Sugarbiosynthesis, Lipoic Acidmetabolism, ABC Transporters and Nirogen Metabolism. After functional screening of metabolic pathways, lipoic acid metabolism, alginate transport in ABC transport and biological nitrogen fixation were identified as the main functionally differentiated pathways.



These metabolic pathways on the figure were found in the metabolic pathways of both different lifestyle microbes, while those labelled in green are enzymes or pathways that are significantly expressed in FL and those labelled in red are enzymes or pathways that are significantly expressed in PA.

Figure 3: Pathway diagram of the difference between FL and PA

ISSN 2616-5872 Vol.6, Issue 3: 20-29, DOI: 10.25236/AJEE.2024.060303

4. Differential Metabolic Pathways and Some Unknown Genes

4.1 Lipoic Acid Metabolism

Lipoic acid is an eight-carbon saturated fatty acid with two sulfur atoms attached between the 6- and 8-position carbon atoms, which participates in the key reactions of central carbon metabolism and oxidation of dissociated sulfur [36-39], and is both water-soluble and fat-soluble, and has a strong antioxidant property, which is known as the "all-purpose antioxidant" because it can scavenge reactive oxygen species, chelate heavy metal ions, and regenerate other antioxidants, such as vitamin C, glutathione, and sulfur-oxygen-reducing proteins [40].

In prokaryotes there are two pathways for lipoic acid, the de novo synthesis pathway and the remedial synthesis of lipoic acid, which complement each other (Fig. 3) [41]. The lipoic acid de novo synthesis pathway refers to the de novo synthesis of lipoic acid using octanoyl ACP (acyl carrier protein) as a substrate and the completion of the enzyme protein lipoic acid modification, while the lipoic acid remediation synthesis pathway refers to the direct obtainment of lipoic acid by the bacterium from the growth environment and the completion of the enzyme protein lipoic acid modification. When lipoic acid is available in the growth environment, bacteria first use exogenous lipoic acid to complete the enzymatic protein modification; when the environment lacks lipoic acid, bacteria synthesise lipoic acid through the de novo synthesis pathway to supply the cells with lipoic acid.

The lipoic acid de novo synthesis pathway uses octanoyl ACP, an intermediate product of fatty acid anabolism, as a substrate, and transfers the octanoyl group to the lipoic acid structural domain of the E2 subunit of α-ketoacid dehydrogenase catalyzed by the enzyme lactobionyltransferase (LipB), which is further catalysed by the enzyme lipoic acid synthetase (LipA), which connects the two sulphur atoms between carbon atoms 6 and 8 of the octanoyl group to form the lipoic acid acylated protein subunit [41 -43].

LipB, a monomeric protein of approximately 29 kDa (PDB code 2QHV), is able to catalyse the transfer of the octanoyl group and the generation of a thiolipid bond with cysteine at position 169 (Cys169) in LipB using octanoyl-ACP as a substrate, which is then further transferred to the E2 subunit (or the GcvH protein) to form an amide bond linked to a lysine in the thiooctanoic acid structural domain [42-44].

The lipoic acid remedy synthesis pathway is dependent on the lipoic acid ligase LplA. The reaction is catalysed in two steps, firstly LplA catalyses the binding of lipoic acid to ATP to generate the activated intermediate product lipoic acid-AMP and the release of pyrophosphate; and then LplA catalyses the transfer of the lipoic acid moiety from lipoic acid-AMP to the lipoic acid structural domain of the α-ketoacid dehydrogenase complex E2 subunit (or GcvH) to generate an amide bond with the ε-amino group in the conserved lysine residue, with the accompanying release of AMP [44,45] (Figure 3). And the content of LplA of free microorganisms was significantly higher than that of PA obtained in the intergroup variability analysis, which indicated that FL possessed a stronger synthetic ability of lipoic acid metabolism than PA in extreme environments, and because FL did not attach to particulate matter for survival, and the products of lipoic acid metabolism would participate in pyruvate oxidation, which would in turn produce Acetyl-CoA to participate in the tricarboxylic acid cycle to provide energy for the cells (Figure 3), which undoubtedly greatly enhances the ability of FL to survive in the oligotrophic water environment of the seafloor.

