



Invisible Threats to Oceans (InTO): Assessing the combined toxicity effects of emerging anthropogenic pollutants on microbial communities

Dissertation submitted in fulfilment of the requirements for the degree of
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Certificate of originality

I hereby confirm that this PhD thesis is an original piece of work conducted independently by the undersigned and all work contained herein has not been submitted for another degree.

All research material has been duly acknowledged and cited.

Signature of candidate:

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Charlotte Lee
[27/02/2025]

General Abstract

Marine environments near populated and tidal convergence zones accumulate pollutants, many of which are toxic to marine life. The impact of these pollutants on marine microorganisms threatens the biochemical balance of the oceans and can induce cascading changes throughout the food chain, prompting urgent investigation. Plastics are of particular concern due to their persistence in the ocean, their accumulation of persistent organic pollutants (POPs), and their role in carrying and transporting marine microbial communities termed ‘plastispheres’. In this thesis, the combined impact of plastics and POPs on these marine microbial communities was therefore studied to reveal the impact of this interaction on essential microbial metabolic processes.

In **Chapter 2**, marine microbial communities extracted from beached plastics were grown on pristine low-density polyethylene (LDPE) for three and seven days. Metagenomic analysis revealed increased microbial abundance over time, and a prevalence of potential pathogens. Metaproteomic analysis identified proteins involved in bacterial attachment, cell-cell interaction, biofilm maintenance, and antioxidant production, along with limited proteins related to pollutant biodegradation and virulence. In **Chapter 3**, the secretome of the representative marine bacterium *Epibacterium mobile* was studied after short exposure to the organic UV-filters benzophenone-3 (BP3), and ethylhexyl methoxycinnamate (EHMC), a more commonly used UV-filter. *E. mobile* was exposed to each UV-filter separately, and the proteins secreted into the medium were examined. Analysis of protein yields and contamination highlighted challenges inherent to the analysis of the secretome. However, regardless of technical limitations, growth and metaproteomics data showed that EHMC elicits a markedly different response in *E. mobile* than BP3. Finally, in **Chapter 4**, a nascent marine plastisphere was exposed to 5 mg/L EHMC, and analysed for metabolic impacts of co-pollution on the plastisphere. Metagenomic analysis of the plastisphere after EHMC exposure revealed a decline in growth and activity of a genera associated with pollutant biodegradation, and an increase in the growth and activity of other genera containing pathogenic species. EHMC exposure also triggered a stress response and the regulation of nitrogen respiration proteins, suggesting an inhibition of aerobic processes. In the marine environment, co-pollutants like EHMC may therefore impact plastic persistence by inhibiting core metabolic processes, as well as increasing the prevalence of potentially pathogenic bacteria, further threatening public health. Furthermore, this research indicates that combined pollution amplifies toxicity towards marine microorganisms, posing additional risks to microbial communities, and their biogeochemical processes.

This thesis demonstrates that combined pollution represents a significant, multifaceted threat to marine environments. Left unaddressed, these interactions could increase the hazards associated with the marine environment, compromise essential ecosystem services, and perpetuate the accumulation of pollutants. Continued efforts to mitigate pollution and monitor its effects on microbial communities will be critical to safeguarding the health of marine ecosystems and, by extension, the planet.

Declarations

- i) This research was conducted in accordance with the ethical guidelines of the University of Stirling. Formal ethical approval for this project (ID 0502) was obtained from Stirling Ethical Review Manager, under approval reference number EC 2021 0502 1420, dated 11/02/2021.
- ii) I confirm that this thesis does include content created using assistance from artificial intelligence (AI) tools. Current 'declarations' page excepting, where AI was used, it was employed for research, language refinement, and sentence/paragraph structuring purposes only. All outputs were critically reviewed and edited to ensure academic integrity. No AI-generated content has been presented as original thought or analysis.
- iii) I consent to this thesis being submitted for analysis using plagiarism detection software, such as Turnitin or any other software approved by the University of Stirling. I understand that such checks are conducted to ensure academic integrity and originality of the work.
- iv) Where this thesis includes collaborative work, I have clearly indicated my individual contributions in each relevant chapter. Co-authors of any published or submitted work have been appropriately acknowledged, and their contributions are detailed in the thesis.

Signed:

Charlotte E Lee



Date: **27/02/2025**

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This thesis is dedicated to my mother, Lesley Anne Lee.

Abbreviations

AMR	Antimicrobial Resistance
ARG	Antibiotic Resistance Gene
ASW+G	Artificial Seawater + vitamins, trace metals, and glucose (*Also ASW)
BHB+v	Bushnell Haas Broth + vitamins, and trace metals
CIF	Collision Induced Fragmentation
D3/D7	Day 3/7
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DDA	Data-Dependent Acquisition
DDT	Dichlorodiphenyltrichloroethane
DIA	Data-Independent Acquisition
DMSO	Dimethyl Sulfoxide
EC₅₀	Effective Concentration [for] 50[%]
EPS	Exopolymeric Substances
FC	Fold Change
FDR	False Discovery Rate
HGT	Horizontal Gene Transfer
LC₅₀	Lethal Concentration [for] 50[%]
LC-MS/MS	Tandem Liquid Chromatography-Mass Spectroscopy
LDPE	Low-Density Polyethylene
LiSC	Life Science Compute Cluster
MAG	Metagenome-Assembled Genome
mPies	MetaProteomics In Environmental Sciences
mRNA	messenger RNA
MS	Mass Spectrometer / Mass Spectroscopy
NO₃-	Nitrate
O₂	Oxygen
OD	Optical Density
PAH	Polycyclic Aromatic Hydrocarbon
PCB	PolyChlorinated Biphenyls
PCP	Personal Care Product
PET	PolyEthylene Terephthalate
PI	Precursor Ion
POP	Persistent Organic Pollutant
PVC	PolyVinyl Chloride
ROS	Reactive Oxygen Species
SWATH	Sequential Window Acquisition of All Theoretical Mass Spectra
TCA	Tricarboxylic Acid (cycle)
TOF	Time-Of Flight
TT	Transmembrane Transport
UV	UltraViolet
WWTP	Waste Water Treatment Plant
>D3/>D7	Protein(s) upregulated on D3/D7
UV-filters	
4-MBC	4-methylbenzylidene camphor
BP3	BenzoPhenone-3 / Oxybenzone
EHMC	EthylHexyl MethoxyCinnamate

Metals

Ag	Silver
Al	Aluminium
As	Arsenic
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
Fe²⁺	Iron (II)
Hg	Mercury
Mn	Manganese
Ni	Nickel
Pb	Lead
Zn	Zinc

Heterotrophic bacteria

Acn	<i>Acinetobacter</i>
Mar	<i>Marinomonas</i>
Pse	<i>Pseudomonas</i>
Rho	<i>Rhodobacter</i>
Shw	<i>Shewanella</i>

Proteins

ABC	ATP-Binding Cassette (transporter)
AcrA	Acriflavine resistance protein A
AD	Alcohol Dehydrogenase
ADI	Arginine Deiminase
Agg	Agglutination protein
AGPR	N-acetyl-gamma-glutamyl-phosphate reductase
AHB	Aconitate Hydratase B
Ahp(C)	Alkyl hydroperoxide reductase (C)
AidA	Adhesin
ALDH	Aldehyde Dehydrogenase
AlgF	Alginate biosynthesis protein
ALS	Acetolactate Synthase
CAT	Catalase
CK	Carbamate Kinase
ClpA	ATP-dependent Clp protease binding subunit
CopA	Copper resistance protein A
CP	Cytosol aminopeptidase
CSD	Cold Shock Domain-containing protein
CsgG	Curli assembly protein
Dsb	Thiol:disulfide interchange protein
Fhp	Flavo-hemoprotein
FLA	Flagellin
gaABC	Glutamate/Aspartate ABC transporter
GATase	Glutamine amidotransferase
GMD	GDP-mannose 6-dehydrogenase
Grx	Glutaredoxin

GSHs	Glutathione synthase
HtpG	Chaperone HtpG
HU	Transcriptional regulator HU
IsCS	Cysteine desulfurase
Kat	Catalase peroxidase
LP	Lipoprotein
LPS	Lipopolysaccharide
NOr	Nitrous-Oxide reductase
Omp	Outer membrane protein
OprF	Outer membrane porin F
OTC	Ornithine carbamoyltransferase
PilZ	Pilus assembly protein
PK	Pyruvate Kinase
RecA	SOS-response protein
SOD	Superoxide Dismutase
TelA	Toxic anion resistance protein
TerD	Tellurium resistance protein
Tfp	Type IV Pili
TolB/C	Tol-Pal system protein
TRAP	Tripartate ATP-independent Periplasmic (transporter)
Trx	Thioredoxin
THSS/TVISS	Type- 11/VI Secretion System

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Publications and Communications

Publications

- [1] Lozano C, **Lee C**, Wattiez R, Lebaron P & Matallana-Surget S (2021) Unraveling the molecular effects of oxybenzone on the proteome of an environmentally relevant marine bacterium. *Science of The Total Environment*, 793, p. 148431.
- [2] Duncan E, Omeyer L ... **Lee C** ... Matallana-Surget S ... & Godley B (2022) Research priorities to inform solutions to marine plastic pollution in Southeast Asia. *Science of The Total Environment*, 841, Art. p. 156704.
- [3] **Lee C**, Messer LF, Holland SI, Gutierrez T, Quilliam RS and Matallana-Surget S (2024) The primary molecular influences of marine plastisphere formation and function: Novel insights into organism -organism and -co-pollutant interactions. *Critical Reviews in Environmental Science and Technology*, 54:2, pp. 138-161.
- [4] Messer, LF, **Lee CE**, Wattiez R, and Matallana-Surget.S (2024) Novel Functional Insights into the Microbiome Inhabiting Marine Plastic Debris: Critical Considerations to Counteract the Challenges of Thin Biofilms Using Multi-Omics and Comparative Metaproteomics. *Microbiome* 12, no. 1: 36.
- [5] **Lee CE**, Messer LF, Wattiez R, & Matallana-Surget S (2025) Decoding Microbial Plastic Colonisation: Multi-Omic Insights into the Fast-Evolving Dynamics of Early-Stage Biofilms. *Proteomics*, 25: e202400208.
- [6] **Lee CE**, Messer LF, Wattiez R, & Matallana-Surget S (2025) The Invisible Threats of Sunscreen as a Plastic Co-Pollutant: Impact of a Common Organic UV Filter on Biofilm Formation and Metabolic Function in the Nascent Marine Plastisphere. *Journal of Hazardous Materials*.

