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Metagenome-Assembled Genomes and Metatranscriptome Analysis of Perfluorooctane Sulfonate-Reducing Bacteria Enriched From Activated Sludge

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) exhibit a widespread distribution across diverse global ecosystems throughout their lifecycle, posing substantial risks to human health. The persistence of PFAS makes biodegradation a challenging yet environmentally friendly solution for their treatment. In the authors' previous study, a bacterial consortium capable of reducing perfluorooctane sulfonate (PFOS) was successfully enriched from activated sludge. This study aimed to investigate the array of genes associated with PFOS reduction via biosorption and biotransformation to elucidate the metabolic pathways. Two metagenome-assembled genomes (MAGs) based on 16S rRNA sequences that share 99.86% and 97.88% similarity with *Hyphomicrobium denitrificans* and *Paracoccus yeei*, respectively were obtained. They were found to contain several genes encoding enzymes that potentially regulate biofilm formation of biosorption and facilitate the desulfonation and defluorination processes of biotransformation. Transcriptomic analysis demonstrated the high expression levels of these genes, including alkanesulfonate monooxygenase, catechol dioxygenase, (S)-2-haloacid dehalogenase and putative cytochrome P450, suggesting their involvement in PFOS biotransformation. The expression of these genes supports the presence of candidate metabolites of PFOS biotransformation detected in the previous study. These findings emphasise the significant potential of bacterial consortia and the crucial role played by genes encoding enzymes in facilitating the remediation of PFOS contaminants.

1 | Introduction

Per- and polyfluoroalkyl substances (PFAS), a large class of synthetic organic compounds, exhibit chemical and thermal stability due to their unique perfluoroalkyl moiety, combining a hydrophobic fluorocarbon tail with a hydrophilic or hydrophobic alkyl functional group (O'hagan 2008). Two notable PFAS are perfluorooctanoic acid and perfluorooctane sulfonate (PFOS), which are recognised as emerging contaminants due to their persistent environmental presence, bioaccumulation and toxicity (Fenton et al. 2021; ATSDR 2021). These compounds are detected globally throughout their lifecycle (Cordner et al. 2021; Guida et al. 2023). Their widespread occurrence is reported in wastewater, groundwater, surface water, tap water and even indoor air and dust, posing potential exposure risks to humans (Boone et al. 2019; Schwanz et al. 2016; Takemine et al. 2014; Sunderland et al. 2019).

Biological treatment is an environmentally friendly and cost-effective method for destroying various pollutants, including PFAS, although the process is time consuming (Chetverikov et al. 2017; Kanaly and Harayama 2000; Peng et al. 2013; Takagi 2020). Several studies have highlighted the efficacy of specific bacterial strains in PFAS biotreatment. *Pseudomonas aeruginosa* was shown to degrade and transform PFOS into shorter chains such as perfluorobutane sulfonate and perfluorohexane sulfonate without producing fluoride (Kwon et al. 2014). Similarly, *Pseudomonas plecoglossicida* was found to degrade PFOS, producing perfluoroheptanoic acid and fluoride (Chetverikov et al. 2017). In another genus, *Acidimicrobium* sp. strain A6 demonstrated the ability to degrade PFOS into perfluorobutane sulfonate and heptafluorobutyric acid (Huang and Jaffé 2019). These findings suggest that bacterial strains degrade PFOS by reducing perfluorinated carbons and defluorination. While these bacterial strains' degradation ability has been proposed based on the detection of byproducts, a comprehensive study of metabolic activity at the molecular level during the degradation process has not been conducted. The formation of individual byproducts during PFAS biodegradation could result from different metabolic processes inherent to specific bacterial strains, which can be influenced by growth conditions and energy sources.

In the biodegradation process, microbes and their enzymes serve as workhorses for the catalytic destruction or transformation of PFAS through diverse metabolic processes. The identification and characterisation of potential genes associated with PFAS biodegradation will lead to a better understanding of the metabolic pathways. Several genes have been proposed to be involved in the biotransformation of less persistent PFAS, such as 6:2 fluorotelomer alcohol, 6:2 fluorotelomer sulfonate and 6:2 fluorotelomer carboxylic acid (Hu and Scott 2024). Previous findings suggest that the desulfonation of sulphur-containing PFAS involves sulfonate-sulphur utilisation genes (Méndez et al. 2022; Yang et al. 2022; Kahnert et al. 2000; Koch et al. 2005). The subsequent defluorination through the cleavage of carbon–fluorine bonds is considered even more challenging due to the high Gibbs energy input required (Bentel et al. 2019). Nevertheless, some studies on the defluorination of these compounds have reported the involvement of several potential genes encoding enzymes, such as alkane monooxygenase, haloacid dehalogenases and cytochrome P450s (Yang et al. 2022; Kim et al. 2012; Lewis et al. 2016). However, there has been no research on whether these genes can also facilitate the biotransformation of PFOS, which is more persistent than the aforementioned PFAS. Filling this knowledge gap is necessary in order to understand the metabolic pathways of PFOS biotransformation.

In our previous study, we obtained a PFOS-reducing bacterial consortium enriched from the activated sludge of a full-scale wastewater treatment plant and identified several candidate metabolites of PFOS in the consortium (Sorn et al. 2023). This study aimed to clarify the array of genes involved in PFOS reduction through biosorption and biotransformation in the bacterial consortium using metagenome and metatranscriptome analyses. After metagenome-assembled genomes (MAGs) of PFOS-reducing bacteria were reconstructed using a hybrid sequencing approach, candidate genes were listed from the MAGs. Furthermore, metatranscriptome analysis was performed to reveal highly expressed genes along with the reduction of PFOS. The findings of this study provide a crucial foundation to elucidate the metabolic pathways of PFOS biotransformation.