4.2 Alkalose Transport

As a natural sugar, alginose, also known as fungal sugar, muscovado sugar, is a non-reducing disaccharide composed of two glucose molecules, with the molecular formula of C12H22O11, and often exists as a dihydrate compound with the molecular formula of C12H22O11-2H2O.[46] The earliest discovery of alginate was made by Wigger, who, in his study of the ergot fungus of rye, after allowing the solution to stand for some time, found that a number of colourless, non-reducing, slightly sweet sugar crystals formed in the walls of the vessel [46, 47]. Subsequently, it was found to be widespread in plants, animals and microorganisms in nature, and Elbein summarised the distribution of alginate content in a wide range of organisms, with nearly 80 species of plants, algae, fungi, yeasts, bacteria, insects and invertebrates listed [48].

Alginose is a typical stress metabolite that can form a unique protective film on the cell surface

ISSN 2616-5872 Vol.6, Issue 3: 20-29, DOI: 10.25236/AJEE.2024.060303

under harsh environmental conditions such as high temperature, high cold, high osmotic pressure, and drying and water loss, which effectively protects the biomolecular structure from destruction, thus maintaining the life processes and biological characteristics of living organisms. [49, 50]

In this paper, we found that FL was significantly more competent than PA for alginate transport (Fig. 3), suggesting that compared to epiphytic microorganisms adsorbed to live on tiny particulate matter, free microorganisms in the seawater column of the oligotrophic and harsh Mariana Trench seawater ensure the survival of their cells in the seafloor environment by enhancing the mechanism of alginate-associated transport, which protects the cellular structure from the extreme environmental impacts.

4.3 Biological Nitrogen Fixation

The biological nitrification process is a central component of the Earth's nitrogen cycle, and the ammonia oxidation process (NH3 → NO2-) is a key step in the nitrification process [51]. In the FL microbial community studied here, the nitrogen fixation-related enzyme (nifD, K02586) was significantly expressed compared to PA, and this enzyme can convert N2 molecules to NH3 and further participate in the ammonia oxidation process (Figure 3). The process of ammonia oxidation is mainly mediated by ammonia-oxidising microorganisms (AOM) [51], which are classified into ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA), and Nitrosopumilus, a genus of nitrifying pygmies in the phylum Chikungunya, is the prototypical microorganism of ammonia-oxidising archaea [52]. Nitrosopumilus spp. accounted for 10 times more abundance in FL than in PA (0.075%-0.007%). Further studies also revealed that the macrogenomes of FL microorganisms from the Marinaia Trench all contained three known genera of ammonia-oxidising bacteria in the β-amoeba phylum, Nitrosococcus, Nitrosomonas, and Nitrosospira [53], which corresponded to the information of these potential ammonia-oxidising microbial species information, these results suggest that ammonia oxidation processes may be important metabolic processes in FL communities.