Oral Communications

- [7] *Comparative Metaproteomics of the Plastispheres Isolated from Transparent and Coloured Plastic Debris Obtained from joint Sampling Programmes*. Marine Alliance for Science and Technology for Scotland (MASTS) – Scottish Funding Council (SFC) Saltire Emerging Researcher Scheme (SERS) webinar with **Charlotte Lee**, 12/04/2023.
- [8] *'I am an environmental biotechnologist because...' talking head recording*. Environmental Biotechnology Network (EBNet): **Charlotte Lee**, 04/01/2024.

Posters

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Chapter 1

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General Introduction

Marine microorganisms constitute >70% of total marine biomass equalling to approximately 2.2 million species (1, 2). As such, the world's oceans contain a broad array of microbial genera responsible for nitrogen cycling, carbon sequestration, and produces $\geq 50\%$ of global O_2 (3-5). Owing to their microscopic size ($\leq 100\mu m$) and total biomass (6), these organisms form the core of the marine, and indeed terrestrial food chain due to the inherent links that the two networks share (7). Crucially, another consequence of their size is a unique sensitivity to toxins like pollution (8). As organisms that have thrived and adapted for millennia, an extinction of essential microbial species is not predicted (9). However, shifts in marine microbial community composition and function may arise due to pollutant metabolism and growth in specialised heterotrophs, a toxic impact on microbial metabolism and growth, and further unpredicted outcomes (10). Such shifts could drastically impact the ecosystem services which are essential to human life (11). Consequentially, in depth study of marine pollution, particularly the most abundant pollutants (i.e. plastic, UV-filters; *Section 1.*) is required to understand how some of the ocean's most influential organisms may be affected.

1. The primary molecular influences of marine plastisphere formation and function: Novel insights into organism -organism and -co-pollutant interactions

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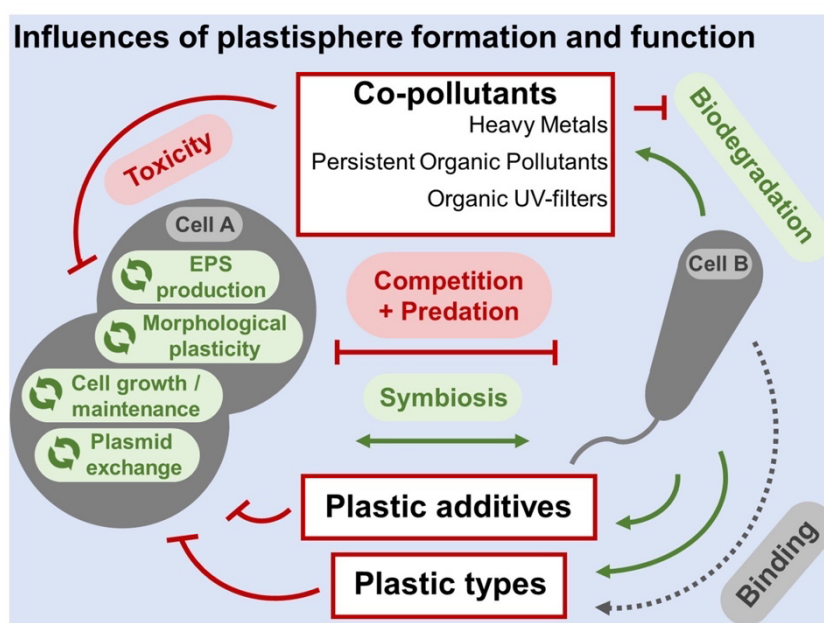
This paper serves as the foundation for the information presented here, although the text and content have been revised slightly for inclusion in this thesis.

Abstract

Marine plastic pollution is rapidly colonized by a biofilm of microorganisms associated with the control of biogeochemical cycles, plastic biodegradation, and potentially pathogenic activities. An extensive number of studies have described the taxonomic composition of this biofilm, referred to as the ‘plastisphere’, and previous reviews have reported on the influence of location, plastic type, and plastic-biodegradation ability on plastisphere formation. However, few studies have investigated the metabolic activity of this complex biofilm and how microbial pathogenicity and bioremediation could be regulated in this ecosystem. In this review, we highlight the understudied molecular and abiotic factors influencing plastisphere formation and microbial functioning beyond taxonomic description. In this context, we critically discuss the impacts of (i) organism-organism interaction, (ii) microbial cell wall composition, and (iii) commonly encountered plastic-bound co-pollutants (heavy metals, persistent organic pollutants, UV filters). For the first time, we review the anticipated impact of lipophilic organic UV-filters – found in plastic additives and sunscreens – on the plastisphere due to their reported affinity for plastics, persistence, and co-location at high concentrations in touristic coastal environments. Herein, we integrate the findings of 34 global studies exploring plastisphere composition, 35 studies quantifying co-pollutant concentrations, and draw upon 52 studies of cell-cell and -substrate interaction to deduce the inferred, yet still unknown, metabolic interactions within this niche. Finally, we provide novel future directions for the advancement of functional plastisphere research applying advanced molecular tools to new, and appropriate research questions.

Highlights

- Data was compiled from 97 plastispheres across 34 different studies and an additional 87 studies relating to the impacts of plastisphere components on microbial species.
- The biotic and abiotic factors influencing microbial adhesion to different plastic polymers, including cell wall composition, and plastisphere location are considered.
- The impacts of heavy metals and organic co-pollutants – and for the first time, organic UV-filters – on plastisphere formation and function are reviewed and discussed.
- A change in direction from novel research questions to the use of state-of-the-art methodologies are recommended for the advancement of functional plastisphere research.



Key Words

Plastisphere dynamics; Marine pollution; Microbial ecotoxicology;
Bacterial metabolism; Plastic colonisation; Plastic biodegradation.

1.1 Introduction

Throughout the last six decades, plastic has accumulated in the world's oceans to now represent more than 80% of all marine litter (12, 13). Leaching from maritime activity, wastewater treatment plants (WWTP) and other municipal effluents (14, 15), plastics have become one of the largest man-made threats to ocean life. The fragmentation of plastic also releases 1.15–2.41 Mt of microplastics (<5 mm size) into the world's seas and oceans each year (16), which is projected to persist in the marine environment for centuries (17). These plastics and microplastics contain synthetic additives, and can further aggregate anthropogenic leachate such as persistent organic pollutants (POPs) and heavy metals (18), which are environmentally stable from years to decades (19). As plastics are highly persistent and only removed through sedimentation or photodegradation (12, 20), the accumulation of these marine contaminants on plastic increases their persistence and dispersal throughout the ocean (21, 22). Microplastics can then bioaccumulate throughout the food chain (23), introducing potential chemical hazards to all other organisms (15, 22).

In the ocean, plastics are exposed to ultraviolet-radiation (UV-R), temperature fluctuations, and ocean hydrodynamics (12). This can induce the formation of reactive oxygen species (ROS) (24), the release of chemical additives – namely plasticisers (25, 26) – and increase plastic charge and porosity (18). This abiotic plastic weathering is facilitated by the formation of biofilms (27), termed 'plastispheres' (28), which specifically and rapidly adhere to the surface of plastics (29). These plastispheres are perhaps most likely to form on the sea surface microlayer (SML) – the micrometres thick primary interface between the ocean and atmosphere (30) – due to the inherent buoyancy of many polluting plastics (*Section 1.2.2*) and their subsequent accumulation in oceanic 'garbage patches' (31). Concerningly, this SML is also where some of the ocean's most prevalent biogeochemical cyclers accumulate to perform essential gas exchanges, and photosynthesis which aid in maintaining the ocean's natural population (32). The interaction between these microorganisms and plastic has therefore attracted global interest, with increasing research now demonstrating which microorganisms most strongly associate with the plastic (*Sections 1.2; 1.3*). However, the impact of the anthropogenic chemicals which aggregate in and around the plastic-bound biofilm (22) on the well-studied formation (33), and composition (34, 35) of the plastisphere, as well as its capacity for plastic biodegradation (36, 37), and pathogenicity (22, 35, 37) has not been reviewed. Mammo and co-workers (2020) (15) and Amaral-Zettler (2022) (34) have

discussed the role of co-pollutants in the development of antibiotic resistance in pathogens, however this is not the only process which may be impacted by chemical-microorganism interactions. The activity of pathogens may also be inhibited by co-pollutants, while plastic-biodegrading organisms, or microorganisms important to biogeochemical cycling may also be affected (25, 38-40). Other aspects of the plastisphere, including the chemical composition of the plastic (35, 41-43), and organism-organism interactions within the biofilm (44, 45) may also impact these microorganisms and their metabolic processes. Greater exploration of these complex interactions is therefore required to understand the impact of plastisphere colonisation on microbial metabolism.

In this review, we outline the selection pressures within the marine plastisphere and use studies examining the impact of plastisphere-related factors on single-organisms to synthesise how they may impact plastisphere metabolism. We elaborate on the role of location and plastic type in selecting for the early plastisphere's primary microorganisms; topics presented within previous reviews (15, 33, 34, 36, 37, 46). For the first time, we also introduce organism-organism and organism-co-pollutant interaction as other essential parameters that are fundamental to the formation and functioning of the plastisphere. The impacts of chemical and heavy metal pollutants on the plastisphere have also gained little attention (22, 33, 36), and is therefore a major focus of this review. Lipophilic contaminants such as the organic ultraviolet (UV)-filters used in plastics and personal care products (19, 47) have a greater capacity than most POPs to bind to plastic and impact the plastisphere. These organic UV-filters also accumulate in marine coastal environments (47) due to the increasing number of tourists in densely populated coasts (22, 48), and are therefore discussed in this review as co-pollutants of critical importance for plastisphere research. For the first time, this review discusses the core molecular interactions which must be considered when studying plastisphere formation, metabolism, and related processes, and provides instructions for future research in the field.

