Taxonomic and Functional Metagenomic Profile of Sediment From a Commercial Catfish Pond in Mississippi

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Metagenomic analyses of microbial communities from aquatic sediments are relatively few, and there are no reported metagenomic studies on sediment from inland ponds used for aquaculture. Catfish ponds in the southeastern U.S. are eutrophic systems. They are fertilized to enhance algae growth and encourage natural food production, and catfish are fed with commercial feed from spring to fall. As result, catfish pond sediment (CPS) contains a very dense, diverse microbial community that has significant effects on the physiochemical parameters of pond dynamics. Here we conducted an in-depth metagenomic analysis of the taxonomic and metabolic capabilities of a catfish pond sediment microbiome from a southeastern U.S. aquaculture farm in Mississippi using Illumina next-generation sequencing. A total of 3.3 Gbp of sequence was obtained, 25,491,518 of which encoded predicted protein features. The pond sediment was dominated by Proteobacteria sequences, followed by Bacteroidetes, Firmicutes, Chloroflexi, and Actinobacteria. Enzyme pathways for methane metabolism/methanogenesis, denitrification, and sulfate reduction appeared nearly complete in the pond sediment metagenome profile. In particular, a large number of Deltaproteobacteria sequences and genes encoding anaerobic functional enzymes were found. This is the first study to characterize a catfish pond sediment microbiome, and it is expected to be useful for characterizing specific changes in microbial flora in response to production practices. It will also provide insight into the taxonomic diversity and metabolic capabilities of microbial communities in aquaculture. Furthermore, comparison with other environments (i.e., river and marine sediments) will reveal habitat-specific characteristics and adaptations caused by differences in nutrients, vegetation, and environmental stresses.

Keywords: metagenome, sediment, aquaculture pond, catfish, eutrophic, nitrogen metabolism, sulfur metabolism, methanogenesis

INTRODUCTION

Channel catfish production is the largest aquaculture industry in the United States with total sales of $380 million in 2017 (USDA, 2018). Aquaculture is one of the most rapidly growing food production sectors (Food Agriculture Organization of the United Nations and Fisheries and Aquaculture, 2014), but it faces several production and environmental challenges to maintain its
economic viability (Schreier et al., 2010). Fish production ponds are rich in dissolved nutrients due to intensive feeding and fecal waste. Unconsumed feed, fish feces, and senescent phytoplankton are deposited into aquaculture sediments, which can enhance the microbial flora in the sediments and lead to anoxic conditions (Holmer and Kristensen, 1992). Medicated feed, such as Terramycin (oxytetracycline), Romet-30 (sulfadimethoxine-ormetoprim), and Aquaflo (florfenicol), as well as fertilizers may also impact pond sediment microflora. In turn, changes in sediment microflora impact fish health in aquaculture systems.

Fish excrete nitrogen in the form of ammonia, which is toxic to fish; nitrification by chemolithoautotrophic bacteria in pond sediments is an important process that prevents toxic buildup. The microbes contributing to nitrification consist of two functional groups: the ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) that convert toxic ammonia to nitrite, and the nitrite-oxidizing bacteria (NOB) that oxidize nitrite to the less toxic nitrate. AOB include the genera Nitrosomonas, Nitrosococcus, and Nitrosospira (Kowalchuk and Stephen, 2001). AOA include Nitrospumilus from marine water (Könneke et al., 2005). The NOB group includes genera Nitrobacter, Nitrospira, and Nitrospona (Wang et al., 2014). Pond sediments also have potential to serve as a reservoir for fish pathogens. Therefore, it is important to understand microbial flora in aquaculture pond sediments because of its impact on the pond ecosystem, especially in nutrient cycling, concentrations of organic and inorganic nutrients and toxins, and effects on fish health.

Metagenomic analysis allows assessment of mixed environmental microbial communities by directly sequencing DNA from environmental samples (Dinsdale et al., 2008). This approach provides a picture of the diversity and microbiome structure present in the environment (Simon and Daniel, 2009), and it enables studies to understand how microbial diversity is modulated in response to environmental or anthropogenic impacts (Larsen et al., 2012). Thus, it is particularly appropriate for assessing the taxonomic and functional microbial diversity in a pond sediment biome and monitoring community changes over space and time (Nogales et al., 2011).

Previous culture-independent studies investigating sediment microbial phylogenetic structure showed that microbial communities are indicators of both the physicochemical status of freshwater sediments (Logue et al., 2008; Gibbons et al., 2014) and ecological degradation (Feris et al., 2009). A few metagenomes have been published from deep-sea sediment (Kimes et al., 2013), mangrove (Andreote et al., 2012), and river systems (Staley et al., 2013), and recent studies have examined the response of fish gut-associated microbial communities from aquaculture systems in response to lifestyle and dietary preference (Wu et al., 2010; Xing et al., 2013). Signatures of bacterial composition were found in shrimp farming (Sousa et al., 2006), and pyrosequencing was used to explore bacterial diversity and detect potential fish pathogens during production of Scophthalmus maximus (turbot) and Solea senegalensis (sole) (Martins et al., 2013).

In pond sediments, the microbiome is critical for maintenance of homeostasis conditions, including toxin removal and cycling of carbon, nitrogen, and phosphorus (Brock, 1970; Leung et al., 1994). In particular, nitrogen is very important in aquaculture as a nutrient and potential toxicant, and it is an essential requirement for phytoplankton and bacterial growth in anaerobic conditions. In the current study, we present a description of the microbiome found in sediment from a catfish research pond maintained under commercial production conditions. Our results are culture-independent and based on metagenomic sequence of total DNA extracted directly from the pond sediment and analyzed by Illumina sequencing. We describe the microbial taxa present in catfish pond sediment and the potential metabolic processes that appear to be occurring in this ecosystem affecting carbon, nitrogen, and sulfur cycling. This analysis also provided a fundamental baseline profile of the catfish pond sediment microbiome for comparison with sediments from other environments.

**MATERIALS AND METHODS**

**Sediment Sampling, DNA Extraction, and Sequencing**

Sediment sampling was performed in October 2012 (water temperature approximately 20°C) in sediments from a 0.8-hectare aquaculture research pond stocked with approximately 6,000–8,000 catfish (Ictalurus punctatus) with average size of 0.5 kg at the Delta Research and Extension Center, Stoneville, MS, United States. The pond was maintained at typical stocking and feeding parameters used for catfish aquaculture. Three samples were collected at 10 am using a modification of a previously published method (Schneegurt et al., 2003). In brief, ∼500 g per sample of pond sediment was collected at 5–10 cm of sediment depth ∼8 m from the pond bank in a water depth of ∼1.2 m using a sterile spatula and immediately transferred to sterile 50 ml tubes kept on ice.