2 | Materials and Methods

2.1 | Genome Sequencing, Hybrid Assembly and Annotation

In this study, we used the PFOS-reducing bacterial consortium that was enriched from activated sludge in our previous study, where PFOS was reduced to 14% of the initial concentration at 2 mg/L in 3 weeks and further to 25% in 6 weeks, along with the production of several candidate metabolites (Sorn et al. 2023). The bacterial consortium was cultivated on a PFOS-containing medium with 2 mg/L of PFOS and 0.4% (vol/vol) of methanol as carbon sources for 5 days. Total genomic DNA was extracted using a Qiagen Genomic DNA buffer set with Qiagen Genomic-tip 100/G (Qiagen, USA) according to the manufacturer's instructions. The DNA sample was analysed for hybrid genome assembly using both short-read and long-read sequencing techniques.

A sequencing library for short reads was prepared using the MGIEasy FS DNA Library Prep Set and the MGIEasy DNA Adapters-96 (Plate) Kit (MGI Tech Co. Ltd.), following the instruction manual. Short reads of the genomic DNA were sequenced on DNBSEQ-G400 (MGI Tech Co. Ltd.) using 2×200-base pair (bp) paired-end reads. A sequencing library of long reads was prepared using a Ligation Sequence Kit (Oxford Nanopore Technologies). The long reads of the genomic DNA were sequenced on GridION (Oxford Nanopore Technologies). The resulting summary statistics of genomic DNA and sequenced bps are displayed in Table S1. Short reads with quality scores lower than 20 and less than 127 bp were removed using sickle version 1.33, while low-quality long reads with less than 1000 bp were removed using Filtlong version 0.2.0. The high-quality short and long reads were de novo assembled using Unicycler version 0.4.7 and then annotated using Prokka version 1.14.5. The whole genome of the bacterial consortium and average nucleotide identity (ANI) were identified using the Microbial Genomes Atlas (MiGA) webserver and illustrated using Proksee (Grant et al. 2023; Rodriguez-R et al. 2018). The completed draft genomes are available in the DNA Data Bank of Japan's (DDBJ) BioProject collection with the accession number PRJDB17782. Phylogenetic analysis was conducted by comparing 16S rRNA gene sequences retrieved from the MiGA webserver to the BLAST (Basic Local Alignment Search Tool) search databases hosted by the National Center for Biotechnology Information. Multiple sequences were aligned using MUSCLE (MUltiple Sequence Comparison by Log-Expectation), and the phylogenetic tree was constructed with the neighbour-joining method in MEGA version 11 (Tamura et al. 2021). The obtained MAGs were identified as either chromosomes or plasmids using Bandage (Ver 0.8.1) (Wick et al. 2015).

2.2 | Metatranscriptome and Gene Expression Analyses

A total of 5 mL of the pre-cultured consortium described in Section 2.1 was harvested and centrifuged at 10,000*g* for 15 min

at 4°C. The pellet was collected, rinsed twice with sterilised 0.85% NaCl solution to remove PFOS residues, and resuspended in 25 mL of liquid medium containing 0.4% (vol/vol) methanol as an energy supply. PFOS was spiked into the treatment assay at a final concentration of 2 mg/L to assess its effect on gene expression compared to the untreated control assay. The assays were incubated at 30°C under shaking conditions (120 rpm) in triplicate. After 3 weeks of incubation, 5 mL of bacterial culture was collected for gene expression analysis, corresponding to an early stage when PFOS reduction is actively occurring (Sorn et al. 2023). The collected samples were centrifuged at 10,000*g* for 5 min, and all of the supernatant was carefully removed before rinsing the pellet twice with a sterilised 0.85% NaCl solution. RNAprotect bacteria reagents (Qiagen, USA) were added to the pellet directly, which was then vortexed for 5 s, incubated at room temperature for 5 min and centrifuged for 10 min at 5000*g*. The supernatant was decanted and completely removed by gently tapping onto a paper towel for 10 s. Finally, the pellet was frozen using liquid nitrogen for 30 s before being stored at −80°C. The stored sample was defrosted at room temperature before the RNA was extracted using a ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research, USA) according to the manufacturer's protocol.

The total RNA quality was assessed using a 5200 Fragment Analyser system and an Agilent HS RNA Kit (Agilent Technologies). DNase treatment was carried out using RNA Clean & Concentrator-5 with DNase I (Zymo Research), followed by rRNA removal using riboPool (siTOOLs Biotech). The sequencing library was prepared using an MGIEasy RNA Directional Library Prep Set (MGI Tech Co. Ltd.) following the manufacturer's manual. The prepared library and circularised DNA were prepared using the MGIEasy Circularization Kit (MGI Tech Co. Ltd.). The DNA nanoball (DNB) was manually prepared using the DNBSEQ-G400R High-throughput Sequencing Kit (MGI Tech Co. Ltd) and sequenced on DNBSEQ-G400 using 2×200-bp paired-end reads. Low-quality reads and sequences shorter than 100 bp were removed using sickle version 1.33 after trimming the adapter sequences using Cutadapt version 4.0. The high-quality reads were subjected to de novo transcriptome assembly using Trinity version 2.11.0 and mapped using bowtie2 version 2.4.2 after annotation by Prokka version 1.14.6. Differential expression genes based on tag count data were analysed using the TCC package in R, while the normalisation of count data for two triplicate datasets was completed using the edgeR Bioconductor package (Sun et al. 2013). The metabolic pathways of regulated genes were analysed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Kanehisa and Goto 2000). The expressed genes are available in the DDBJ Genomic Expression Archive (GEA) database with the accession number E-GEAD-690. For gene homology analysis, nucleotide sequences of the genes potentially associated with PFOS biotransformation were translated into amino acid sequences using Prokka 1.14.6. Alignment of the amino acid sequences was performed using CLUSTAL O (1.2.4) with those reported in previous studies (Yang et al. 2022; Li et al. 1998; Ridder et al. 1999; Schmidberger et al. 2007; Rye et al. 2009; Chan et al. 2022; Harris et al. 2022), then their key residues were identified using the UniProt database.