1.2 Influences of plastisphere formation

1.2.1 *Plastisphere location*

The world's oceans, though interconnected, are biogeochemically defined by broad variations in nutrient and light availability, temperature, pressure, salinity (49, 50), and even pollution (Section 1.4.2; 1.4.3; 1.4.4). This creates many distinct niches within the water column to which each microbial species must adapt by the constitutive expression of traits increasing their survival (51, 52). This cultivates unique communities at each location specifically adapted to their associated biogeochemical pressures (51). As a result, once plastic is introduced into a particular region, only the microorganisms suited to that region can bind to it (49, 50). The current consensus is that this initial binding is indiscriminate, so members of the plastisphere will largely reflect the community composition of this region (34, 37). In most marine regions, the SAR11 clade (Alphaproteobacteria) dominates the pelagic microbial community, while *Prochlorococcus*, *Synechococcus* (Cyanobacteria) (53), *Rhodobacteraceae* (Alphaproteobacteria), *Flavobacteria* (Bacteroidetes), and other Alphaproteobacteria, Bacteroidetes, and Gammaproteobacteria are also regarded dominant classes (43, 50, 54). However, only biofilm-forming (Section 1.2.3) species able to withstand the plastisphere's competition (Section 1.2.4) and co-pollutant toxicity (Section 1.4) will be enriched in the plastisphere. *Rhodobacteraceae* and *Flavobacteria* are still dominant on plastic in many locations, though *Prochlorococcus*, *Synechococcus*, and the SAR11 clade, along with many other SAR and OM1 clades are not (31, 43). The dominant bacteria in these biofilms are instead a mix of the other less-dominant classes found in those regions (31, 43, 50, 54, 55). We compiled data from 97 plastispheres across 34 different studies which investigated plastisphere composition after a plastic's long or short exposure to endemic seawater communities (Figure 1.1). Using taxonomic class as an indicator of plastisphere composition, these studies show that the heterotrophs Gammaproteobacteria, Alphaproteobacteria, and Bacteroidetes are the most dominant bacterial taxa on plastic regardless of location (Figure 1.1). Warmer climates (Figure 1.1c-f), however, are revealed to produce more heterogeneous plastispheres than temperate climates (Figure 1.1a-b; 1g-h) due to the high species richness (56) and accelerated microbial growth (57). Indeed, 13.9% - 49.3% of these plastispheres represent microbial classes rarely observed in cooler climates (i.e. Actinobacteria, Sphingobacteria) (Figure 1.1c-f). This community-level variation between plastispheres is likely to increase when lower-level taxonomic ranks (e.g., family, genus) are investigated

(58). This would also facilitate ecological interpretation of the plastisphere's functional ecology as known taxa are linked to their metabolic roles.

Coons and co-workers (2021) (49) studied this effect across four global regions, finding biogeographical location to impact plastisphere community composition more than any other factor. In another cross-hemisphere study of three distinct global regions, consistencies were identified between plastispheres of each location, though phylogenies were mostly distinct (50). Amaral-Zettler and co-workers (2021) (59) also examined plastic debris across four locations in the Mediterranean Sea, noting a gradual succession of plastisphere communities between regions. A greater distinction may be seen between plastispheres originating from different non-marine sources (i.e. soils, freshwater, wastewater) (33, 58), though this has not been studied in depth. The marine plastisphere is also occupied by archaea, algae, fungi, and protozoa (28, 31, 54). The distribution of these additional kingdoms is also impacted by biogeography (51), meaning their abundance and taxonomic diversity would also depend on location. Early plastic colonisation is therefore highly opportunistic, reliant on the present microbial communities capable of exploiting this niche (*Section 1.2.3*) (60) which is dependent on the region's temperature, salinity, and even co-pollutant concentrations (*Section 1.4.2; 1.4.3; 1.4.4*).

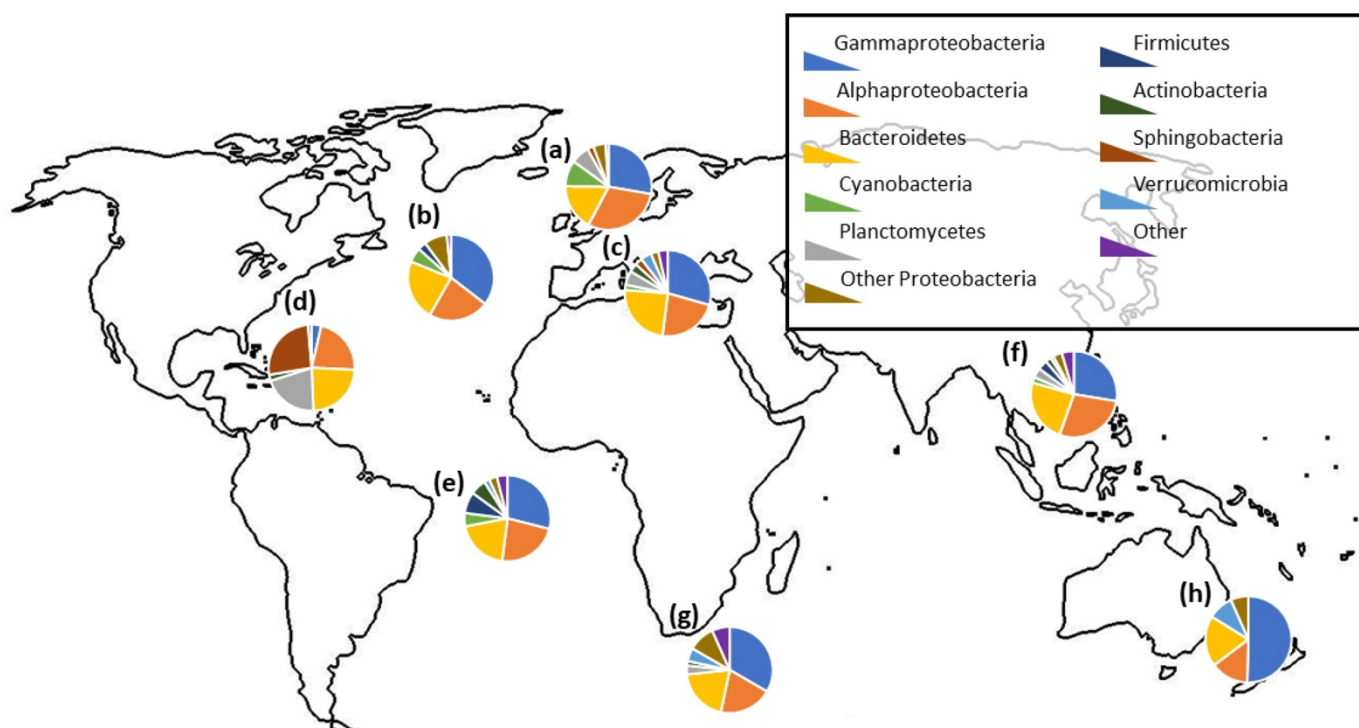


Figure 1.1 Bacterial classes present on plastics in different ocean regions.

Data was collected from 34 studies of plastisphere composition. With the exception of Scales and coworkers' (2021) (50), and Coons and coworkers' (2021) (49) studies, the community on one plastic type at one time point was considered a single plastisphere. In the aforementioned studies, data provided for bacterial abundances were given per location, so each location was also considered a 'plastisphere' here. Within the 34 studies, a total of 97 plastispheres were used to build pie charts: 13 (a), 10 (b), 18 (c), 16 (d), 7 (e), 22 (f), 4 (g), and 7 (h). Each lineage within each plastisphere was rated via a five-point scoring system: 10 for the most abundant, 8 for the 2nd, 6 for the 3rd, 4 for the 4th, and 2 for the 5th most abundant. To provide the final data for the pie charts, scores from all represented lineages were then totalled for each location. Microorganisms representing 6th most abundant communities or fewer were omitted, and total scores ranged from 0 to 184 per bacterial class per location. All charts presented here contain data retrieved from a mix of coastal, beach, and oligotrophic locations (Supplementary Table1).

1.2.2 Plastic type

All plastics consist of hydrocarbon chains, some of which contain aromatic benzene rings (polystyrene, polyethylene terephthalate: PET), methyl groups (polyethylene, polyamide, PET, polypropylene), or other functional groups (e.g. carbonyls, ketones, esters) (61). These groups increase polymer branching which separate the hydrocarbon chains within the polymer matrix, therefore producing either aliphatic (loosely packed) (e.g. polyethylene and polypropylene), or crystalline (tightly packed) plastics (e.g. polystyrene and polyvinyl chloride: PVC) (61). Within minutes of immersion in seawater, dissolved organic matter (DOM) and abiotic nanoparticles (i.e. trace metals) collect on the surface of submerged

materials (62). Marine microorganisms are then drawn to their surface through hydrophilic interaction, electrostatic interaction, or Van der Waals force, facilitated by functional groups expressed in the ecocorona (Figure 1.2) (62, 63). Plastic charge, crystallinity, hydrophobicity, buoyancy, and surface roughness are therefore often used to explain differences in plastisphere diversity (54, 64, 65).

Frère and co-workers (2018) (43), and Miao and co-workers (2020) (41) found that polystyrene and PVC -bound communities were significantly different from aliphatic communities. Zhang and co-workers (2022) (42) found microbial diversity to vary between all tested plastic types. A meta-analysis of extant plastisphere studies (35) also revealed that aliphatic plastic attracts different microorganisms to other plastics, likely due to the varied, sometimes aromatic functional groups that these other polymers contain (61). Chemical additives also vary between plastic types (*Section 1.4*) [64], further contributing to the differences observed between plastispheres (41, 43). However, the influence of plastic-type on microbial community formation appears less significant than the influence of location (49, 50), and location-dependent factors such as salinity (58). The surface chemistry of different plastics also converges after biofilm formation, accounting for the overlap in biofilm diversity observed in the latter stages of plastisphere development (54, 66). This reduces the role that plastic type may have in late plastisphere colonisation, though early biofilm formation is still dependent on the chemical composition of the base substrate (42, 54, 65), which can vary between plastics (61). Plastic type may not influence plastisphere formation as much as location, though it is still important for our understanding of why the plastisphere's microorganisms are there, and thus requires further investigation.

1.2.3 Attachment strategy

Microorganisms anchor themselves to substrates, including plastic, to form a biofilm which provides protection from environmental stressors, nutrient limitation, and predation (67). This ability of microorganisms to bind to plastic is a potential selection pressure of the early plastsphere, as only a limited number of microbial species express morphologies suited to substrate binding. *Hyphomonas*, for example, are often found in the plastsphere (28, 60), possibly due to their uncommon expression of prosthecae, which are used for substrate binding (28). Quorum-sensing as performed by some *Rhodobacteraceae* – the most abundant Alphaproteobacteria in the plastsphere (28, 31, 68) – may also allow for better navigation to and throughout the plastsphere, allowing these lineages to more rapidly colonise and persist. Negatively-charged lipopolysaccharide (LPS) or capsular colonic acid expressed by Gram-negative bacteria also strengthens a bacterium's ionic, hydrogen, and covalent-bond interactions with plastic, and thus their final attachment (64). For example, *Sphingomonas*, an Alphaproteobacteria (28, 50, 69) expresses Glycosphingolipids (70), a more negatively charged alternative to LPS, possibly contributing to its abundance in the plastsphere (28, 50, 69). No study has directly explored the impact of cell wall composition on plastsphere adhesion, though inferences can be made of the plastsphere's most effective adhesion strategies based on its most common microorganisms, and the strategies they use.