Genomic DNA was extracted from each sediment sample separately using MoBio PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, United States) according to manufacturer’s protocol with 5.0 g of sediment per extraction. A NanoDrop (Thermo Scientific, Wilmington, DE, United States) spectrometer was used to quantify extracted DNA and to assess DNA quality. Illumina library preparation and sequencing followed manufacturer’s protocols (Illumina, San Diego, CA, United States). Briefly, genomic DNA was sheared by sonication and separated by electrophoresis on a 1% agarose gel. Gel slices corresponding to ∼300 and ∼500 bp were excised and purified using QIAquick Gel Extraction Kit (Qiagen). The blunt-ended DNA fragments were A-tailed using the Quick Blunting Kit (New England BioLabs) and purified. Sheared DNA was ligated to sequence adapters. All ligated libraries were enriched using standard PCR (11–12 cycles) with Illumina paired end primers before library quantification and validation. One microgram DNA was used in library construction. The amplified libraries were pooled in an equimolar ratio and sequenced using an Illumina HiSeq 2000 (Illumina, San Diego, CA, United States) using paired end reads of 100 bases. Raw reads containing three or more “N” bases or contaminated by adapter (>15 bp overlap)
were removed by Trimmomatic (Bolger et al., 2014), and the filtered clean reads were used for metagenomic analysis.

**Taxonomic Distribution and Functional Analysis of Metagenomic Sequence**

The taxonomic analysis was performed using BLASTX against the SEED and Pfam databases (Altschul et al., 1997) on the MG-RAST server1 using a cut-off E-value of 1e-5, minimum identity of 60%, and a minimum alignment length of 15 bp (Meyer et al., 2008). BLASTX was also conducted using MetaGenome Analyzer software (MEGAN v5) with the lowest common ancestor (LCA) algorithm used to visualize results (Huson et al., 2007). Using BLASTX and BLASTN, reads were compared against the NR and NT NCBI databases. Analysis was performed comparing distinct hierarchical levels, and a directed homogeneity test was used to identify significant differences in sample comparisons. Multiple testing correction analysis was not applied, and all unassigned reads were ignored.

Statistical analysis was performed using results from the MG-RAST annotation system, and results were visualized using Statistical Analyses of Metagenomic Profiles (STAMP) (Parks and Beiko, 2010) to detect biologically relevant differences in the relative proportion of sequences. Paired metagenomic samples were used for the analysis, and statistical significance of the differences between samples was assessed by the Two-sided Fisher’s Exact test. Story’s false discovery rate (FDR) was used for multiple test correction as recommended by STAMP. Results with q-value (<0.05) were considered significant, and unclassified reads were removed from the analysis.

Functional classification was conducted using BLASTX (cut-off E-value of 1e-5) against COGs (Tatusov et al., 2001), which was downloaded from hierarchical classification in MG-RAST server using NCBI database. BLASTX and subsystem analysis were used against the SEED-NR database in MG-RAST for functional sequence annotation with the same parameters as a taxonomic distribution. A functional analysis using the SEED (Overbeek et al., 2005) and KEGG (Kanehisa et al., 2004) databases was conducted using MG-RAST sever. Each sequence was associated with its SEED functional role using the best BLAST score to protein sequences without known functional roles. A similar procedure was used to match each sequence to a KEGG orthology (KO) accession number. Results were matched with each protein’s RefSeq database, and relative abundance was used to identify enzymes in important metabolic pathways. Matches with alignment scores higher than 80 were retained.

To the best of our knowledge, there is no previous information about structure and function of bacterial communities in aquaculture pond sediments. Therefore, the microbial community from aquaculture sediment was compared to freshwater sediment from the Tongue River in Southeastern Montana (MG-RAST ID 4481977.3) (Gibbons et al., 2014) and deep-sea sediment of the Gulf of Mexico (MG-RAST ID 4465489.3) (Kimes et al., 2013). A classification was used to determine the sample that most closely clustered to the taxonomic composition or metabolic potential of the sediment metagenome (E-value of 1e-5, the minimum identity of 60%, and a minimum alignment length of 15 bp).

**RESULTS AND DISCUSSION**

**Sequence Generation**

Whole community microbial DNA from catfish pond sediment (CPS) was sequenced, making this study the first metagenomic survey of a catfish aquaculture environment (MG-RAST ID 4583113.3). Of the 29,278,265 sequences (totaling 2,927,826,500 bps) that passed quality control, 3,690,631 sequences (11.2% of total) were identified as artificial duplicate reads (ADRs), which are nearly identical sequences that result from sequencing two or more copies of the exact DNA fragment (Niu et al., 2010). Of the sequences without rRNA genes, 26,172,903 contained predicted protein features, 4,424,138 (16.9%) of which were assigned an annotation using at least one protein database, and 21,703,765 (82.9% of features) contain predicted proteins with unknown function. A total of 2,855,527 sequences (64.5% of annotated proteins) were assigned to functional categories (Supplementary Table S1). At the domain level, Bacteria (96.8%) dominated, while the Archaea (1.2%) and the Eukaryota (1.5%) contributed substantially less to the CPS community.