3 | Results and Discussion

3.1 | MAGs of PFOS-Reducing Bacterial Consortium

The PFOS-reducing bacterial consortium was comprised of seven contigs, two of which were circular chromosomes defined as MAGs. MAG1 was 3,634,313 bp in length and contained 3462 protein-coding genes, while MAG2 was 3,184,520 bp in length and contained 3081 protein-coding genes. Whole genome-based ANI analysis revealed that MAG1 and MAG2 were closely related to *Hyphomicrobium denitrificans* strain ATCC 51888 NC 014313^{T} (ANI 95.2%) and *Paracoccus limosus* strain GCA 009711185 1 (JCM 17370^{T}) (ANI 88.09%), respectively (Table 1). In addition, five MAGs, including five plasmids, were obtained (Table S2); however, they were shorter than 432,548 bp, and most genes on these other MAGs were also present in MAG1 and MAG2.

Furthermore, taxonomic identification using 16S rRNA gene sequences indicated that MAG1 and MAG2 shared maximum similarity with *H. denitrificans* strain 1NES1 (99.86%) and *Paracoccus yeei* strain CCUG 32053 (97.88%), respectively, with query coverages of 100%. The resulting phylogenetic tree is shown in Figure 1. These genome identities align with the findings of the previous study based on microbial community analysis targeting V3-V4 regions of 16S rRNA, which detected *Paracoccus* (72%) and *Hyphomicrobium* (24%) as the genera with the highest abundances (Sorn et al. 2023). Both *Paracoccus* and *Hyphomicrobium* genera are known as methylotrophic bacteria, which can dissimilate methanol as their energy supply by methanol dehydrogenase (Dziewit et al. 2015; Schär et al. 1985). *Paracoccus* and *Hyphomicrobium* were abundant in the bacterial consortium due to our methanol-enriched cultivation process. Methanol is widely used as a co-substrate in bioremediation to stimulate environmental microbial communities for the degradation of micropollutants such as trichloroethylene, chlorobenzene, hydrocarbons and diphenyl ether (Kao and Prosser 1999; Zhao et al. 2019; Guo et al. 2021). In this study, methanol was used to maintain the activity of the

TABLE 1 | Sequence quality of MAG1 and MAG2 retrieved from metagenomic analysis using MiGA.

	MAG1	MAG2
Total length (bp)	3,634,313	3,184,520
ANI (%)	95.2 (*Hyphomicrobium denitrificans*)	88.09 (*Paracoccus limosus*)
N50 (bp)	3,634,313	3,184,520
G + C content (%)	60.7667	66.4918
Predicted proteins	3462	3081
Completeness (%)	99.1	100
Contamination (%)	0.9	0.9
Quality (%)	94.6	95.5

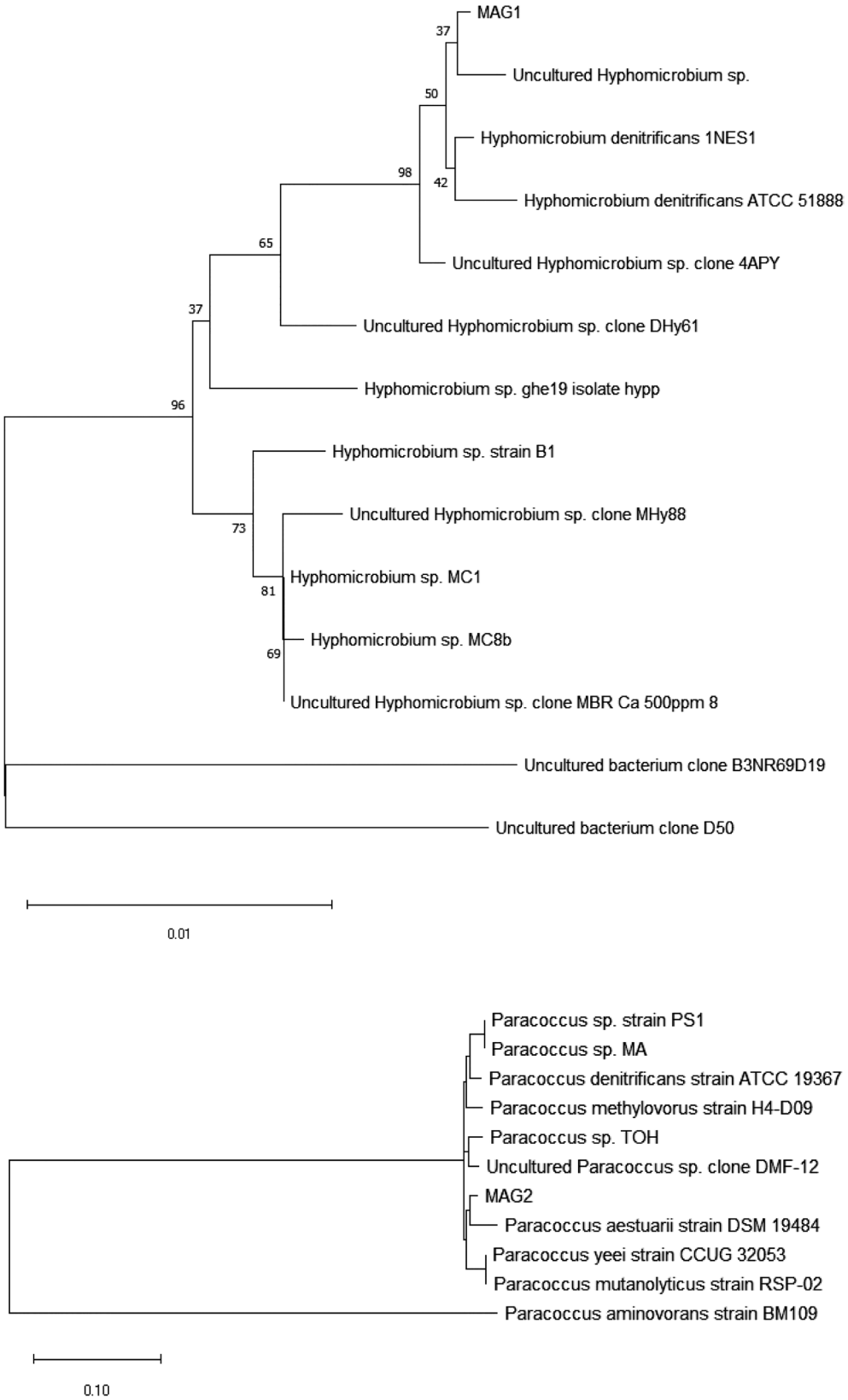


FIGURE 1 | Phylogenetic trees of MAG1 and MAG2 based on 16S rRNA sequences analysed using MEGA11.