The process of ammonia oxidation releases electrons and generates energy that can be used to fix CO2, form organic compounds, and sustain cell growth, among other things. However, there are important differences in carbon sequestration between AOA and AOB, with AOA sequestering carbon through the 3-hydroxypropionic acid/4-hydroxybutyric acid (3HP/4HB) pathway [54-55], while AOB sequesters carbon through the Calvin cycle [56]. In the macrogenomic sequence of surface sediments from the Mariana Trench, we successfully annotated the genes encoding enzymes such as methylmalonyl-CoA mutase (mcmA) and acetyl-CoA C-acetyltransferase (atoB), which are required for the carbon sequestration pathway of 3HP/4HB. genes, and the high relative abundance of these genes in the whole macrogenome suggests that the carbon sequestration process of the 3HP/4HB pathway has an important role in sediment microbes. Also, this result corresponds to the results of high abundance of the Chiguria phylum in the microbial community composition of abyssal surface sediments. At the same time, in-depth analyses also revealed the presence of all genes required for a complete Calvin cycle in the abyssal surface sediment macrogenome (Fig. 4), and the discovery of this metabolic pathway also corresponds to the community composition analyses that revealed potential AOB taxa such as Nitrosococcus, Nitrosomonas, and Nitrosospira. Nitrosospira) as potential AOB taxa. In addition, the genes encoding carbonic anhydrase, which converts HCO3- to CO2 in seawater or sediment pore water, were found in the macrogenomic data of FL and PA [57], providing substrate for the Calvin cycle. The discovery of these high abundance of functional genes and potential microbial populations related to autotrophic carbon fixation suggests that autotrophic carbon fixation may be a more active microbial process in the Mariana Trench, which can provide organic carbon compounds with higher activity to the organic carbon pools in the abyssal microbes, whereas FL showed more nitrogen fixation, which demonstrates that heterotrophic microorganisms free-living in the water column have higher nitrogen fixation capacity and are in oligotrophic environments of the seafloor are able to search for needed metabolic energy more autonomously.

ISSN 2616-5872 Vol.6, Issue 3: 20-29, DOI: 10.25236/AJEE.2024.060303

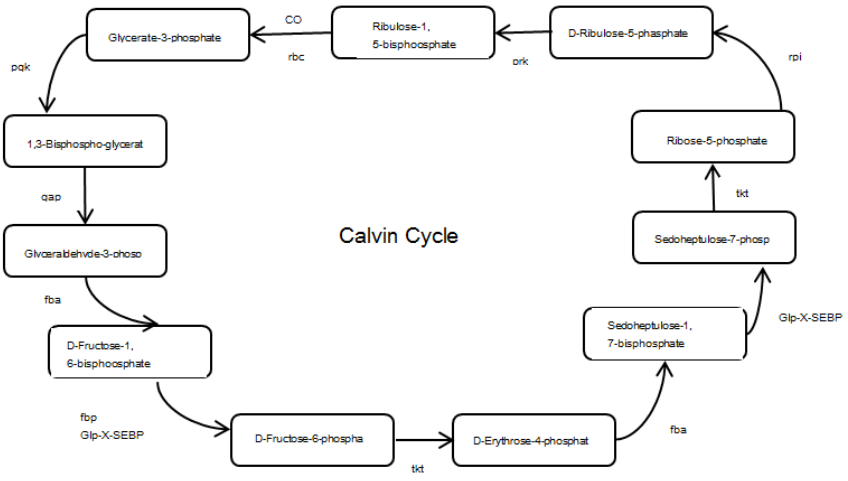


Figure 4: Metabolic pathways of the Calvin cycle

4.4 High Percentage of New Species and Metabolic Potential

The presence of a large number of high-quality sequences not annotated to species at the genus level in the macrogenomic sequences obtained (FL: 43.2%, PA: 44.47%), probably due to the lack of their reference sequences in the current species databases [34], suggests that microorganisms annotated to species in the present study bear a poor resemblance to the downloaded currently stored species databases, i.e., the Mariana Trench, which is where a large number of currently unstudied species are found to be living. microbial communities are present with a large number of currently unstudied species. This finding is in line with previous studies on the population structure of abyssal microorganisms [22], and supports the hypothesis that "geographic isolation and some different environmental extremes can lead to microbial specificity" [58, 59]. However, it remains to be investigated whether these unknown sequences belong to unstudied species. In addition, this paper identified a large number of gene coding sequences that were not annotated in existing functional databases, suggesting that perhaps more potential functions exist for the microbes studied in this paper [57]. What is more, the annotation results of microbial metabolic functions can only indicate that certain metabolic potentials may exist for the current coding sequences, but whether these metabolic potentials are actually present in microbial communities still needs to be verified by biological research methods such as experimental cultures [60].