**Taxonomic Profiles**

The numbers of sequences affiliated with each bacterial taxon in CPS were similar with other sediment samples, with a dominance of Proteobacteria (46.0%) and an abundance of Bacteroidetes (15.9%), Firmicutes (8.6%), Chloroflexi (5.1%), and Actinobacteria (5.0%) (Table 1). Minor groups represented at the phylum level included Cyanobacteria, Verrucomicrobia, Planctomycetes, Chlorobi, and Acidobacteria. The four most abundant classes

**TABLE 1** | Bacterial classifications and abundance in the CPS metagenome.

| Taxonomic group       | Number of reads | Abundance (%) | Number of genera |
|-----------------------|-----------------|---------------|------------------|
| Proteobacteria (phylum)| 2,971,341       | 46.0          | 66               |
| Delta proteobacteria  | 1,408,697       | 21.8          | 66               |
| Betaproteobacteria    | 596,878         | 9.2           | 87               |
| Gammaproteobacteria   | 501,097         | 7.8           | 165              |
| Alphaproteobacteria   | 415,930         | 6.4           | 119              |
| Epsilonproteobacteria | 36,824          | 0.6           | 13               |
| Bacteroidetes (phylum)| 1,027,970       | 15.9          | 8                |
| Bacteroidia           | 415,272         | 6.2           | 29               |
| Flavobacteria         | 254,455         | 3.9           | 10               |
| Cytophagia            | 184,562         | 2.9           | 10               |
| Spingobacteria        | 130,637         | 2.0           | 6                |
| Firmicutes (phylum)   | 557,836         | 8.6           | 70               |
| Clostridia            | 360,863         | 5.6           | 43               |
| Bacilli               | 167,236         | 2.6           | 3                |
| Chloroflexi (phylum)  | 331,544         | 5.1           | 4                |
| Chloroflexi           | 132,079         | 2.1           | 106              |
| Actinobacteria (phylum)| 326,195       | 5.0           | 4                |
| Actinobacteria        | 326,195         | 5.0           | 106              |

1http://metagenomics.anl.gov/
of bacteria were *Deltaproteobacteria, Bacteroidia, Clostridia,* and *Actinobacteria* according to MG-RAST analysis (Table 1).

The *Proteobacteria* associated with each sample were examined more closely to evaluate the potential of both aerobic and anaerobic biodegradation. In aquaculture ponds and lakes, the top sediment layer down to a few millimeters is typically aerobic, but below this depth sediment is anaerobic (Boyd and Tucker, 1998). The high occurrence of *Deltaproteobacteria* in CPS, which is not commonly observed in metagenomes from water or sediment samples (Figure 1), might be related to the catfish habitat, where eutrophic and anaerobic conditions could drive selection for specific microbial groups such as sulfate associated bacteria (Harrison et al., 2009). The *Deltaproteobacteria* in our sample was mostly comprised of a branch of strictly anaerobic genera containing many of the known sulfate- and sulfur-reducing bacteria including *Desulfovibrio, Desulfobacter, Desulfovoccus, Desulfonema,* and *Desulfuromonas* spp. High organic loads and anaerobic conditions in pond sediments yield ideal conditions for sulfate reduction and sulfide production (Boyd and Tucker, 1998). Many of the *Deltaproteobacteria* were also species involved in methane transformation, which was paralleled by the presence of other bacteria with anaerobic physiology such as ferric iron-reducing *Geobacter* spp. (Supplementary Table S2).

*Deltaproteobacteria,* in particular, are capable of fumarate addition to both aromatic and aliphatic hydrocarbons, hence activating the anaerobic hydrocarbon biodegradation pathway. The increases in *Deltaproteobacteria* correlated with an increase in other protein-coding genes involved in anaerobic degradation of hydrocarbons, such as benzylsuccinate synthase (BSS), acetyl-CoA acyltransferase, and benzoyl-CoA reductase (Kimes et al., 2013). Anthropogenic hydrocarbon loading from catfish feed may enrich for microbial species with anaerobic hydrocarbon degradation capabilities. Other types of *Proteobacteria,* including *Beta-,* *Gamma-,* and *Alphaproteobacteria* had relatively lower representation compared with microbial populations from other aquatic sediments from river or deep sea environments (Figure 1). Moreover, the CPS had a higher prevalence of *Epsilonproteobacteria* than the river and deep-sea sediments. *Epsilonproteobacteria* are prevalent in the digestive tracts of animals and serve as symbionts or pathogens; their energy metabolism involves oxidizing reduced sulfur, formate, or hydrogen coupled with the reduction of nitrate or oxygen (Takai et al., 2005).

The phylum *Bacteroidetes* is very diverse and includes *Cytophaga, Flexibacter,* and *Bacteroides* (Woese, 1987; Woese et al., 1990). The *Bacteroidetes* phylum is comprised of four classes: *Bacteroidia, Flavobacteria, Cytophage,* and *Sphingobacteria,* which include around 7,000 different species (Bergey et al., 2011). The *Bacteroidetes* phylum in CPS included the *Flavobacteria,* which has many aquatic species (Table 1), and it also contained opportunistic human pathogens (Bernardet and Nakagawa, 2006), including the genera *Elizabethkingia, Weeksella* and *Capnocytophaga* (Kim et al., 2005; Leadbetter, 2006) (Supplementary Table S2). *F. psychrophilum, F. columnare,* and *F. branchiophilum* are some *Bacteroidetes* species that have economic impacts on freshwater fish, causing infections that can have severe effects on farmed and wild fish (Hawke and Thune, 1992; Loch and Faisal, 2015). *Flavobacterium* infections were first reported a century ago in aquaria (Davis, 1922).

Previously, *Firmicutes* was observed as a dominant phylum in the digestive tract of many marine and freshwater fish species (Austin, 2006; Wu et al., 2012). In the present study, *Firmicutes* was found to be prevalent in CPS. In particular, *Clostridium* sp. was the most abundant genus within *Firmicutes* and represented 5.6% of the identified sequences (Table 1), making it more abundant in CPS compared to marine and river sediments (Figure 1). *Clostridium* sp. are commonly found in human and animal guts and can form endospores, allowing survival under unfavorable environments (Davies et al., 1995; Mueller-Spitz et al., 2010). *Clostridium* sp. contribute to hydrolytic enzyme production, suggesting a possible role in degradation of organic matter. In eutrophic marine cage aquaculture sediments, metabolism is dominated by anaerobic decomposition (Holmer and Kristensen, 1992); therefore, enrichment of *Clostridium* sp. may be a good indicator of the impact of organic matter in aquatic sediments. Interestingly, some lactic acid bacteria were detected in the *Firmicutes* phylum in CPS, including the genera *Lactococcus, Streptococcus,* and *Enterococcus* (Supplementary Table S2). Lactic acid bacteria are generally considered to be non-pathogenic (Ringo and Gatesoupe, 1998). However, some species including *Lactococcus garvieae, Streptococcus shiloi,* and *Streptococcus difficile* were reported as fish pathogens (Eldar et al., 1994, 1996).