enriched microbial consortium and facilitate co-metabolic processes, promoting the expression of enzymes capable of directly degrading PFOS. Furthermore, its cost-effectiveness at a practical scale supports its application for PFOS bioremediation in real contaminated environments. The MAGs obtained in this study also harboured methanol dehydrogenases (*mdh*, *moxF_1*, *moxF_2*, *moxF_3*, *moxF_4*, *moxF_5*, *moxG*, *moxI*), suggesting their capability of methanol dissimilation. *Paracoccus* and *Hyphomicrobium* have often been reported to be capable of degrading various xenobiotics, including

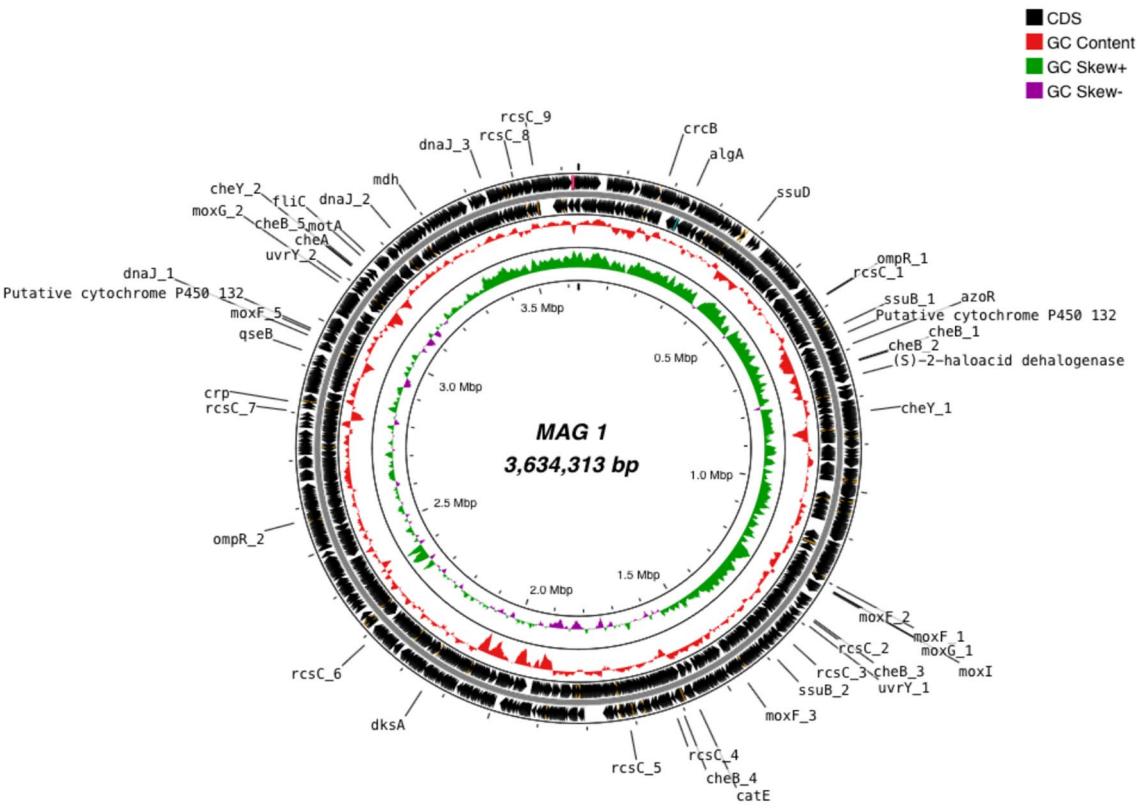
pesticides, polycyclic aromatic hydrocarbons, organic solvents (e.g., N-methyl-2-pyrrolidone, N,N-dimethylformamide and pyridine), antibiotics, dyes and hazardous gaseous compounds (Dziewit et al. 2015; Higgins et al. 1993; Al-Rashed et al. 2021; Puri et al. 2022; Li et al. 2021), although there have been no reports on the bioremediation of PFAS by these genera. Importantly, the obtained MAGs included genes that were reported to be involved in the metabolism of PFOS (Hu and Scott 2024; Méndez et al. 2022; Yang et al. 2022; Kahnert et al. 2000; Koch et al. 2005; Kim et al. 2012; Lewis et al. 2016), as illustrated Figure 2. Genes associated with organic sulphur metabolism, such as alkanesulfonate monooxygenase (*ssuD*), ATP-binding cassette (ABC) transporters (*ssuB_1* and *ssuB_2*) and catechol dioxygenase (*catE*), were identified in both MAGs. Genes encoding alkanesulfonate permease proteins (*ssuC_1* and *ssuC_2*) and a sulphate transporter (*cysZ*) were exclusively found in MAG2. Putative cytochrome P450 132, which belongs to the cytochrome P450s superfamily and is potentially implicated in desulfonation, was identified in both MAGs. MAG1 exhibited genes encoding for enzymes associated with defluorination, such as (S)-2-haloacid dehalogenase, azoreductase (*azoR*) and alkane 1-monooxygenase 2 (*alkB2*), along with a fluoride ion transporter found inside the cells (*crcB*). In addition, both MAGs were likely capable of biofilm formation, because they possessed an array of genes (e.g., *envZ*/*ompR*, *rscC*, *uvrY* and *qseC*/*qseB*) that are involved in biofilm development by regulating bacterial motility and quorum sensing, reviewed by Birgit M. Prüß (Prüß 2017). Many studies have highlighted that biofilms could serve as effective PFAS biosorbents from observation of natural biofilms in rivers and lakes and lab-scale experiments (Munoz et al. 2018; Zhang et al. 2022; Butzen et al. 2020; Fitzgerald et al. 2018).