In summary, heterotrophic microorganisms (e.g., γ-metazoans) are the dominant taxa in the microbial community of the Mariana Trench. In addition, the presence of a large number of genes related to various carbohydrates, hydrocarbons and nitrogen fixation in this macrogenomic sequence suggests that abyssal microbes have the metabolic potential to utilise a wide range of organic matter. This study also shows that the relative abundance of genes related to lipoic acid metabolism, alginate transport and nitrogen fixation in free microorganisms in the Mariana Trench is much higher than that in epiphytic microorganisms, suggesting that autotrophic carbon fixation and autotrophic nitrogen fixation processes may be important in carbon turnover in abyssal free microorganisms. The results confirm the diversity of abyssal microbial metabolism, investigate the species composition of epiphytic and free-living microorganisms and their metabolic characteristics and differences in the Mariana Trench, and demonstrate the potential impact of abyssal microorganisms on elemental cycling (e.g., carbon and nitrogen cycles) in the deep ocean, which is of great significance to the exploration of the life processes of abyssal microorganisms and the internal mechanisms that drive the biogeochemical cycling in the abyssal ocean [61].

5. Conclusion

By comparing the biome functions of two different lifestyles of microorganisms, this paper found that free microorganisms in the deepest part of the Mariana Trench are more resistant to extreme environments, and the metabolisms of free microorganisms in alginate transport and lipoic acid metabolism were found to be significantly higher than those of particulate attached microorganisms,

ISSN 2616-5872 Vol.6, Issue 3: 20-29, DOI: 10.25236/AJEE.2024.060303

which demonstrated that free microorganisms are able to survive more stably in oligotrophic environments, and that the free microorganisms are important in maintaining the composition and function of the abyssal trench biosphere. This finding indicates that free-living microorganisms can survive more stably in oligotrophic environments, suggesting that free-living microorganisms are important in maintaining the composition and function of the abyssal trench biosphere.

This study demonstrates the metabolic differences between free and particle-attached microorganisms in order to graphically explore how microorganisms survive and thrive in extreme environments. The results contribute to a deeper understanding of the distribution of marine microbial community structure and metabolic capacity in different environments, and improve the understanding of biogeochemical cycling and potential microbial-driven mechanisms in abyssal trenches.