The highest proportion of archaean reads within the CPS metagenome was *Euryarchaeota* (87.6%), which was composed of several classes: *Archaeglobi* (1,159 reads), *Halobacteria* (3,001 reads), *Methanobacteria* (1,956 reads), *Methanococci* (2,334 reads), *Methanomicrobia* (12,467 reads), and *Thermococci* (1,593 reads) (Figure 2). *Methanosarcina* was the most abundant genus and accounted for 31% of the total archaeal sequences. This genus includes many methanogens involved in both acetotrophic and hydrogenotrophic methanogenesis, which is a type of anaerobic respiration that generates methane and is considered the terminal step in organic decomposition. Most *Methanosarcina* are non-motile and mesophilic, and they are unique among archaeal bacteria in that most can utilize multiple substrates as electron acceptors, including methanol (Kandler and Hippe, 1977). Thus, *Methanosarcina* is among the most adaptable and flexible of the methanogens (Maeder et al., 2006).

The eukaryotic sequences represented 23 phyla from the *Animalia,* *Fungi,* *Plantae,* and *Protista.* The *Animalia* phylum *Chordata* (20.76 – 28.52%) showed predominant abundance in the three sediments, followed by *Aithropoda, Ascomycota,* and *Steptophyta.* *Chordata* was the most abundant in deep-sea sediment, but the *Aithropoda* was lower compared to CPS and river sediment. The *Bacillariophyta, Cnidaria,* and *Echinodermata* phyla had greater abundance in CPS compared to river sediment, and *Apicomplexa* was in greater abundance in river sediment (Figure 3).

**Functional Categories**

Environmental DNA from CPS had matches in 24 COG and 28 KEGG functional categories, respectively.
The dominant COG functions were prokaryotic, with high abundance of sequence reads in energy production and conversion as well as amino acid transport and metabolism. In particular, CPS had a high number of sequences in signal transduction mechanisms, carbohydrate transport and metabolism, inorganic ion transport and metabolism, and general function prediction. A lower percent of reads was found for functions associated with eukaryotic organisms (RNA procession and modification, chromatin structure and dynamics, cell motility, and cytoskeleton and extracellular structures) (Figure 4A). The most abundant KEGG functional categories were carbohydrate metabolism, clustering-based subsystems, miscellaneous, amino acids and derivatives, and protein metabolism (Figure 4B).

The metabolic potential of the CPS metagenome was compared with two freshwater and deep-sea sediment metagenomes publicly available on the MG-RAST server. A heat map showed that the CPS metagenome is similar to the deep-sea sediment metagenome. CPS had more sequences within phosphorous metabolism, protein metabolism, and membrane transport than the other aquatic sediments (Figure 5). Phosphorus is relatively abundant in catfish ponds because producers often fertilize ponds to encourage algal blooms, and protein is also relatively abundant due to application of commercial feeds.

Based on our taxonomic analysis, we expected to detect genes encoding enzymes involved in methanogenesis, which is considered the final step in decomposition. As expected, we detected methanogenesis genes encoding an F420-dependent $N(5),N(10)$-methylenetetrahydromethanopterin reductase (EC 1.5.99.11), $N(5),N(10)$-methylenetetrahydromethanopterin cyclohydrolase (EC 3.5.4.27), formylmethanofuran dehydrogenase (EC1.2.99.5), CoB–CoM heterodisulfide reductase (EC1.8.98.1), coenzyme F420 hydrogenase (EC...
The transformation of methane and methanol into formaldehyde and then formate in CPS was suggested by metabolic reconstruction, mainly from the activity of particulate methane monoxygenase (EC 1.14.13.25), methanol dehydrogenase (EC 1.1.99.8), and S-formylglutathione hydrolase (EC 3.1.2.12). We also detected genes encoding enzymes involved in formaldehyde fixation, including enzymes for enzymes that incorporate methane into organic compounds via the serine pathway or the ribulose monophosphate pathway, indicating 1.12.98.1), N5-methyltetrahydromethanopterin (EC 2.1.1.86), and the enzyme responsible for the last step of methanogenesis, methyl coenzyme M reductase (EC 2.8.4.1) (Table 2).
that the CPS microbiome is able to metabolize methane as their source of carbon and energy to survive. Compared to the metagenome of freshwater sediment from the Tongue River, both CPS and the freshwater sediment microbiomes encode processes for formaldehyde fixation; the distinct sediments differ only in the particular pathways used. In CPS, methane metabolism is encoded by the *Gammaproteobacteria* using the ribulose monophosphate pathway to assimilate carbon; in addition, part of the *Alphaproteobacteria* utilized the serine pathway of carbon assimilation. Oxidation of formate yields carbon dioxide, and a high abundance of genes encoding proteins involved in the conversion of carbon dioxide into carbon monoxide and later into acetyl-CoA was detected (Figure 6A). Formate also contributes to oxidative stress response; oxidase stress catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1.7) were detected in CPS, both of which are antioxidant enzymes that contribute to limiting oxidative damage by reactive oxygen species (ROS) such as H$_2$O$_2$ (Table 2).

Analysis of nitrogen metabolism revealed genes encoding nitrogen immobilization and mineralization in CPS (Figure 6B). Sequences encoding nitrogen fixation were detected, including atmospheric nitrogen fixation using a nitrogenase (EC 1.18.6.1) that converts nitrogen gas to ammonia. A high abundance of nitrification species was detected in the metagenome (Supplementary Table S3) such as *Nitrosomonas* spp., *Nitrobacter* spp., and *Nitrooccus* spp. These species oxidize ammonia to nitrate, preventing toxic buildup of ammonia that can affect fish health. Genes encoding nitrification such as hydroxylamine oxidoreductase (EC 1.7.3.4) for oxidation of hydroxylamine were found. Genes encoding denitrification enzymes, including nitrate reductase (EC 1.7.99.4), nitrite reductase (EC 1.7.1.4, EC 1.7.7.1 and 1.7.2.1), cytochrome c552 precursor (EC 1.7.2.2), nitric-oxide reductase (EC 1.7.99.7) and nitrous-oxide reductase (EC 1.7.99.6) were observed (Table 2). Denitrification is the dissimilatory reduction of nitrate into nitric oxide, dinitrogen oxide, and nitrogen. The balance among these pathways is affected greatly by environmental conditions including oxygenation, temperature, nitrate concentration, and organic matter content in the sediment (Saunders and Kalff, 2001).