3.2 | Metatranscriptome Analysis of Genes Potentially Associated With PFOS Metabolism

Metatranscriptome analysis was conducted to compare gene expression under the presence of PFOS (PFOS and methanol) to control (methanol only). Metatranscriptome analysis of the bacterial consortium identified 3455 genes (99.8% of the genes in MAG1) and 3073 genes (99.6% of the genes in MAG2) with fragments per kilobase of transcript per million mapped reads (FPKM) values greater than 0. A total of 2378 genes in MAG1 and 2647 genes in MAG2 exhibited FPKM values above 256 ($Log_2FPKM \geq 8$) and were categorised as highly expressed genes. Approximately 50% of them (1792 of 3455 MAG1 genes and 1332 of 3073 MAG2 genes) were hypothetical proteins whose functions were unknown. After exposure to PFOS, there was a notable increase in the number of downregulated genes in the bacterial consortium, with a total of 618 downregulated genes that had a false discovery rate (FDR) < 0.01 and 358 genes with an FDR < 0.001 (Figure 3). Compounds identified among the downregulated genes included cold shock protein A (*cspA*), cold shock protein (*scoF*), cypemycin N-terminal methyltransferase (*cypM*), transcriptional regulator (*mraZ*), glutamate-cysteine ligase (*gshA*), ESAT-6-like protein (*esxB*), 10 kDa chaperonin (*groS*) and other unknown genes labelled as hypothetical proteins. Even using the KEGG database (Figure S1), the limited understanding of functional genes, including the other downregulated genes found in this study (Table S3), made elucidating the functional genes associated with PFOS metabolic pathways challenging.

Among the highly expressed genes in MAG1 and MAG2, several genes were potentially associated with PFOS metabolism (Figure 4A). In MAG1, the expression of azoreductase (*azoR*), catechol dioxygenase (*catE*), (S)-2-haloacid dehalogenase (*had*) and putative cytochrome P450 132 (*CYP132*) was higher in the PFOS condition. Although none of the upregulated genes exhibited $p < 0.05$ nor $q < 0.05$ (Table S4), *CPY132*, *catE* and *azoR* exhibited $p < 0.10$, suggesting these genes were possibly upregulated in the presence of PFOS. In MAG2, the expression of catechol dioxygenase, a sulphate transporter (*cysZ*), alkane monooxygenase (*alkB2*), an ABC transporter (*ssuB_1*), alkanesulfonate permease proteins (*ssuC_1* and *ssuC_2*) and alkanesulfonate monooxygenase (*ssuD*) was higher in the presence of PFOS. Since PFOS was the only substance that contained fluorine in the culture medium, these highly expressed genes on desulfonation and defluorination were assumed to be associated with the observed reduction of PFOS via biotransformation and/or biosorption. According to the gene homology analysis, the similarity of amino acid sequences of these genes ranged from 23.2% to 47.3%. Among the identified sequences in the UniProt database, the key residue information was available only for haloacid dehalogenases (HADs). The amino acid sequence of the identified *had* gene was aligned with three structurally characterised crystal HADs and four defluorinating HADs in the literature (Figure 5) (Yang et al. 2022; Li et al. 1998; Ridder et al. 1999; Schmidberger et al. 2007; Rye et al. 2009; Chan et al. 2022; Harris et al. 2022). The results revealed a low to moderate level of identity (25.54%–35.41%), but most of the key residues of HADs were highly conserved in the newly identified *had*, except for the oxyanion hole. The detailed components and positions of key residues were provided in Table S4.

In addition, several genes associated with bacterial mobility and biofilm development also showed higher expression in the presence of PFOS in both MAGs (Figure 4B). In MAG1, the bacterial chemotaxis system-associated genes *cheB* and *cheY* and transcriptional regulatory protein *ompR*, involved in initiating surface attachment in the early stage of biofilm formation, were highly expressed (Xu et al. 2021; Prigent-Combaret et al. 2001). Moreover, stress responses through the expression of *uvrY*, *phoP*, *dksA* and CRP-like cAMP-activated global transcriptional regulator *crp*, which may be involved in the adaptation of bacteria to biofilm environments, were observed (Suzuki et al. 2002; Yin et al. 2019; Azriel et al. 2015; Soutourina et al. 1999). The expression of sensor histidine kinase *rcsC*, a part of the Rcs phosphorelay system regulating exopolysaccharide (EPS) production, and chaperone protein *dnaJ* was remarkably high. These genes are important in forming the biofilm matrix and ensuring proper protein function for early biofilm development (Ferrieres and Clarke 2003; Shi et al. 1992). In MAG2, the expression of *envZ*/*ompR*, *fliC*, *motA* and *motB* was observed. These genes are involved in enhancing biofilm stability, promoting surface colonisation and facilitating bacterial movement towards the surface for initial attachment (Xiao et al. 2022; Ling et al. 2021; Guttenplan and Kearns 2013). In addition, the high expression of *algA* and *qseC*/*qseB* could contribute to strengthening the biofilm matrix by producing alginate and enabling quorum sensing