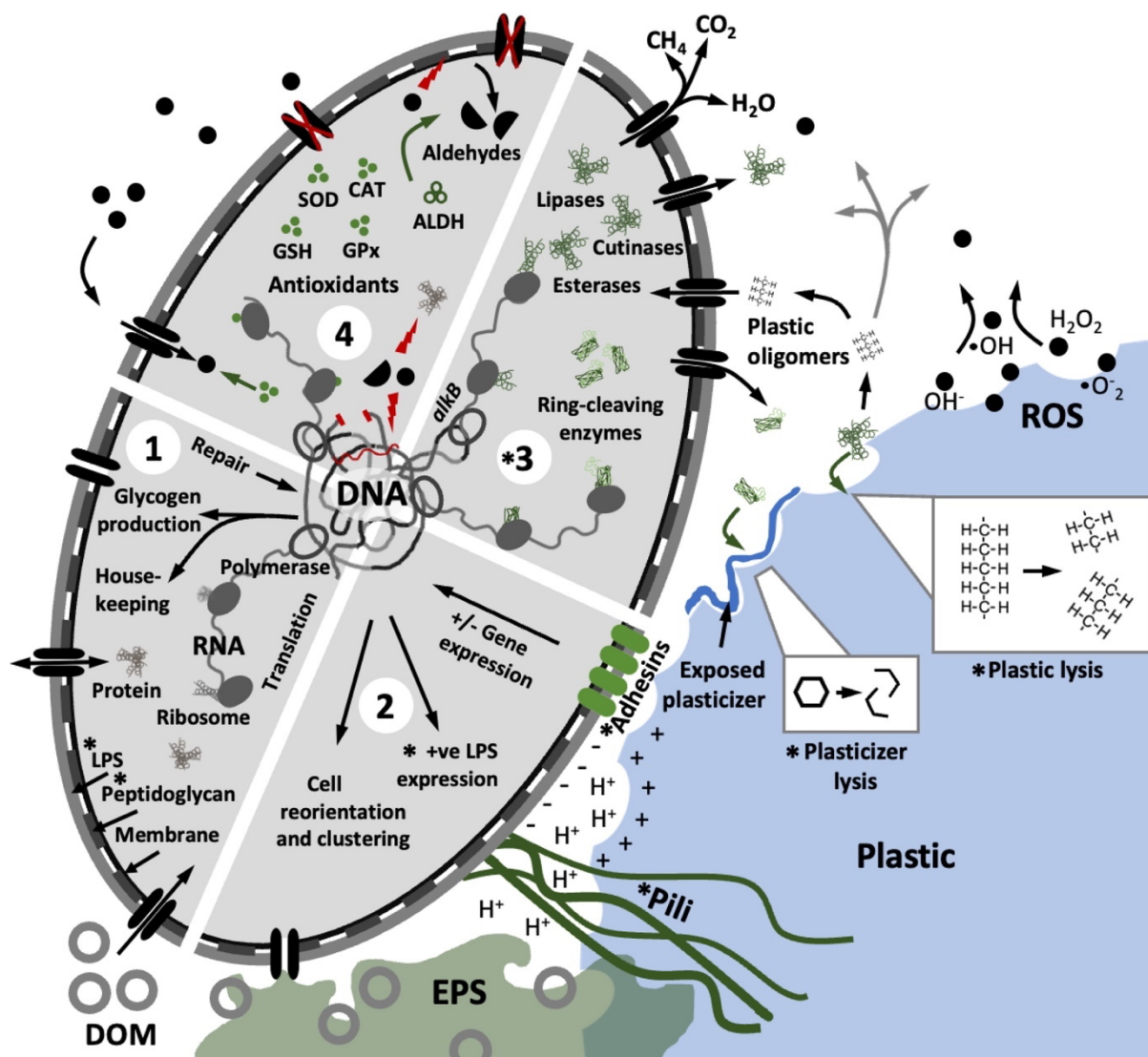


Figure 1.2 Inferred molecular interactions between marine microorganisms and plastics.

1 Neutral interaction: Homeostatic bacteria. **2** Adhesion: Bacteria are drawn toward plastic through electrostatic interaction. Gene expression then shifts so they can adhere to the plastic using pili, adhesins, and by secreting EPS, and orientate themselves within the establishing biofilm. **3** Biodegradation: Ring-cleaving enzymes are expressed which liberate plasticizers from the plastic, making it more susceptible to depolymerization via the enzymatic action of lipases, cutinases, and esterases. The resultant plastic oligomers and DOM-like plasticizers are released from the plastic allowing this and other bacteria to utilize them as a carbon source. CH₄, CO₂ and H₂O are also released as a byproduct. **4** Toxicity: ROS released from plastic weathering react with the cell membrane (releasing aldehydes), proteins (resulting in their misfolding), and DNA (causing mutations and double-strand breaks). Aldehydes also damage DNA and proteins. Cells produce antioxidants and ALDHs to mitigate this damage, which diverts energy away from maintaining homeostasis and normal cell functioning.

The mechanisms depicted herein are synthesized from the literature and demonstrated for a Gram-negative bacterium, although the concept applies to other microorganisms. Processes and structures unique to bacteria marked with a '*'. Green – Molecules involved in cell response to binding Red - Disruption to cell; MP - Microplastic; DOM - Dissolved organic matter; ROS - Reactive oxygen species; LPS - Lipopolysaccharide; EPS – Exopolymers substance; CAT - Catalase; SOD - Superoxide dismutase; GSH - Glutathione; GPx - Glutathione peroxidase; ALDH - Aldehyde dehydrogenase; alkB - Alkane hydroxylase gene.

In the plastisphere, one of the dominant prokaryotic classes, Gammaproteobacteria is represented by species of *Alteromonadales*, *Pseudomonadales*, *Vibrionales*, and *Oceanospirillales* (69, 71, 72). All Gammaproteobacteria are Gram-negative, meaning they express LPS, facilitating their attachment to plastic (64), while *Pseudomonadales* and *Vibrionales* also express type IV pili (Tfp), which can anchor them to the debris (Figure 1.2.2) (73). The Gram-negative Planctomycetes and Cyanobacteria, mixed-Gram Firmicutes, and Gram-positive Actinobacteria also bind frequently, which in the case of Actinobacteria, may be due to the production of sprawling filamentous structures specifically for substrate binding (Figure 1.1) (74). Despite being less abundant than Proteobacteria and Bacteroidetes, these are some of the most active microorganisms in the plastisphere, particularly Cyanobacteria (58, 75), which secrete EPS for protection and microbial aggregation (Figure 1.2.2) (69, 76, 77). Arthropods, sponges, cnidaria, nematodes, and a range of protists, also significantly contribute to plastisphere diversity (28, 31, 54). However, it is not known how many present eukaryotes, such as the protozoan *Radiolaria*, bind to plastic (28). Archaea and fungi are also rarely observed (29, 59, 66) considering their ability to bind to substrates using archaella and hyphae (31, 54, 73). Cell morphology therefore seems to play a lesser role in plastisphere adhesion for eukaryotes and archaea compared to bacteria. It is important to note that bacteria are generally primary colonisers, while eukaryotes and archaea are secondary colonisers (31, 45). It is therefore likely that cell structure, alongside location, impacts the constitution of the early prokaryotic plastisphere more than the developed plastisphere.

1.2.4 Organism-organism interaction

In the environment, a combination of top-down and bottom-up selection pressures shape the composition of biofilm communities (65). The number of grazers and pathogens which negatively impact bacterial populations (28, 78) may therefore be depleted in the plastisphere through the joint secretion of toxins and EPS by bacteria and algae (67, 79).

Pseudoalteromonas sp. also produce metalloproteases that specifically target peptidoglycan, and consequentially, Gram-positive bacteria (80). Gram-positive Actinobacteria then produce antibiotic enzymes (81) which are likely used to compete with and protect against Gram-negative bacteria. This enzyme production, if widespread, may contribute to the lower abundance of Gram-positive bacteria in early plastispheres (Figure 1.3) (72). However, there is no direct evidence of these enzymes within the plastisphere (68, 75) despite their common use in bacteria (Figure 1.3.2b) (30, 70). Many of the most abundant bacteria have also been

found to be dormant on plastic (68, 75), suggesting that this antagonism would not be a selection pressure in these plastispheres.

Regardless of their activity, microorganisms constitutively express negatively charged and adhesive proteins (Figure 1.2.2) (27, 64) such as those which allow Bacteroidetes, Actinobacteria, and Alphaproteobacteria to attach to plastic (*Section 1.2.3*) (15, 69, 73). Attracted by bacterial hydrophilicity (62, 71, 79), diatoms are drawn to the plastisphere during early stages of microbial succession (Figure 1.3) (57). All diatoms accommodate an associated phycosphere in which bacteria (namely *Rhodobacteraceae* and *Flavobacteria* species) assemble for mutual gain (79). Members of these phycospheres are then immobilised against stress through the exchange of dissolved organic carbon (DOC) and horizontal gene transfer (Figure 1.3.1a; 1b) (82). This may explain why *Rhodobacteraceae* and *Flavobacteria* are two of the three most well represented (71), metabolically active (68) bacterial lineages in the plastisphere (Figure 1.3), although no published work has so far characterised active symbiotic interactions within the plastisphere. Diatom abundance remains consistent as the plastisphere population stabilises (28, 54, 60, 83), also supporting the notion that the plastisphere biofilm is an inclusive community (45).

By examining the understood interactions between species found in the plastisphere, we can therefore predict that the presence and abundance of species during early succession will impact that of the latter species (57, 62, 84) as in other communities. This concept of inclusion versus inhibition is commonly used when exploring selection pressures of newly created niches. However, this is not explored in the study of plastisphere progression and may be one of the reasons for plastisphere variation between locations.