The predominant type of sulfur metabolism encoded in the CPS generates the reductive form of sulfite and hydrogen sulfide (H$_2$S) (Figure 6C). Most of genes observed were involved
TABLE 2 | Numbers of represented gene variants in the CPS metagenome for different functions.

| Metabolism Function | Methane Function | No of sequence reads | No of genes |
|---------------------|------------------|----------------------|-------------|
| Particulate methane monooxygenase (EC 1.14.13.25) | Oxidation of ammonia, methane, halogenated hydrocarbons, and aromatic molecules | 20 | 11 |
| Methanol dehydrogenase (EC 1.1.99.8) | Methanol to formaldehyde. | 271 | 41 |
| S-formylglutathione hydrolase (EC 3.1.2.12) | S-formylglutathione to formic acid and glutathione | 73 | 44 |
| F420-dependent reductase (EC 1.5.99.11) | CO₂ to methane | 209 | 25 |
| N(5), N(10)-methenyltetrahydromethanopterin cyclohydrolase (EC 3.5.4.27) | CO₂ to methane | 30 | 12 |
| Formylmethanofuran dehydrogenase (EC1.2.99.5) | CO₂ and methanofuran to N-formylmethanofuran. | 229 | 77 |
| CoB–CoM heterodisulfide reductase (EC1.8.98.1) | Reduction of the heterodisulfide of the methanogenic thiol-coenzymes, coenzyme M, and coenzyme B | 5,015 | 274 |
| Coenzyme F420 hydrogenase (EC 1.12.98.1) | CO₂ to methane | 59 | 22 |
| N5-methyltetrahydromethanopterin (EC 2.1.1.86) | Transfer of the methyl group from N5-methyltetrahydromethanopterin to coenzyme M | 83 | 44 |
| Methyl-coenzyme M reductase (EC 2.8.4.1) | Methyl-coenzyme M and coenzyme B to methane (anaerobic oxidation). | 73 | 25 |
| Nitrogen | Nitrogenase (EC 1.18.6.1) | Nitrogen to ammonia | 2,226 | 172 |
| Nitrate reductase (EC 1.7.99.4) | Nitrate to nitrite | 3,573 | 456 |
| Nitrite reductase (1.7.1.4, EC 1.7.7.1 and 1.7.2.1) | Reduction of nitrite | 1,517 | 270 |
| Cytochrome c552 precursor (EC 1.7.2.2) | Nitrite to ammonia | 2,418 | 104 |
| Nitric-oxide reductase (EC 1.7.99.7) | Nitric oxide to nitrous oxide | 3,067 | 134 |
| Nitrous-oxide reductase (EC 1.7.99.6) | Nitrous oxide to dinitrogen | 1,027 | 49 |
| Hydroxylamine reductase (EC 1.7.3.4) | Hydroxylamine to ammonia and water | 4 | 3 |
| Sulfur | Sulfate adenylyltransferase (EC 2.7.7.4) | Transfer of the adenylyl group from ATP to inorganic sulfate, generating adenosine 5′-phosphosulfate and pyrophosphate. | 7,133 | 884 |
| Adenylyl sulfate kinase (EC 2.7.1.25) | Catalyze the synthesis of activated sulfate | 2,144 | 276 |
| Phosphoadenylyl sulfate reductase (EC 1.8.4.8) | Reduction of activated sulfate into sulfite | 413 | 122 |
| Adenylyl sulfate reductase (EC 1.8.99.2) | Adenosine 5′-phosphosulfate (APS) to sulfite and AMP | 1,186 | 50 |
| Sulfite reductase (EC 1.8.99.1, 1.8.12 and 1.8.7.1) | Sulfite to sulfide | 1,559 | 305 |
| Other | Oxidase stress catalase (EC 1.11.1.6) | Hydrogen peroxide to water and oxygen | 5,846 | 454 |
| Peroxidase (EC 1.11.1.7) | Oxidation of organic compounds | 5,183 | 269 |

In conversion of sulfate into adenylylsulfate and to sulfite and H₂S, including sulfite adenylyltransferase (EC 2.7.7.4), adenylylsulfate kinase (EC 2.7.1.25), phosphoadenylyl sulfate reductase (EC 1.8.4.8), adenylylsulfate reductase (EC 1.8.99.2), and sulfite reductase (EC 1.8.99.1, 1.8.12 and 1.8.7.1) (Table 2). Enzymes mediating reduction of sulfate and adenylylsulfate into H₂S dominated sulfur metabolism in CPS (Figure 6C). Generated H₂S can influence the reductive carboxylate cycle (CO₂ assimilation) and might be released by volatilization, producing the typical smell of mangrove swamps (Andreote et al., 2012). Sulfate-reducing bacteria obtain energy by oxidizing organic matter or hydrogen using sulfate (or other sulfur molecules) as electron acceptors, yielding H₂S. They are prevalent in environments such as swamps and standing waters that have low oxygen. Sulfur-reducing bacteria and some archaea are similar, but they use elemental sulfur as an electron acceptor, and they also produce H₂S. During catabolism of organic matter, hydrogen sulfide is also released by other anaerobic bacteria when sulfur-containing amino acids are digested. In CPS, Deltaproteobacteria was the most abundant, possibly indicating the importance of sulfate reduction in this environment (Wrighton et al., 2014).

Overall, the metabolism of carbon, nitrogen, and sulfur are interlinked within the microbial population, and this is particularly true for the metabolism of sulfur and carbon. The abundance of organic matter in the anaerobic environment of CPS yields an optimal environment for several anaerobic bacteria such as sulfate-reducing bacteria and methanogens (Dar et al., 2008). These groups share similar environmental niches, and their relative abundance is controlled by substrate availability (Oremland and Polcin, 1982). Simple substrates are important for methanogens, while sulfate-reducing bacteria are capable of degrading more complex substrates, including long chain and aromatic hydrocarbons (Muyzer and Stams, 2008).
FIGURE 6 | Part of a SEED-based functional analysis of the CPS metagenome. (A) Carbon fixation and methane metabolism; (B) nitrogen metabolism; and (C) sulfur metabolism. Blue boxes are proteins that were represented in CPS.
Genes encoding resistance to antibiotics and toxic compounds (RATC) represents a subset of virulence genes that made up 3.45% of the classified metagenome in CPS. By comparison, genes encoding RATC proteins generally make up ∼2–2.24% of the classified metagenome in other aquatic ecosystems. Compared to other aquatic sediments, the CPS metagenome encoded a higher proportion of proteins in copper homeostasis, cobalt-zinc-cadmium resistance, multidrug resistance efflux pumps, and resistance to fluoroquinolones; however, the CPS metagenome encoded a lower proportion of arsenic resistance, beta-lactamase, erythromycin resistance, methicillin resistance, and resistance to vancomycin (Table 3). In particular, genes encoding cobalt-zinc-cadmium energy-dependent efflux pump had the highest proportion in the RATC category in CPS. By contrast, the fish gut microbiome encodes a much higher proportion of proteins in copper homeostasis, multidrug resistance efflux pumps, and resistance to fluoroquinolones; however, the CPS metagenome compared to other aquatic sediments, and it demonstrates capability of anaerobic hydrocarbon metabolism.