References

[1] Mulder T. Gravity processes and deposits on continental slope, rise and abyssal plains. Developments in Sedimentology. 2011, 63: 125-148.
[2] Schrope M. Journey to the Bottom of the Sea. Scientific American, 2014, 310(4): 60-69.
[3] Lloyd K G, May M K, Kevorkian R T, et al. Meta-Analysis of Quantification Methods Shows that Archaea and Bacteria Have Similar Abundances in the Subseafloor. Applied and Environmental Microbiology, 2013, 79(24): 7790-7799.
[4] Fang J S, Zhang L. Exploring the deep biosphere. Science China, 2011, 54(2): 157-165.
[5] Taira K, Kitagawa S, Yamashiro T, et al. Deep and Bottom Currents in the Challenger Deep, Mariana Trench, Measured with Super-Deep Current Meters. Journal of Oceanography, 2004, 60(6): 919-926.
[6] Glud R N, Wenzhofer F, Middelboe M, et al. High rates of microbial carbon turnover in sediments in the deepest oceanic trench on Earth. Nature Geoscience, 2013, 6(4): 284-286.
[7] Luo M, Glud R N, Pan B, et al. Benthic carbon mineralization in hadal trenches: insights from in situ determination of benthic oxygen consumption. Geophysical Research Letters, 2018, 45(6): 2752-2760.
[8] Yayanos A A, Dietz A S, Van Boxtel R. Obligately Barophilic Bacterium from the Mariana Trench. Proceedings of the National Academy of Sciences of the United States of America, 1981, 78(8): 5212-5215.
[9] Kato C, Li L, Nogi Y, et al. Extremely barophilic bacteria isolated from the Mariana Trench, Challenger Deep, at a depth of 11,000 meters. Applied and environmental microbiology, 1998, 64(4): 1510-1513.
[10] Pathom-Aree W, Stach J, Ward A C, et al. Diversity of actinomycetes isolated from Challenger Deep sediment (10,898 m) from the Mariana Trench. Extremophiles, 2006, 10(3): 181-189.
[11] Eloe E A, Shulse C N, Fadrosh D W, et al. Compositional differences in particle-associated and free-living microbial assemblages from an extreme deep-ocean environment. Environmental Microbiology Reports, 2011, 3(4): 449-458.
[12] Nunoura T, Hirai M, Yoshida-Takashima Y, et al. Distribution and Niche Separation of Planktonic Microbial Communities in the Water Columns from the Surface to the Hadal Waters of the Japan Trench under the Eutrophic Ocean. Frontiers in Microbiology, 2016, 7(270): 1261.
[13] Nunoura T, Takaki Y, Hirai M, et al. Hadal biosphere: Insight into the microbial ecosystem in the deepest ocean on Earth. Proceedings of the National Academy of Sciences of the United States of America, 2015, 112(11): 1230-1236.
[14] Tarn J, Peoples Lm, Hardy K, et al. Identification of Free-Living and Particle-Associated Microbial Communities Present in Hadal Regions of the Mariana Trench. Frontiers in Microbiology, 2016, 7: 665.
[15] Liu R L, Wang L, Liu Q F, et al. Depth-Resolved Distribution of Particle-Attached and Free-Living Bacterial Communities in the Water Column of the New Britain Trench. Frontiers in Microbiology, 2018, 9: 625.
[16] Peoples L M, Sierra D, Oladayo O, et al. Vertically distinct microbial communities in the Mariana and Kermadec trenches. PLoS ONE, 2018, 13(4): e0195102
[17] Wang Y, Gao Z M, Li J, et al. Hadal water sampling by in situ microbial filtration and fixation (ISMIFF) apparatus. Deep Sea Research Part I: Oceanographic Research Papers, 2019, 144: 132-137.
[18] Nunoura T, Nishizawa M, Hirai M, et al. Microbial Diversity in Sediments from the Bottom of the Challenger Deep, the Mariana Trench. Microbes and Environments, 2018, 33(2): 186-194.
[19] Hiraoka S, Hirai M, Matsui Y, et al. Microbial community and geochemical analyses of