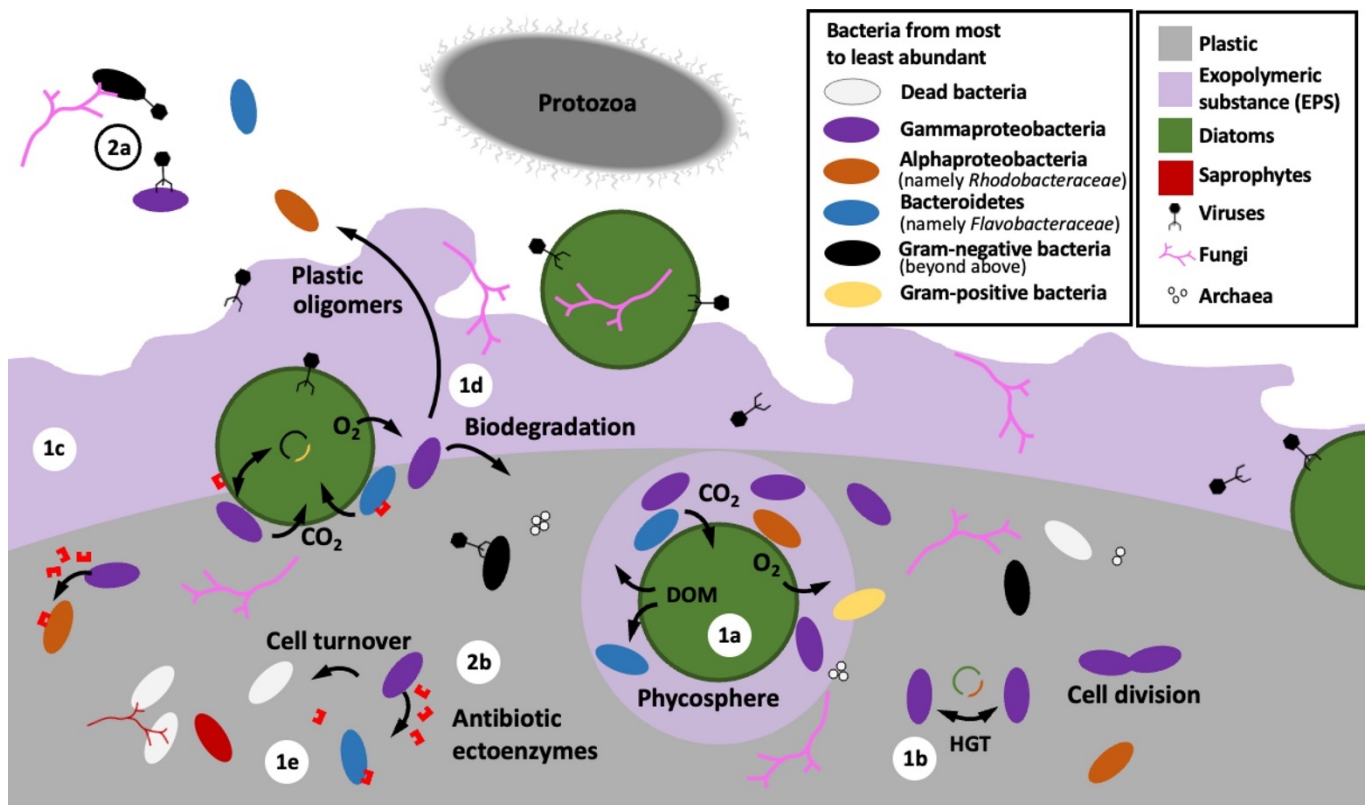


Figure 1.3 Predicted organism-organism interactions within the marine plastisphere.

The plastisphere is a diverse microbial community, primarily consisting of bacteria, then diatoms, fungi, and Archaea. Eukaryotes other than diatoms and fungi are also noted as plastisphere constituents, but are not included here.

1 Symbiosis: **a** Bacteria are often found within the phycosphere, allowing bacteria and diatoms to cooperate by exchanging essential supplies and **b** genetic material (via HGT). **c** Both also benefit from their mutual production of EPS which may trap and remove grazers (protozoa) and parasitic organisms (viruses and fungi) from the plastisphere. **d** Oligomers produced by plastic degradation, and **e** the dead microorganisms produced by the biofilm's high cell turnover also fuel heterotrophic, and saprophytic growth inside and outside the plastisphere. **2 Competition:** **a** Parasitism by viruses and fungi may occur within the plastisphere. **b** To reduce resource competition, bacteria can release antibiotics, lysing nearby cells, increasing the amount of nutrients (in the form of lysate) available, and reducing the number of other microorganisms competing for the same resource.

DOM - Dissolved organic matter; HGT - Horizontal gene transfer. Estimates for most abundant bacteria from (Supplementary Table1).

1.3 Plastisphere function

1.3.1 Plastic biodegradation

The discovery of microorganisms able to utilise plastic polymers as a source of carbon is still ongoing, though this research has already identified plastic-biodegrading species in many global ecosystems, including the ocean (71, 72, 85). To biodegrade plastic, aromatic plasticisers (e.g. phthalates) stabilising the polymer matrix (26), are first removed by physical weathering (12), or through the specialised bacterial expression of ring-cleaving enzymes, such as homogentisate 1, 2- dioxygenase (Figure 1.2.3) (31, 86). By oxidising and hydrolysing plastic's chemical chains using lipases, esterases, and cutinases, plastic-degrading bacteria then depolymerise the plastic surface into oligomers (Figure 1.2.3) (86), a process also performed by some fungi and archaea (87, 88). With no shortage of water or oxygen to fuel these processes in the ocean, considerable concentrations of CO₂, H₂O, N₂ and CH₄ are produced by the plastic-utilising microorganisms, alongside the hydrocarbon oligomers (Figure 1.2.3) (89). Many of these hydrocarbon oligomers are further broken down within the plastisphere, or are directly transported into the cell with the aid of biosurfactants (90). The remaining hydrocarbons are then captured in the water column, namely by Alphaproteobacteria which use esterases and hydrolases to liberate carbon-rich monomers (Figure 1.3.1d) [76]. This process continues until the plastic ceases to be a viable energy source; up to six months on polystyrene (92).

The polymers polyethylene, PET, and polystyrene can support several species of fungi (88) and bacteria (71) that express enzymes associated with this specialised metabolism. *Rhodococcus ruber* from marine sites, and *Pseudomonas spp.* from soil biodegrade polyethylene by expressing laccase and alkane hydroxylase to liberate small chain hydrocarbons (40, 93). The freshwater Actinobacterium *Arthrobacter sp. KI72* expresses hydrolases and oxidoreductases associated with polyamide metabolism (94). *Ideonella sakaiensis* from sediments and the fungus *Humicola insolens* both secrete cutinases associated with PET depolymerisation (85, 95). The *Oceanospirillales*, *Alcanivorax borkumensis* forms thick, uniform colonies on polyethylene after two months (71). And in the marine environment, *Thioclava dalianensis*, and *Bacillus aquimaris* express hydrolases, oxidoreductases, and other uncharacterised enzymes associated with PET depolymerisation (72). Capable of accessing these secure carbon stores, such bacteria are likely to outcompete

others on their respective plastics, particularly during early plastisphere succession (69). This may explain why microbial diversity has been found to differ on plastics compared to organic substrates (42, 65, 96). Although conflicting results from Kirstein and co-workers (2018) (54), and Woodall and co-workers (2018) (66) reported no effect of substrate type on microbial diversity. It is also unlikely that plastic biodegradation significantly influences plastisphere formation as metabolism is reduced upon binding to plastic (41, 97). In fact, in the first studies of plastisphere protein expression, no plastic-biodegrading microorganisms or metabolic biproducts of degradation have so far been found associated with an active plastisphere (68, 75). It is therefore suggested that when metabolically active, early colonisers instead use DOM (84, 98) or photosynthesis (31, 35, 57, 65, 68) for growth, while latter colonisers, including saprophytes (Figure 1.3.1e), use cell detritus (65, 84). Factors other than a microorganism's use of plastic polymers (*Section 1.2; 1.4*) must also impact the presence and activity of each microbial species in the plastisphere. As a valuable route for pollutant bioremediation, this should be considered in ongoing study of the plastisphere and plastic biodegradation.

1.3.2 Pathogens in the plastisphere

Horizontal gene transfer (HGT) is the exchange of genetic information between microorganisms for the benefit of the recipient, and often the entire microbial community (38). In the plastisphere, this increases the resistance of all microorganisms capable of HGT to stressors, including antibiotics (82, 99). Potentially pathogenic bacteria have been of particular interest concerning the development of this antibiotic resistance (82, 99-102). For example, *Vibrionaceae* can dominate a marine plastisphere (68, 69), including *V. cholerae* (102), *V. parahaemolyticus* (54), *V. anguillarum* (69), and *V. vulnificus* (100) which are often associated with water-borne diseases (102). As antibiotic resistance is promoted on the surface of plastic (99-101), the virulence of these potentially pathogenic bacteria may also be increased. Concerningly, microplastics facilitate the transport of pathogenic bacteria throughout the water column and up the food chain once consumed by marine organisms (15, 103), and are therefore considered a significant risk to public health (22). However, the virulence of these bacteria on plastic has not yet been confirmed (68, 75), and needs to be further investigated.

1.4 Influence of ROS and co-pollutants

1.4.1 Plastic toxicity

Plasticisers, other POPs, and heavy metals induce toxicity in marine species by infiltrating essential tissues and disrupting cell homeostasis (74, 104, 105). Upon ocean weathering and photodegradation, the plastic itself also induces stress through the formation of polymer radicals, leading to carbon-chain scission and the production of ROS (106). Due to their lack of tissue barriers, microorganisms in the plastisphere are directly exposed to the ROS that plastics produce (24), making them more likely than larger organisms to be negatively impacted by oxidative stress (Figure 1.2.4; Figure 1.4.3).

Plastic-induced oxidative stress affects microbial metabolism and physiology in a plastic- and species- specific manner (Figure 1.2.4; Figure 1.4.3). For instance, exposure to plastic reduces nitrogen cycling and cellular rearrangement in *Halomonas alkaliphila* (107), and impairs membrane function, photosynthesis, and DNA integrity in the marine diatom *Phaeodactylum tricornutum* (108). Polyethylene and polypropylene ROS increase EPS production and expression of apoptotic markers in the freshwater algae *Chlamydomonas reinhardtii* (109), and polystyrene impairs membrane integrity, and promotes microcystin toxin production in the freshwater cyanobacterium *Microcystis aeruginosa* (110). However, 200 mg/L of polystyrene increases the growth of *Clostridiaceae*, *Geobacteraceae*, *Dethiosulfovibrionaceae*, *Desulfobulbaceae*, and *Anaerolineaceae*, while decreasing the growth of *Cloacamonaceae*, *Porphyromonadaceae*, *Gracilibacteraceae*, and other *Anaerolineaceae* (111). *Phaeodactylum tricornutum* is also unaffected by 500 mg/L of polyethylene (112), which is 10x the plastic toxicity threshold in other algal species (113, 114). These studies suggest that plastic type may influence plastisphere composition (Section 1.2.2). However, as extant plastic toxicity has exclusively been explored in model bacteria, the wider implications of plastic-induced ROS on the plastisphere remain unknown. Metabolomics, proteomics, or specialised redox (reduction and oxidation) assays would reveal if microorganisms of the plastisphere experience oxidative stress and reduced functionality after binding (Section 1.5.2).