Function | No. of sequences† | No. of hits‡
--- | --- | ---
Adaptation to d-cysteine (catalyze the transformation of d-cysteine into pyruvate, H2S, and NH3) | 63 | 41
Aminoglycoside adenyltransferases (confers resistance to kanamycin, gentamicin, and tobramycin) | 13 | 12
Arsenic resistance | 5,506 | 598
Beta-lactamase (inactivates beta-lactam antibiotics including penicillins and cephalosporins) | 5,912 | 1,112
Bile hydrolysis | 97 | 47
BlaR1 family regulatory sensor (controls expression of beta-lactamase) | 9,705 | 1,257
Cadmium resistance | 195 | 65
Cobalt-zinc-cadmium resistance | 58,584 | 3,252
Copper homeostasis | 12,481 | 1,788
Erythromycin resistance | 352 | 147
Mercury resistance operon | 360 | 67
Methicillin resistance in Staphylococci in Staphylococci | 3,029 | 656
MexE-MexF-OprN multidrug efflux system | 499 | 67
Multidrug resistance efflux pumps | 23,910 | 1,881
Resistance to vancomycin | 129 | 64
Resistance to chromium compounds | 519 | 106
Resistance to fluoroquinolones | 21,073 | 1,744
The mdt/ABCD multidrug resistance cluster | 954 | 204
Zinc resistance | 5,771 | 204

†The number of sequences that contain a given annotation. ‡No. of hits refer to the number of unique database sequences that were found in the similarity search without double counting.

DNA gyrase subunit B gene and topoisomerase IV subunit A were most frequently associated with *Clostridia, Actinobacteria, Bacteroidetes* and with *Proteobacteria* in the CPS metagenome, but whether they carry mutations associated with resistance is not currently known. Beta-lactamase genes were most frequently associated with *Alpha* and *Gammaproteobacteria*, which encode resistance to beta-lactam antibiotics (penicillins and cephalosporins). The true risk to public health from antimicrobial use and subsequent resistance in aquaculture is speculative. However, the presence of antibiotic resistance genes and elements in pond sediments is a threat to public health if the resistance genes are transferrable to clinically significant pathogens.

Of these RATC categories, beta-lactamase resistance, multidrug resistance efflux pumps, fluoroquinolone resistance, cobalt/zinc/cadmium resistance, and acriflavine resistance genes are present in other metagenomes such as lake sediment, soil, feces, and marine environment (Durso et al., 2012). This broad distribution across agricultural, environmental, and human-associated samples indicates that these mechanisms are generally distributed and suggests they are functionally important in diverse habitats.

CONCLUSION

Catfish pond sediment is a highly eutrophic, nutrient-rich, and diverse ecosystem that has significant effects on the physiochemical parameters of pond dynamics. This study is a pacesetting metagenomics analysis using Illumina sequencing for sediment in an intensive aquaculture system, and it revealed significant coupling between phylogeny and functional potential. The community structure suggests that the distribution of particular taxa is driven by their metabolic capabilities in response to the environment. For example, *Deltaproteobacteria* was the most abundant class, which is unique in the CPS metagenome compared to other aquatic sediments, and it demonstrates capability of anaerobic hydrocarbon metabolism. Functionally, our analysis revealed that the metagenome likely has significant impacts on nitrogen, phosphorus, and sulfur dynamics in catfish production ponds.

Further work to assess pond sediment could yield a complete description of the potential metabolic pathways in the CPS metagenome. The current work establishes a critical baseline for the CPS metagenome for comparison with other distinct environments. Also, future work could assess the effects of environmental changes (for example, feeding changes or antimicrobial use) in catfish production ponds on the sediment metagenome.

AUTHOR CONTRIBUTIONS

ML and MM supervised the study. ML, MM, and AK designed the experiments. HA, SN, SP, DP, and MM performed the experiments. SN, SP, and ML analyzed and interpreted the data. All authors wrote and approved the manuscript.
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REFERENCES

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., et al. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402. doi: 10.1093/nar/25.17.3389

Andreote, F. D., Jimenez, D. J., Chaves, D., Dias, A. C., Luviottzo, D. M., Dini-Andreote, F., et al. (2012). The microbiome of Brazilian mangrove sediments as revealed by metagenomics. PLoS One 7:e38600. doi: 10.1371/journal.pone.0038600

Austin, B. (2006). The bacterial microflora of fish, revised. ScientificWorldJournal

Bergey, D. H., Whitman, W. B., Krieg, N. R., and Staley, J. T. (2011). Bergey’s Manual of Systematic Bacteriology, Vol. 4. New York, NY: Springer.

Bernardet, J.-F., and Nakagawa, Y. (2006). An introduction to the family flavobacteriaceae. Prokaryotes 455–480. doi: 10.1007/0-387-30747-8_16

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics 30, 2114–2120. doi: 10.1093/bioinformatics/btu170

Boyd, C. E., and Tucker, C. S. (1998). Pond Aquaculture Water Quality Management. Boston, MA: Kluwer Academic Publishers, 700. doi: 10.1007/978-1-615-5407-3_8

Brock, T. D. (1970). Biology of Microorganisms. Englewood Cliffs, NJ: Prentice-Hall.