ISSN 2616-5872 Vol.6, Issue 3: 20-29, DOI: 10.25236/AJEE.2024.060303

transtrench sediments for understanding the roles of hadal environments. The ISME journal, 2020, 14: 740-756.
[20] Cui G J, Li J, Gao Z M, et al. Spatial variations of microbial communities in abyssal and hadal sediments across the Challenger Deep. Peer J, 2019, 7: e6961.
[21] Peoples L M, Grammatopoulou E, Pombrol M, et al. Microbial Community Diversity Within Sediments from Two Geographically Separated Hadal Trenches. Frontiers in Microbiology, 2019, 10: 347.
[22] Liu R L, Wang Z X, Wang L, et al. Bulk and active sediment prokaryotic communities in the Mariana and Mussau trenches. Frontiers in Microbiology, 2020, 11: 1521.
[23] Eloe E A, Fadrosh D W, Mark N, et al. Going Deeper: Metagenome of a Hadopelagic Microbial Community. PLoS ONE, 2011, 6(5): e20388.
[24] Liu J W, Zheng Y F, Lin H Y, et al. Proliferation of hydrocarbon-degrading microbes at the bottom of the Mariana Trench. Microbiome, 2019, 7(1): 47.
[25] Gao Z M, Huang J M, Cui G J, et al. In situ meta-omic insights into the community compositions and ecological roles of hadal microbes in the Mariana Trench. Environmental Microbiology, 2019, 21: 4092-4108.
[26] Zhang X, Xu W, Liu Y, et al. Metagenomics Reveals Microbial Diversity and Metabolic Potentials of Seawater and Surface Sediment From a Hadal Biosphere at the Yap Trench. Frontiers in Microbiology, 2018, 9: 2402.
[27] León-Zayas R, Peoples L, Biddle J F, et al. The metabolic potential of the single cell genomes obtained from the Challenger Deep, Mariana Trench within the candidate superphylum Parcubacteria (OD1). Environmental Microbiology, 2017, 19(7): 2769-2784.
[28] León-Zayas R, Novotny M, Podell S, et al. Single Cells Within the Puerto Rico Trench Suggest Hadal Adaptation of Microbial Lineages. Applied and Environmental Microbiology, 2015, 81(24): 8265-8276.
[29] Wei Z F, Li W L, Huang J M, et al. Metagenomic studies of SAR202 bacteria at the full-ocean depth in the Mariana Trench. Deep Sea Research Part I Oceanographic Research Papers, 2020, 165: 103396.
[30] Huang J M, Wang Y. Genomic differences within the phylum Marinimicrobia: From waters to sediments in the Mariana Trench. Marine Genomics, 2019, 50: 100699.
[31] Bolger A M, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, 2014, 30(15):2114-2120.
[32] Peng Y, Leung H C, Yiu S M, et al. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics, 2012, 28(11):1420-1428.
[33] Hyatt D, Chen G L, Locascio P F, et al. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010, 11(1): 119.
[34] Menzel P, Ng K L, Krogh A. Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nature Communications, 2016, 7: 11257.
[35] Kanehisa M, Sato Y, Morishima K. BlastKOALA and GhostKOALA: KEGG Tools for Functional Characterization of Genome and Metagenome Sequences. Journal of Molecular Biology, 2016, 428(4): 726-731.
[36] Cronan J E. Assembly of Lipoic Acid on Its Cognate Enzymes: An Extraordinary and Essential Biosyn-thetic Pathway. Microbiol Mol Biol Rev. 2016; 80(2):429–450. https://doi.org/10. 1128/ MMBR. 00073-1PMID: 27074917.
[37] Cao X, Koch T, Steffens L, Finkensieper J, Zigann R, Cronan JE, et al. Lipoate-Binding Proteins and Specific Lipoate-Protein Ligases in Microbial Sulfur Oxidation Reveal an Atypical Role for an Old Cofac- tor. elife. 2018; 7:e37439. https://doi.org/10.7554/eLife.37439 PMID: 30004385.
[38] Spalding M D, Prigge ST. Lipoic Acid Metabolism in Microbial Pathogens. Microbiol Mol Biol Rev. 2010;74(2):200–228. https://doi.org/10.1128/MMBR.00008-10 PMID: 20508247.
[39] Dunn M F. Vitamin Formation from Fatty Acid Precursors. In: Geiger O, editor. Biogenesis of Fatty Acids, Lipids and Membranes. Handbook of Hydrocarbon and Lipid Microbiology. Cham: Springer Nature Switzerland AG; 2019. p. 259–271.
[40] Liao D F, Chen J W, Xie P, Zhu Z Q, Xu R. Studies on the antioxidation effects of alpha-lipoic acid and dihydrolipoic acid. Journal of East China Normal University: Natural Science, 2007(2): 87–92, 136.
[41] Cronan J E. Biotin and lipoic acid: synthesis, attachment, and regulation. EcoSal Plus, 2008, 3(1): 10.1128. DOI:10.1128/ecosalplus.3.6.3.5.
[42] Jordan S W, Cronan J E. The Escherichia coli lipB gene encodes lipoyl (octanoyl)-acyl carrier protein: protein transferase. Journal of Bacteriology, 2003, 185(5): 1582-1589. DOI:10.1128/JB. 185.5. 1582-1589.2003.