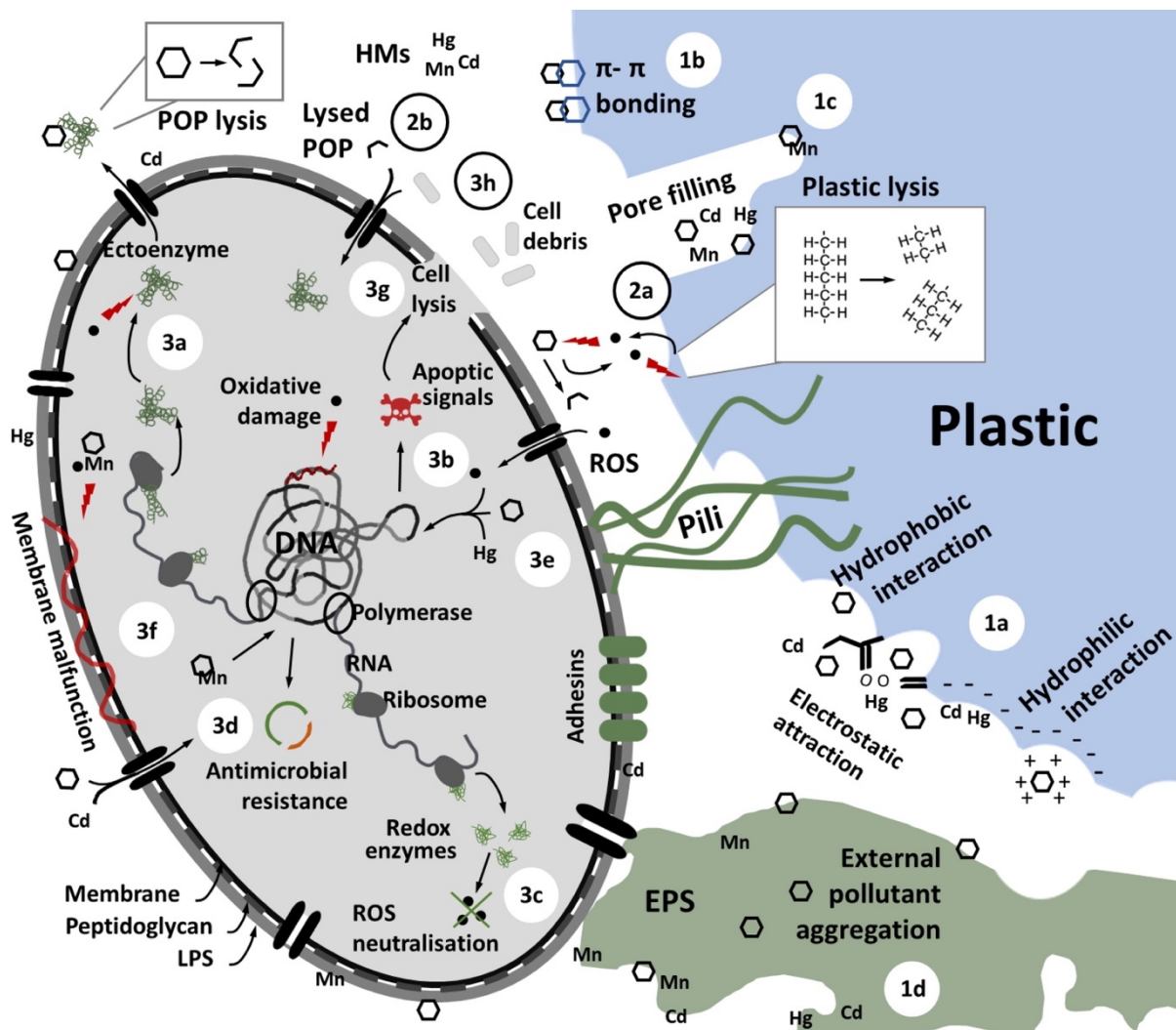


Figure 1.4 Deduced molecular interactions between microorganisms and plastic's co-pollutants.

1 Binding: Pollutants are first attracted to the plastisphere through **a** hydrophilic interaction, hydrophobic interaction, electrostatic attraction, and **b** π - π bonding. **c** These are then retained in plastic pores, **d** and in secreted EPS, keeping most pollutants from entering the cell.

2 POP Lysis: **a** The ROS released from pollutants expedites the POP and plastic degradation driven by microorganisms (Fig. 1.2), **b** which can fuel much cell growth. **3** Stress: ROS also **a** disrupts protein synthesis, **b** and induces apoptosis. In response to this persistent exposure to POPs and HMs, microorganisms will **c** produce antioxidants, and develop a resistance to this stress, **d** also propagating antimicrobial resistance. Persistent stress, and **e** direct exposure to certain POPs and HMs will ultimately lead to **f** structural deformation and **g** cell death.

h However, this ROS-induced cell turnover increases saprophytic cell growth in the plastisphere. Processes are assumed from literature review involving studies of ecotoxicology, bioremediation, and biofilm function. Branched oxygenated structures on plastic - Cationic exchange sites (i.e. carboxyl groups); $\curvearrowright$ - oxidative damage; POP - Persistent organic pollutant; HMs - Heavy metals (Hg, Cd, and Mn used to represent the group); EPS - Exopolymeric substances; ROS - Reactive oxygen species; LPS - Lipopolysaccharide. Regular cellular processes are highlighted in green; pollutant-induced cellular damage and disruption is highlighted in red.

1.4.2 Heavy metals

Plastics, unlike other materials found in the ocean, are a source of heavy metals and anthropogenic chemicals, as well as a substrate on which they can aggregate (18, 22, 61, 98). These pollutants are therefore likely to be a far more effective and persistent vector for pollutant transport than any other marine debris (17, 21, 22, 115). The plastic degradation performed in part by bacteria also exposes O₂ functional groups (Figure 1.4.1a) (115), cationic exchange sites (Figure 1.4.1a) (92), and increases plastic porosity (Figure 1.4.1c) (28). DOM produced in the biofilm then further increases electron abundance, and consequently site polarity (21, 77), attracting more pollutants and microorganisms to the plastisphere.

As cationic substances, heavy metals bind with greatest affinity to polar plastics (e.g. PVC) through electrostatic attraction (Figure 1.4.1a) (21), although are found on a range of plastics, particularly after ocean weathering (76, 77, 116). Fe²⁺ is the most common of these metals in the water column, and when attached to plastics, which also accumulate Al, Mn, Zn, Pb, Cu, Cr, Ni, Co, Cd, Hg, Ag, and As (*Table. 1*). Once bound, the production of ROS from plastic and heavy metals can oxidise co-pollutants (Figure 1.4.2) (117), and cause oxidative stress in affected organisms (Figure 1.4.3) (105).

Microorganisms are exposed to a greater concentration of heavy metals in the plastisphere than in the water column, so are more likely to experience heavy metal toxicity (Figure 1.4.3). Ag, Cu, and As display bactericidal properties (117-119) and have been found on plastic (99, 105). Cu (5 µg/L) precipitates glutathione peroxidase and catalase expression – indicators of oxidative stress – in the microalgal species *Rhodomonas salina* (118, 119). In *Escherichia coli*, Ag elicits a severe disruption of intracellular protein transportation, resulting in membrane destabilisation and adenosine triphosphate depletion, ultimately causing cell death (120). The interaction between heavy metals and plastic also exacerbates Cu toxicity in *Chlorella vulgaris* (121), and Au toxicity in *Tetraselmis chuii* (104). However, heavy metals can also accelerate plastic biodegradation (40) and increase microbial resistance to other pollutants, such as antibiotics in the plastisphere (*Section 1.3.2*) (99, 122). Heavy metals may therefore regulate many metabolic processes within the plastisphere, and require further exploration.

Table 1. Heavy metal concentrations on natural substrates and plastics (ppm)

	<i>Fe</i>	<i>Al</i>	<i>Mn</i>	<i>Zn</i>	<i>Pb</i>	<i>Cu</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Cd</i>	<i>Hg</i>	<i>Ag</i>	<i>As</i>
<i>SSW</i>	^a 94	^a 62.8	^a 2.02	^a 0.15	^a 3.2x10 ⁻²	^b 0.123	^a 1.93	^a 0.71	^b 5x10 ⁻³	^b 5x10 ⁻⁴	^b 7.7x10 ⁻⁴	^a 1.7x10 ⁻⁴	^a 1.28x10 ⁻²
	^b 0.34	-	^b 0.29	^b 5.7x10 ⁻²	^b 5x10 ⁻³	^a 0.102	^b 2.1x10 ⁻²	^b 6.8x10 ⁻²	^c 2.3x10 ⁻⁶	^a 1.1x10 ⁻⁴	^a 1.9x10 ⁻⁴	^c 3.56x10 ⁻⁵	^d 5.7x10 ⁻³
	^c 2x10 ⁻⁵	-	^d 3.5x10 ⁻³	^d 6x10 ⁻³	^d 8.2x10 ⁻⁵	^d 7.6x10 ⁻³	^d 1.2x10 ⁻³	^c 4.13x10 ⁻⁴	-	^d 3.8x10 ⁻⁴	-	-	^b 3.9x10 ⁻³
	-	-	^c 4.6x10 ⁻⁵	^c 3.14x10 ⁻⁴	^c 9x10 ⁻⁶	^c 1.5x10 ⁻⁴	-	-	-	^c 7x10 ⁻⁵	-	-	-
<i>Plastic</i>	^f 97.8	^f 55.8	^d 31.252	^f 2.68	^d 1.318	^f 0.44	^f 237	^f 131	^f 107	^f 1.65	^g <3x10 ⁻⁴	^g <3x10 ⁻⁴	^d 3.7x10 ⁻²
	^g 34.4	^g 7.43	^f 20.5	^d 1.4x10 ⁻²	^f 1.02	^d 0.223	^d 8.4x10 ⁻²	^g 2.9x10 ⁻²	^g 1.3x10 ⁻²	^d 2.3x10 ⁻²	-	-	-
	-	-	^g 0.712	-	^g 0.11	^g 4.7x10 ⁻²	^g 4.25x10 ⁻²	-	-	^g 5x10 ⁻³	-	-	-

Data collected from **a** sediment in Puerto Rico (123), **b** seawater in Danube Delta (124), **c** seawater in Ross sea (125), **d** seawater, and pristine plastics in China coast (116), **e** seawater in Ottawa (126), **f**, **g** pristine polyethylene and beached plastic in the UK (76, 77). Data from f and g namely from heavy metals attached to beached plastics, as they presented greatest concentrations.

Shaded regions express an increase in heavy metal concentration on plastics compared to sediment. SSW - sand and seawater; Fe – Iron; Al – Aluminum; Mn – Manganese; Zn – Zinc; Pb – Lead; Cu – Copper; Cr – Chromium; Ni – Nickel; Co – Cobalt; Cd – Cadmium; Hg – Mercury; Ag – Silver; As – Arsenic.