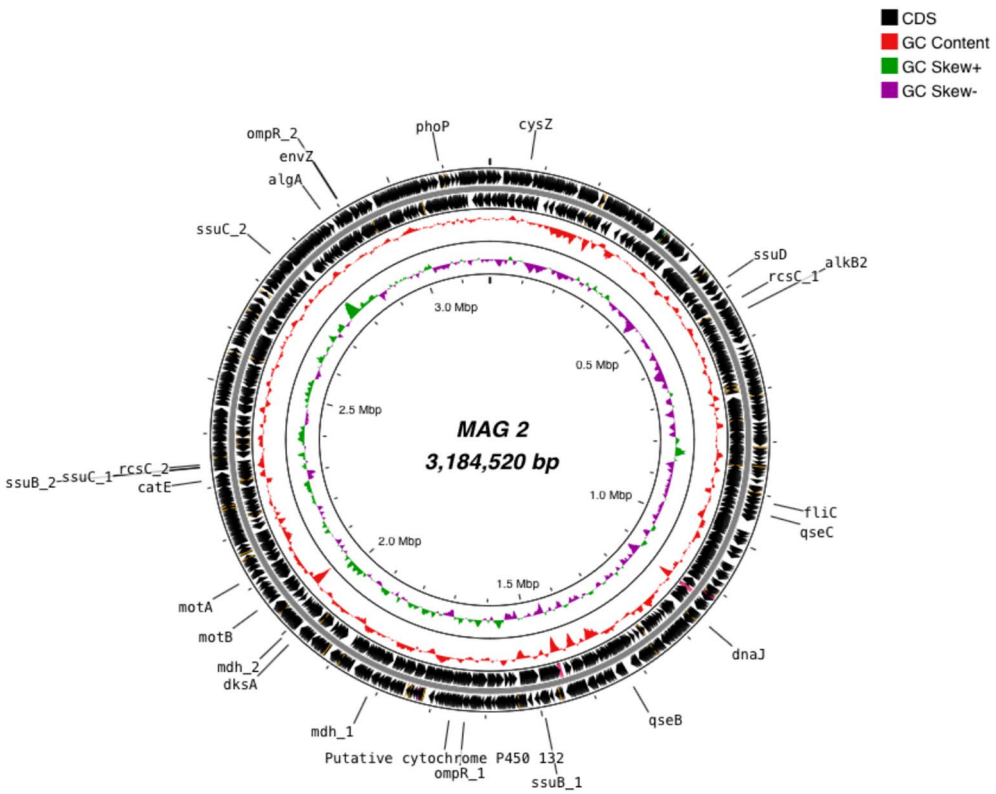


FIGURE 2 | Potential genes encoding enzymes implicated in methanol dehydrogenases, biosorption and biotransformation of PFAS compounds present in MAG1 and MAG2 (illustrated using Proksee).

for communication and biofilm dispersal control (Ma et al. 2012; Wu et al. 2021). Consequently, the increased expression of the array of these genes as responses to PFOS exposure implies that *Paracoccus* and *Hyphomicrobium* in the bacterial consortium are involved in the reduction of PFOS and biofilm formation.

3.3 | Potential Metabolic Pathways of PFOS in Bacterial Consortium

The reduction of PFOS by the bacterial consortium was suggested by Sorn et al. (Sorn et al. 2023) Since several candidate metabolites of PFOS were detected (Figure S2), biosorption and biotransformation were raised as possible mechanisms of the observed reduction of PFOS. According to the candidate metabolites detected in the previous study and the metatranscriptome analysis in this study, a metabolic pathway of PFOS biotransformation, which involves defluorination, desulfonation as well as C—C cleavage, hydrogenation and dehydrogenation, is suggested, as illustrated in Figure 6. The suggested pathway aligns with the typical pathway of PFAS biotransformation reported in other studies (Yu et al. 2020; Zhang et al. 2021). The expression of encoding genes related to alkanesulfonate monooxygenases transcribed in both genomes could be responsible for the cleavage of sulfonate-sulphur (—SO_3^-) in PFOS that resulted in $C_4HF_{10}O_4P$ and perfluorobutanoic acid. However, the incomplete desulfonation was likely due to repression under sulphur-rich conditions in the growth medium, which prevents the high expression of encoding genes related to alkanesulfonate monooxygenases. In this context, desulfonation of PFOS could be enhanced under sulphate starvation, leading to the greater expression of ssuEADCB genes. Each of these gene clusters encodes an ABC-type transport system required for the uptake of aliphatic sulfonates and a desulfonation enzyme. The expression of the sulphate transporter (*cysZ*) encoding gene may facilitate the uptake of sulphate ions into the bacterial cells. Furthermore, the potential metabolites of PFOS detected in the bacterial consortium included 2-3,3,3-trifluoropropylsulfanyl acetic acid, 3-3,3,3-trifluoropropylsulfanyl propanoic acid and $C_4HF_9O_5S$. The formation of these compounds could result from the cleavage of carbon—fluorine bonds within PFOS, facilitated by some of the highly expressed genes observed in the metatranscriptome analysis, such as (S)-2-haloacid dehalogenase, putative cytochrome P450 132 and alkane 1-monooxygenase 2.

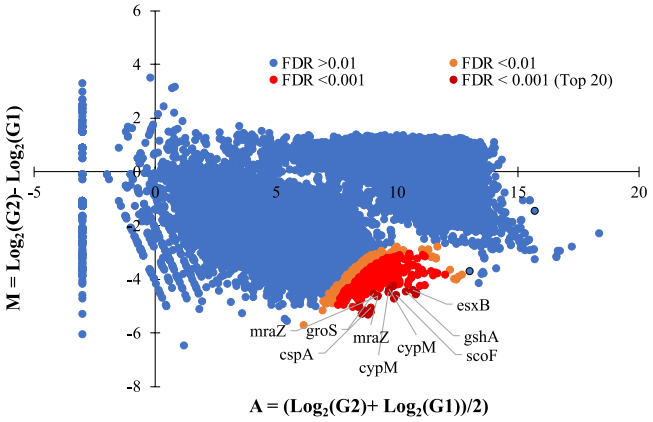


FIGURE 3 | Relative gene expression of bacterial consortium with exposure to PFOS (G2, $n = 3$) and no PFOS exposure (G1, control, $n = 3$).