Collignon, P., and Angulo, F. J. (2006). Fluoroquinolone-resistant Escherichia coli: food for thought. J. Infect. Dis. 194, 8–10. doi: 10.1086/504922

Dar, S. A., Kleerebezem, R., Stams, A. J., Kuenen, J. G., and Muyzer, G. (2008). Competition and coexistence of sulfate-reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. Appl. Microbiol. Biotechnol. 78, 1045–1055. doi: 10.1007/s00253-008-1391-8

Davis, H. S. (1922). A New Bacterial Disease of Fresh-water Fishes. Washington, DC: Government Printing Office. doi: 10.5962/bhl.title.49773

Dinsdale, E. A., Edwards, R. A., Hall, D., Angly, F., Breitbart, M., Brulc, J. M., et al. (2008). Functional metagenomic profiling of nine biomes. PLoS One 3, 629–632. doi: 10.1038/nature06810

Durso, L. M., Miller, D. N., and Wienhold, B. J. (2012). Distribution and quantification of antibiotic resistant genes and bacteria across agricultural and non-agricultural metagenomes. PLoS One 7:e48325. doi: 10.1371/journal.pone.0048325

Eldar, A., Bejerano, Y., and Bercovier, H. (1994). Streptococcus shiiolii and Streptococcus difficilis: two new streptococcal species causing a meningoencephalitis in fish. Curr. Microbiol. 28, 139–143. doi: 10.1007/BF01571054

Eldar, A., Ghittino, C., Asanta, L., Bozzetta, E., Goria, M., Prearo, M., et al. (1996). Enterococcus seriolicida is a junior synonym of Lactococcus garvieae, a causative agent of septicemia and meningoencephalitis in fish. Curr. Microbiol. 32, 85–88. doi: 10.1007/BF00289001

Feris, K. P., Ramsey, P. W., Gibbons, S. M., Frazier, C., Rillig, M. C., Moore, J. N., et al. (2009). Hyporheic microbial community development is a sensitive indicator of metal contamination. Environ. Sci. Technol. 43, 6158–6163. doi: 10.1021/es9005465

Food Agriculture Organization of the United Nations and Fisheries and Aquaculture (2014). The State of World Fisheries and Aquaculture: Opportunities and Challenges. Rome: Food and Agriculture Organization of the United Nations.

Gibbons, S. M., Jones, E., Bearquiver, A., Blackwolf, F., Roundstone, W., Scott, N., et al. (2014). Human and environmental impacts on river sediment microbial communities. PLoS One 9:e97435. doi: 10.1371/journal.pone.0097435

Harrison, B. K., Zhang, H., Berelson, W., and Orphan, V. J. (2009). Variations in archeal and bacterial diversity associated with the sulfate-methane transition zone in continental margin sediments (Santa Barbara Basin, California). Appl. Environ. Microbiol. 75, 1487–1499. doi: 10.1128/AEM.01812-08

Hawke, J. P., and Thune, R. L. (1992). Systemic isolation and antimicrobial susceptibility of Cytophaga columnaris from commercially reared channel catfish. J. Aquat. Anim. Health 4, 109–113. doi: 10.1577/1548-8667(1992)004<0109:SIAASO>2.3.CO;2

Holmer, M., and Kristensen, E. (1992). Impact of marine fish cage farming on metabolism and sulfate reduction of underlying sediments. Mar. Ecol. Prog. Ser. 80, 191–201. doi: 10.3334/meps080191

Huson, D. H., Auch, A. F., Qi, J., and Schuster, S. C. (2007). MEGAN analysis of metagenomic data. Genome Res. 17, 377–386. doi: 10.1101/gr.5969107

Kandler, O., and Hippe, H. (1977). Lack of peptidoglycan in the cell walls of Methanococcina bacterii. Arch. Microbiol. 113, 57–60. doi: 10.1007/BF00428580

Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y., andhattori, M. (2004). The KEGG resource for deciphering the genome. Nucleic Acids Res. 32, D277–D280. doi: 10.1093/nar/gkh063

Kim, K. K., Kim, M. K., Lim, J. H., Park, H. Y., and Lee, S. T. (2005). Transfer of Chryseobacterium meningosepticum and Chryseobacterium michiru to Elizabethkingia gen. nov. as Elizabethkingia meningoseptica comb. nov. and Elizabethkingia michiru comb. nov. Int. J. Syst. Evol. Microbiol. 55, 1287–1293. doi: 10.1099/ijs.0.65341-0

Kimes, N. E., Callaghan, A. V., Aktas, D. F., Smith, W. L., Sunner, J., Golding, B., et al. (2013). Metagenomic analysis and metabolite profiling of deep-sea sediments from the Gulf of Mexico following the Deepwater Horizon oil spill. Front. Microbiol. 4:50. doi: 10.3389/fmicb.2013.00050

Konneke, M., Bernhard, A. E., de la Torre, J. R., Walker, C. B., Waterbury, J. B., and Stahl, D. A. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437, 543–546. doi: 10.1038/nature03911

Kovalchuk, G. A., and Stephen, J. R. (2001). Ammonia-oxidizing bacteria: a model for molecular microbial ecology. Annu. Rev. Microbiol. 55, 485–529. doi: 10.1146/annurev.micro.55.1.485

Larsen, P. E., Gibbons, S. M., and Gilbert, J. A. (2012). Modeling microbial community structure and functional diversity across time and space. FEMS Micribiol. Lett. 332, 91–98. doi: 10.1111/j.1574-6968.2012.02588.x

Leadbetter, E. R. (2006). “The genus Capnocytophaga,” in The Prokaryotes, eds M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, and E. Stackebrandt (New York, NY: Springer), 709–711.

Leung, K., England, L. S., Cassidy, M. B., Trevors, J. T., and Weir, S. (1994). Microbial diversity in soil: effect of releasing genetically engineered micro-organisms. MEC Mol. Ecol. 3, 413–422. doi: 10.1111/j.1365-294X.1994.tb00081.x

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.02855/full#supplementary-material

TABLE S1 | Sequence data from the CPS metagenome.

TABLE S2 | Functional profiles (COG and KEGG) for genes identified in the CPS metagenome.