1.4.3 Persistent organic pollutants

Persistent organic pollutants (POPs) are utilitarian chemical products (74), turned pollutants after leaching into the ocean (Figure 1.5a). These POPs can be additives – core structural elements of plastics (61) – leached after photodegradation (25, 98), or superficially attached to plastics from another source (Figure 1.5b) (18). Non-polar pollutants, such as polycyclic aromatic hydrocarbons (PAHs), mainly attach to plastics through hydrophobic interaction (Figure 1.4.1a) (127). Aromatic pollutants, with a foundation of benzene rings, attach through π – π interaction, connecting to the benzene rings in plastics such as polystyrene and PET (Figure 1.4.1b) (61), while polar plastics (i.e. polyamide and PVC), are bound through electrostatic interaction, hydrogen interaction or hydrophilic interaction (Figure 1.4.1a) (18, 61, 128) .

Many POPs attach through a combination of the above, although non-polar, or hydrophobic interaction, particularly with polyethylene and polypropylene, represents one of the strongest of all interfaces (18, 129). However, hydrophobic PAHs such as phenanthrene are only found on plastics at concentrations reaching 16.4 mg/kg (Figure 1.5b). Conversely, phthalates and polychlorinated biphenyls (PCBs), which bind via π – π interaction, reach 84.6 mg/kg and 18.6 mg/kg on plastic surfaces respectively (Figure 1.5b). Pollutants binding through both of the aforementioned mechanisms, such as the aromatic, hydrophobic dichlorodiphenyltrichloroethane (DDT) have the greatest binding coefficients [96,900 K_d] (130), meaning that they have a great capacity to accumulate on plastics. DDT is therefore found on plastic at concentrations ranging from 0.2 mg/kg (127) to 1600 mg/kg (131). The variation in POP concentrations observed here may be due to environmental concentrations (Figure 1.5a) or sampling strategy.

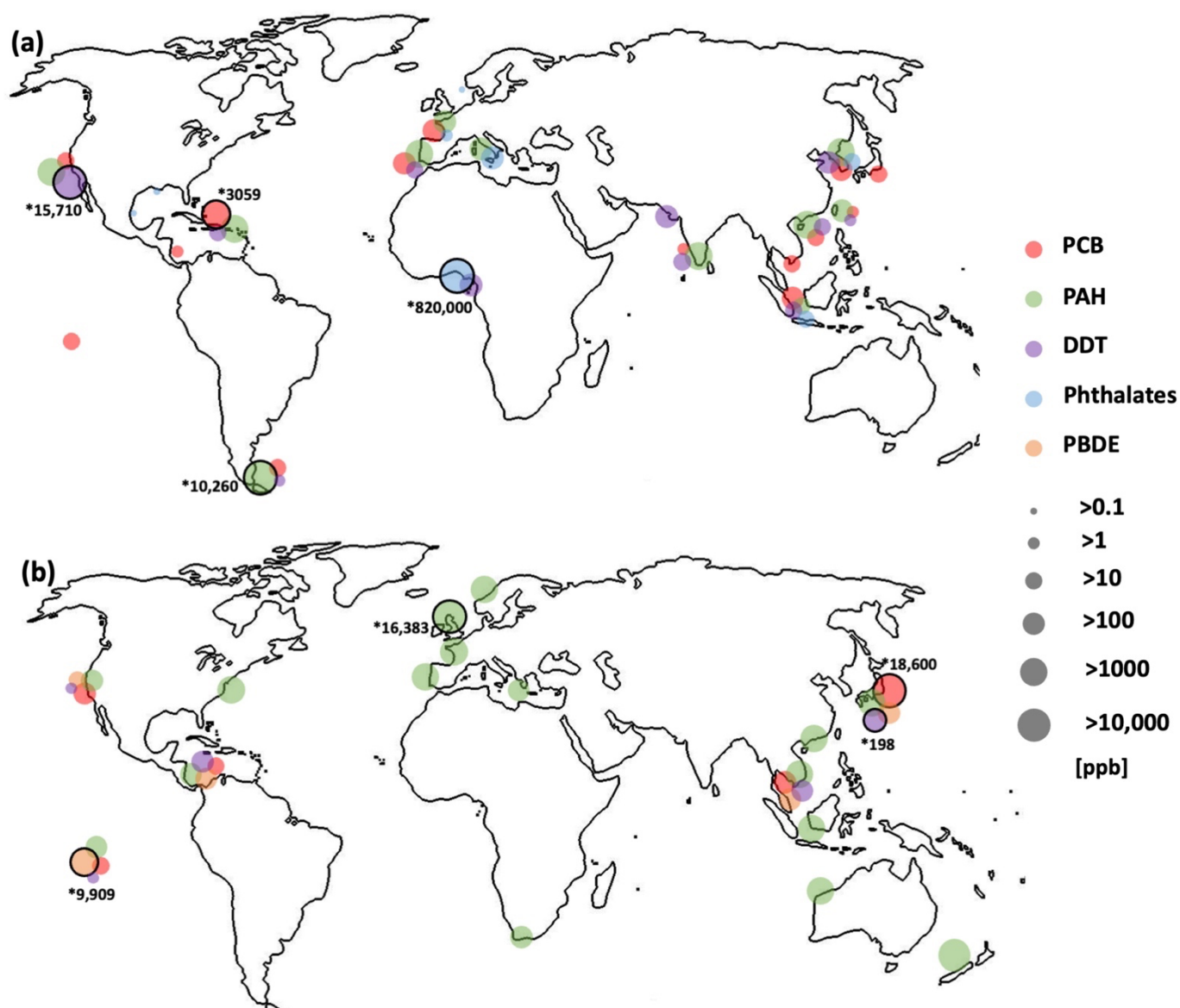


Figure 1.5 Global distribution of persistent organic pollutants.

Persistent organic pollutants have been detected in the ocean (a) and bound to plastics (b).

*(circled)- Highest recorded value for each persistent organic pollutant (POP); ppb - parts per billion;

PCB - polychlorinated biphenyl; PAH - polycyclic aromatic hydrocarbon; DDT -

dichlorodiphenyltrichloroethane; PBDE - polybrominated diphenyl ethers.

POP data: Supplementary Table 2.

Hydrocarbonoclastic microorganisms which can metabolise POPs, such as some Euryarchaeota and Proteobacteria, represent up to 34.4% of the plastisphere (132). By expressing hydroxylases capable of breaking the benzene rings within pesticides, PCBs and PAHs (86, 133), they can use POPs as carbon and energy sources (Figure 1.2.3).

Oceaniserpentilla sp. and *Celeribacter indicus* are the most common species responsible for this (35, 86, 92), though other bacteria can also perform this function (134), as demonstrated by Birnstiel and co-workers (2022) (98), who showed a positive correlation between the release of additives from low-density polyethylene and bacterial growth. This abundance of POPs in the plastisphere may therefore prevent plastic biodegradation in providing a more accessible source of carbon for these communities. However, no study has so far characterised the activity of the plastisphere relating to POP degradation, or which specific additives may be used as a source of carbon.

Rosato and co-workers (2020) (135) demonstrated that a singular POP is not sufficient to influence the community composition of the plastisphere, but the inclusion of multiple additives, such as those used to produce colour, may influence microbial abundance and diversity. When studying the colonisation of yellow, blue, and transparent plastics, Wen and co-workers (2020) (136) found a significantly greater abundance of *Aquabacterium sp.* on blue polyethylene plastics than any other colour. De Tender and co-workers (2015) (55) also noted the selective growth of *Mycobacterium frederiksbergense* on only blue and yellow patches of plastic, and not any other colour, attributing this selection to the presence of anthracene in only these tested plastics. It is therefore probable that these additives will impact the colonisation of the plastisphere; selecting for specialised hydrocarbonoclastic organisms, and selecting against microorganisms affected by POP toxicity.

The electron transfer between plastic and POPs has also been reported to propagate the release of free radicals from plastic (24). These free radicals can react with POPs to form chemical subspecies with higher toxicity (Figure 1.4.2) (137), which may increase oxidative stress in a plastisphere co-polluted by POPs. This would increase cell death, and thus the accumulation of biomass within the plastisphere, and also draw saprophytes to the biofilm, attracted by the abundance of dead microorganisms (Figure 1.3.1e; Figure 1.4.3h) (138). POPs can also be antimicrobial in application, as in the case of triclosan, which is more toxic when bound to plastics (139). In a study of autotrophic (*Halothece sp.* and *Fischerella muscicola*) and heterotrophic (*Cobetia sp.*, *Marinobacterium litorale* and *Pseudomonas azotifigens*) bacteria, the inclusion of additives – particularly Fluoranthene (a PAH) and dioctyl-phthalate – significantly reduced growth of all microorganisms on plastic, apart from *P. azotifigens* (39). The bacterium *Vibrio fischeri*, and the protozoa *Tetrahymena pyriformis* also experience acute toxicity (LC₅₀) when exposed to 11–23 mg/L and 7 mg/L of phthalate respectively (26). This species-specific response to pollutant exposure may be explained by differences in cell morphology (Section 1.2.3) (97), though the physiological and functional mechanisms underpinning these taxonomic differences require further investigation. Nonetheless, it is evident that microbial response to POP exposure is POP-dependent, and microorganism-dependent. POPs may therefore facilitate (98) or inhibit (39) processes such as bioremediation and biogeochemical cycling in the marine plastisphere.