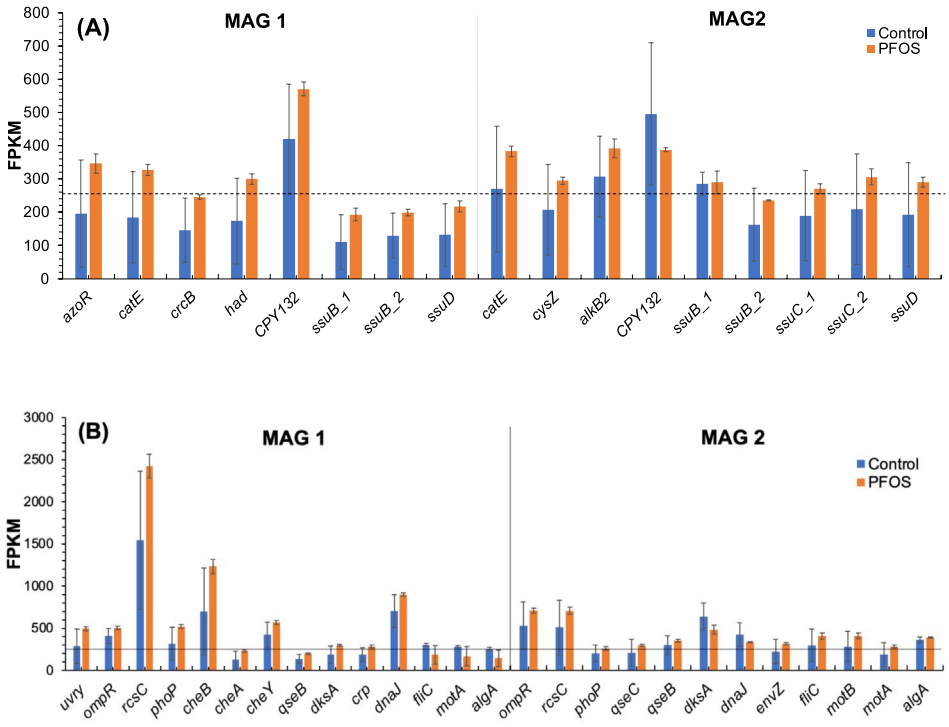


FIGURE 4 | FPKM of expressed genes in samples with ($n = 3$) and without PFOS exposure (the control, $n = 3$) after 3 weeks of incubation. Genes potentially associated with fluorine and sulphur metabolism in MAG 1 and MAG 2 (A), and with biofilm formation in MAG 1 and MAG 2 (B). The horizontal dotted line represents an FPKM value of 256 (Log_2 FPKM = 8).