TABLE S3 | Relative abundances of bacteria by systematic classifications within the CPS metagenome.
Loch, T. P., and Faisal, M. (2015). Emerging flavobacterial infections in fish: a review. J. Adv. Res. 6, 283–300. doi: 10.1016/j.jare.2014.10.009

Logue, J. B., Bürgmann, H., and Robinson, C. T. (2008). Progress in the ecological genetics and biodiversity of freshwater bacteria. Bioscience 58, 103–113. doi: 10.1641/BIO.58.1.3

Maeder, D. L., Anderson, I., Brettin, T. S., Bruce, D. C., Gilna, P., Han, C. S., et al. (2006). The Methanosarcina Barkeri Genome: Comparative analysis with Methanosarcina acetivorans and Methanosarcina mazei reveals extensive rearrangement within methanogenic genomes. J. Bacteriol. 188, 7922–7931. doi: 10.1128/JB.00810-06

Martins, P., Clevy, D. F., Pires, A. C., Rodrigues, A. M., Quintino, V., Calado, K., et al. (2013). Molecular analysis of bacterial communities and detection of potential pathogens in a recirculating aquaculture system for Scophthalmus maximus and Solea senegalensis. PLoS One 8:e80847. doi: 10.1371/journal.pone.0080847

Meyer, F., Paarmann, D., D’Souza, M., Olson, R., Glass, E. M., Kubal, M., et al. (2008). The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9:386. doi: 10.1186/1471-2105-9-386

Mueller-Spitz, S. R., Stewart, L. B., Klump, J. V., and McLellan, S. L. (2010). Freshwater suspended sediments and sewage are reservoirs for enterotoxin-positive Clostridium perfringens. Appl. Environ. Microbiol. 76, 5556–5562. doi: 10.112AEM.01702-09

Nogales, B., Lanfranconi, M. P., Pina-Villalonga, J. M., and Bosch, R. (2011). Anthropogenic perturbations in marine microbial communities. FEMS Microbiol. Rev. 35, 275–298. doi: 10.1111/j.1574-6976.2010.00248.x

Oremland, R. S., and Polcin, S. (1982). Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments. Appl. Environ. Microbiol. 44, 1270–1276.

Parkes, D. H., and Beiko, R. G. (2010). Identifying biologically relevant differences between metagenomic communities. Bioinformatics 26, 715–721. doi: 10.1093/bioinformatics/btq041

Ringo, E., and Gatesoupe, F.-J. (1998). Lactic acid bacteria in fish: a review. Aquaculture 160, 177–203. doi: 10.1016/S0044-8486(97)00299-8

Saunders, D. L., and Kalff, J. (2001). Denitrification rates in the sediments of Lake Memphremagog, Canada USA. Water Res. 35, 1897–1904. doi: 10.1016/S0043-1354(00)00479-6

Schneegurt, M. A., Dore, S. Y., and Kulpa, C. F. Jr. (2003). Direct extraction of DNA from soils for studies in microbial ecology. Curr. Issues Mol. Biol. 5, 1–8.

Schreier, H. J., Mirzoyan, N., and Saito, K. (2010). Microbial diversity of biological filters in recirculating aquaculture systems. Curr. Opin. Biotechnol. 21, 318–325. doi: 10.1016/j.copbio.2010.03.011

Simon, C., and Daniel, R. (2009). Achievements and new knowledge unraveled by metagenomic approaches. Appl. Microbiol. Biotechnol. 85, 265–276. doi: 10.1007/s00253-009-2233-z

Sousa, O. V., Macrae, A., Menezes, F. G., Gomes, N. C., Vieira, R. H., and Mendonca-Hagler, L. C. (2006). The impact of shrimp farming effluent on bacterial communities in mangrove waters. Ceara, Brazil. Mar. Pollut. Bull. 52, 1725–1734. doi: 10.1016/j.marpolbul.2006.07.006

Staley, C., Unno, T., Gould, T. J., Jarvis, B., Phillips, J., Cotter, J. B., et al. (2013). Application of illumina next-generation sequencing to characterize the bacterial community of the Upper Mississippi River. J. Appl. Microbiol. 115, 1147–1158. doi: 10.1111/jam.21233

Takai, K., Campbell, B. J., Cary, S. C., Suzuki, M., Ouda, H., Nounoura, T., et al. (2005). Enzymatic and genetic characterization of carbon and energy metabolisms by deepsea hydrothermal chemolithoautotrophic isolates of Epilithion proteobacteria. Appl. Environ. Microbiol. 71, 7310–7320. doi: 10.112AEM.71.11.7310-7320.2005

Tatusov, R. L., Natale, D. A., Garkavtsev, I. V., Tatusova, T. A., Shankavaram, U. T., Rao, B. S., et al. (2001). The COG database: new developments in phylogenetic classification of proteins from complete genomes. Nucleic Acids Res. 29, 22–28. doi: 10.1093/nar/29.1.22

USDA (2018). USDA, 2018, Catfish production, by the National Agricultural Statistics Service (NASS), Agricultural Statistics Board. Washington, DC: United States Department of Agriculture (USDA).

Wang, X., Wang, C., Bao, L., and Xie, S. (2014). Abundance and community structure of ammonia-oxidizing microorganisms in reservoir sediment and adjacent soils. Appl. Microbiol. Biotechnol. 98, 1883–1892. doi: 10.1007/s00253-013-5174-5

Woese, C. R. (1987). Bacterial evolution. Microbiol. Rev. 51, 221–271.

Woehe, C. R., Yang, D., Mandelco, L., and Stetter, K. O. (1990). The Flexibacter-Flavobacter connection. Syst. Appl. Microbiol. 13, 161–165. doi: 10.1016/S0723-9308(06)80123-3

Wrighton, K. C., Castelle, C. J., Wilkins, M. J., Hug, L. A., Sharon, I., Thomas, B. C., et al. (2014). Metabolic interdependencies between phylogenetically novel fermenters and respiratory organisms in an unconfined aquifer. ISME J. 8, 1452–1463. doi: 10.1038/ismej.2013.249

Wu, S., Gao, T., Zheng, Y., Wang, W., Cheng, Y., and Wang, G. (2010). Microbial diversity of intestinal contents and mucus in yellow catfish (Pelteobagrus fulvidraco). Aquaculture 303, 1–7. doi: 10.1016/j.aquaculture.2009.12.025

Wu, S., Wang, G., Angert, E. R., Wang, W., Li, W., and Zou, H. (2012). Composition, diversity, and origin of the bacterial community in grass carp intestine. PLoS One 7:e30440. doi: 10.1371/journal.pone.0030440

Xing, M., Hou, Z., Yuan, J., Liu, Y., Qu, Y., and Liu, B. (2013). Taxonomic and functional metagenomic profiling of gastrointestinal tract microbiome of the farmed adult turbot (Scophthalmus maximus). FEMS Microbiol. Ecol. 86, 432–443. doi: 10.1111/1574-6941.12174

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