ISSN 2616-5872 Vol.6, Issue 3: 20-29, DOI: 10.25236/AJEE.2024.060303

[43] Cronan J E. The structure of lipoyl synthase, a remarkable enzyme that performs the last step of an extraordinary biosynthetic pathway. Biochemical Journal, 2014, 464(1): e1-e3. DOI: 10. 1042/ bj20141061.

[44] Morris T W, Reed K E, Cronan J E. Lipoic acid metabolism in Escherichia coli: the lplA and lipB genes define redundant pathways for ligation of lipoyl groups to apoprotein. Journal of Bacteriology, 1995, 177(1): 1-10. DOI:10.1128/jb.177.1.1-10.1995.

[45] Kim D J, Kim K H, Lee H H, Lee S J, Ha J Y, Yoon H J, Suh S W. Crystal structure of lipoate-protein ligase A bound with the activated intermediate: insights into interaction with lipoyl domains. The Journal of Biological Chemistry, 2005, 280(45): 38081-38089. DOI:10.1074/jbc. M507284200.

[46] Harding T. S. History of trehalose, its discovery and methods of p reparation. Sugar. 1923, 25: 476–478.

[47] Jiang Xirui, Huo Xingyun, Huang Jihong, Sun Zhongtao. Biofermentation technology. Beijing: China Light Industry Press. 2016, 326-327.

[48] Koch E M. Koch: The p resence of trehalose in yeast. Marine Genomics. 1925, 61: 570–572.

[49] Elbein A. D. The metabolism of a, a - trehalose. Ad2 vances in Carbohydrate Chemistry and Biochemistry. 1974, 30: 227–256.

[50] Yuan Qinsheng. Progress in the application research of alginate. Food and Drugs. 2005, 7 (4): 1-3.

[51] Xu J Y, Mao Y P. Microbiology Letters. From typical nitrifying bacteria to full-process ammonia-oxidizing microorganisms: discovery and research progress. Microbiology Letters, 2019, 46(4): 202-213.

[52] Hong Y G, He X, Wu J P, et al. Research progress on carbon and nitrogen biogeochemical cycle driven by Marine ammonia-oxidizing archaea. Journal of the University of Chinese Academy of Sciences, 2020, 37(4): 433-441.

[53] Gao J F, Fan X Y, Pan K L, et al. Diversity, abundance and activity of ammonia-oxidizing microorganisms in fine particulate matter. Scientific Reports, 2016, 6: 38785.

[54] Walker C B, De La Torre J R, Klotz M G, et al. Nitrosopumilusmaritimus genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. Proceedings of the National Academy of Sciences of the United States of America, 2010, 107(19): 8818-8823.

[55] Tourna M, Stieglmeier M, Spang A, et al. Nitrososphaeraviennensis, an ammonia oxidizing archaeon from soil.Proceedings of the National Academy of Sciences of the United States of America, 2011, 108(20): 8420-8425.

[56] Yu S L, Qiao Y L, Han Y Q, et al. Differencesbetween ammonia-oxidizing microorganisms in phylogeny and physiological ecology. Microbiology China, 2015, 42(12): 2457-2465.

[57] Verhamme D T, Prosser J I, Nicol G W. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. The ISME Journal, 2011, 5(6): 1067-1071.

[58] Liu R L, Wang L, Wei Y L, et al. The hadal biosphere: Recent insights and new directions. Deep-Sea Research Part II: Topical Studies in Oceanography, 2018, 155: 11-18.

[59] Jamieson A J, Fujii T, Mayor D J, et al. Hadal trenches: the ecology of the deepest places on Earth. Trends in Ecology and Evolution, 2010, 25(3): 190-197.

[60] Wang H L, Guo A Y. An Introduction to Metagenome Databases of Environmental Microbiology. Biotechnology Bulletin, 2015, 31(11): 78-88.

[61] Choo Kwang Raymond. Pesticide Pollution Affects the Functional Diversity of Soil Microbial Community. Academic Journal of Environmental Biology, 2020, 1(2): 44-53.