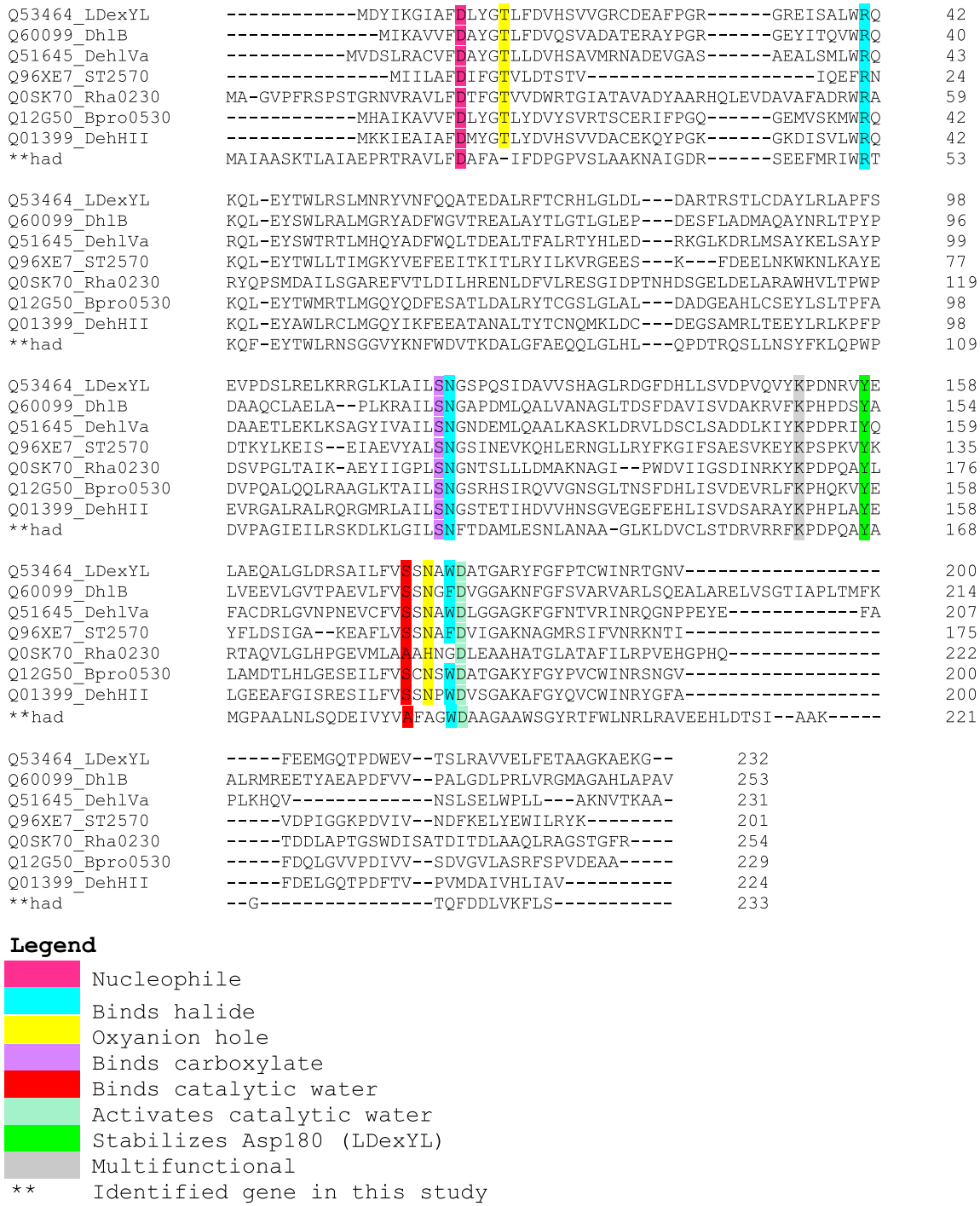


FIGURE 5 | Alignment of amino acid sequences of the identified had gene and HADs identified in past studies (Yang et al. 2022; Li et al. 1998; Ridder et al. 1999; Schmidberger et al. 2007; Rye et al. 2009; Chan et al. 2022; Harris et al. 2022). The sequences of three previously structurally characterised and confirmed HADs (L-Dex YL, DhlB, DehIVa) are aligned with those of the four defluorinating HADs (ST2570, Rha0230, Bpro0530, DehH2) and the gene identified in this study (*had*). The position of the ending residue in each row is numbered.

Moreover, the release of fluoride ions (Figure S3) from defluorination may impact various cellular processes and the viability of bacterial cells. Under the presence of fluoride ions, bacterial survival may be supported through active *crcB*-mediated fluoride transport, which facilitates the efflux of fluoride ions from the bacterial cytoplasm to the external environment. In the bacterial consortium of this study, the crcB gene may encode a fluoride ion channel that helps resist fluoride toxicity from PFOS degradation by exporting fluoride out of the cells. Our analysis revealed several potential genes that were highly expressed along with the reduction of PFOS and that are thus possibly associated with the metabolism

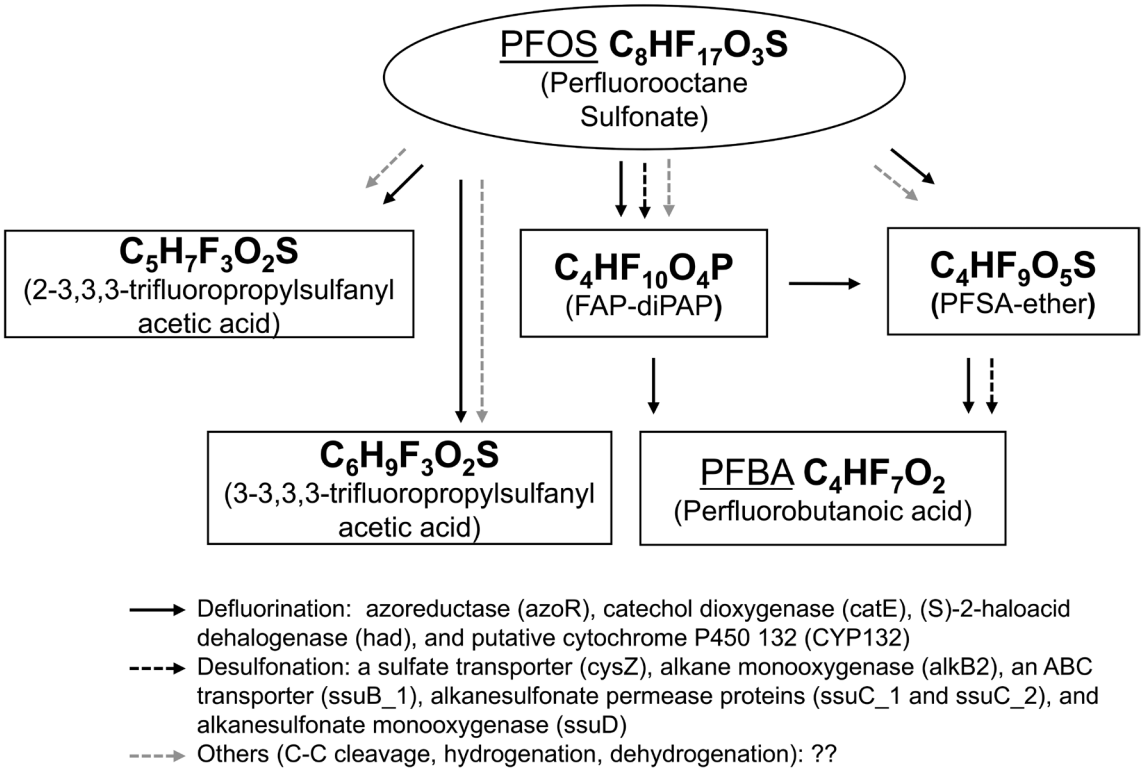


FIGURE 6 | A suggested metabolic pathway of PFOS by the bacterial consortium containing MAG1 and MAG2.

of PFOS. This is the first study to reveal the potential role of genes encoding enzymes in the biological reduction of PFAS in a bacterial consortium from activated sludge. Further research to verify the functions of these genes holds significant promise for research and practical applications in the biological treatment of PFAS. Elucidating the precise roles of these genes would enhance our understanding of the metabolic pathways of PFAS and help optimise bioremediation strategies for PFAS-contaminated environments. Gaining a deeper insight into the genetic mechanisms underlying PFAS metabolism could inform the development of targeted biotechnological solutions that will ultimately contribute to more effective and sustainable approaches for mitigating PFAS pollution.

Author Contributions

Sovannlaksmy Sorn: investigation, methodology, formal analysis, visualization, writing – original draft. **Norihisa Matsuura:** software, writing – review and editing, methodology. **Ryo Honda:** conceptualization, funding acquisition, writing – review and editing, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets supporting this study, including Tables S1–S4 and Figures S1–S3, are available in the Supporting Information, which has been deposited in the Mendeley Data Repository under the DOI: https://10.17632/r7s8xgd2nf.1.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.