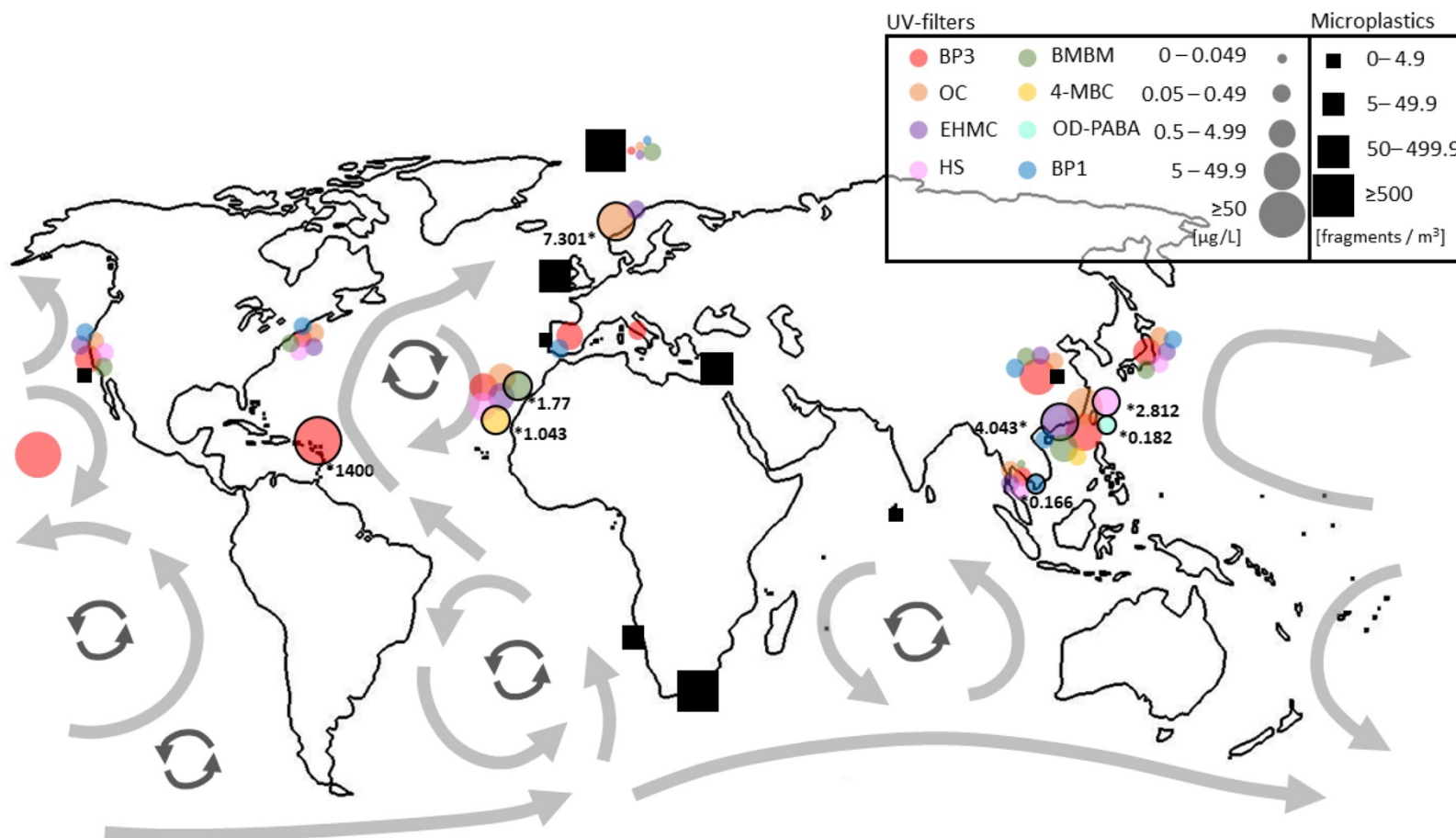


Figure 1.6 Global distribution of UV filters and microplastics.

Large grey arrows represent dominant ocean currents during northern hemisphere winter months and small cyclical arrows represent ocean gyres in which plastic accumulates, forming ‘garbage patches’.

*(circled) - Highest recorded value for each UV-filter (µg/L); BP – benzophenone; EHMC- ethylhexyl methoxycinnamate; OC - octocrylene; 4-MBC - 4-methylbenzylcathinone; HS - homosalate; BMBM - butyl methoxydibenzoylmethane; OD-PABA - ethylhexyl dimethyl p-aminobenzoic acid.

UV-filter data: Supplementary Table 3; Plastics data: (15)

1.4.4 Organic UV-filters

Organic UV-filters are compounds used to protect against UV-A (320-400 nm) and UV-B (290-320 nm) radiation (47). As a component of plastics and personal care products (PCPs), including sunscreens (19, 47), organic UV-filters have become a rising contaminant in municipal effluents, with up to 223 µg/L released in UK effluents alone (140). PCPs, unlike many POP sources, are used by a majority of the general public (47), making these organic UV-filters ubiquitous throughout the ocean (Figure 1.6). Unlike many other pollutants, organic UV-filters co-occur with plastics through their predominant use in coastal regions, where many plastics are found (48). Importantly, these pollutants are also highly hydrophobic, which leads to the formation of slicks on the SML alongside plastics, and in the microhabitat where they may have the greatest negative effect independently (30, 32, 141, 142). The organic UV-filter Benzophenone-3 (BP3), also known as oxybenzone, is used in many PCPs, and has been detected in the ocean at concentrations reaching 1.395 mg/L when measured in close proximity to divers (143). However, ambient concentrations of BP3 typically reach up to 5 µg/L (144), and the concentrations of other organic UV-filters including octocrylene, 4-methylbenzylidene camphor (4-MBC), and ethylhexyl methoxycinnamate (EHMC), reach up to 7.301 µg/L, 1.043 µg/L, and >4 µg/L respectively (Figure 1.6). Significantly, organic UV-filters remain chemically stable in the environment for >2.4 years (19), and sorb to plastics with a coefficient of up to 53,200 K_d (4-methylbenzylidene) (145). PCP-sourced organic UV-filters also bind most strongly [$\text{Log}K_d \leq 4.58$] to the ocean's most abundant plastics, polyethylene and polystyrene (41, 129, 146), which in combination with those leached from the plastic, means many UV-filters are likely present in the plastisphere (Figure 1.4).

BP3 is the lowest molecular weight (228.243 g/mol) UV-filter (141), and as such, has the greatest capacity to surpass the lipid bilayer of cell membranes and disrupt life in the plastisphere. In algae, BP3 induces oxidative stress (147), cytotoxicity (148), as well as impaired metabolic functions (149), and photosynthetic profiles (147). *Isochrysis galbana* growth is reduced after exposure to concentrations as low as 13.8 µg/L of BP3; the lowest threshold for damage of all UV-filters tested with this species (8, 150). While, in a study of 27 bacterial strains, only Gram-negative bacteria were affected by BP3 (48). This was also observed by Liu and co-workers (2015), Mao and co-workers (2017), and Zhang and co-workers (2017) (151-153). BP3 may therefore negatively impact many of the plastisphere's

most abundant bacteria (i.e. Proteobacteria) (Figure 1.3) (69, 83), or facilitate the growth of BP3-degrading bacteria (154). Concerningly, BP3 sorption is greatest on smaller plastics (155), which are more widely distributed than larger plastics (22), equally increasing BP3 distribution and impact on plastisphere communities.

Compared to other UV-filters, octocrylene has a high molecular weight (361.477 g/mol), which may impact its interaction with plastisphere microorganisms (141). While growth of the prymnesiophyte *Isochrysis galbana* was impacted after chronic exposure to as little as 80 µg/L (150), only one of 27 Bacteria (*Arthrobacter aurescens*) was sensitive to 1 mg/L exposure (48). Furthermore, no study has yet demonstrated microbial toxicity to octocrylene at environmentally relevant concentrations, though the purported toxicity of this UV-filter is nonetheless important.

Despite being less concentrated in the ocean (Figure 1.6), 4-MBC is a more toxic, and more adherent (53,200 K_d) organic UV-filter than BP3 (145, 147). 4-MBC is therefore more likely to bind to the plastisphere and affect microbial activity once there. Upon direct exposure, this filter can impair the function of bacterial (137), algal (8), and fungal (156) colonies. However, in the study of 27 marine bacterial isolates (48), no species was affected by 4-MBC exposure. Regardless, it has been shown that upon chlorine-mediated photodegradation, 4-MBC toxicity towards *Vibrio fischeri* is increased 10-fold (137), meaning even low concentrations of 4-MBC may be toxic in the environment.

EHMC toxicity is also exacerbated by photo- and bio- degradation which produces additional toxic photoproducts (157, 158). In the study by Lozano and co-workers (2020) (48), EHMC affected the growth of the most bacterial species across three different phyla: Actinobacteria, Firmicutes, and Alphaproteobacteria (*Arthrobacter aurescens*, *Dietzia maris*, *Halobacillus dabanensis*, *Paenibacillus glucanolyticus*, and *Pelagibacterium halotolerans*), suggesting EHMC may pose a significant threat to a bacterial-dominated plastisphere. Growth of the algal species *Isochrysis galbana* is also inhibited by 74.73 µg/L of the accumulated UV-filter (8). These findings suggest that EHMC sorption to plastic may significantly impact both Eukaryotic and prokaryotic members of the plastisphere and warrants further investigation.

It is evident that UV-filters have a strong affinity for the plastisphere, will impact microbial viability and activity, and often co-occur in high concentrations alongside plastic pollution. This must therefore be considered in future research of microbial associations with plastic.

1.5 Future research / Recommendations

1.5.1 Plastispheres in the ocean

Plastisphere composition, and how it changes over space and time is pivotal to understanding which hazardous (*Section 1.3.2*), biogeochemical (38), or bioremediative (*Section 1.3.1*) processes may be facilitated in the plastisphere. Further study is therefore required to understand how location and plastic type determine the plastisphere's composition (*Section 1.2.1; 1.2.2*) and control the conditions which enrich its primary metabolic processes (*Section 1.3*) (51, 52). Each location, for example, will be a unique combination of temperature, salinity, oxygenation, and plant/animal diversity which will impact nutrient load and microbial diversity in each location, and should be further explored (49-51). It is also highly probable that the DOM which forms the conditioning film on plastics (62) – potentially containing co-pollutants – will change between locations (Figure 1.5; Figure 1.6), thus impacting prokaryotic binding and metabolism in each region. Several more recent studies have also explored the plastisphere's non-prokaryotic community (29, 31, 60, 65). Continued, integrated study of these eukaryotic, fungal, and archaeal species, and their viruses in the plastisphere may therefore reveal their prevalence and key metabolic processes (*Section 1.5.2*) in the next five years. Further study of plastic types would also confirm which factor – plastic type or location – is the prevailing influence of plastisphere formation. Current study of plastisphere location (*Section 1.2.1*), is not matched by present study of plastic type (*Section 1.2.2*), meaning location is currently presumed to be the prevailing influence of plastisphere formation (49, 50, 58), though this may just be due to a lack of evidence regarding the impact of plastic polymers. The impacts of plastic pollution on planktonic microorganisms which do not bind to plastic (31, 42), are also rarely considered when researching marine plastic debris. This is of particular concern due to the extreme shift in planktonic community composition observed after exposure to plastic leachates (25, 98, 130). Further research must therefore be conducted in these areas, particularly focussing on the reasons behind location- and plastic- dependent plastisphere variation, and how leachates impact the surrounding seawater community.

1.5.2 Plastisphere function

As discussed in this review, plastisphere function encompasses the ability of microorganisms to metabolise abiotic elements of the plastisphere (*Section 1.3*), their cellular interaction with each other (*Section 1.2.4*), and effects of co-pollutants (*Section 1.4.2; 1.4.3; 1.4.4*). This can be studied using meta-omics; techniques that enable the elucidation of how plastisphere-driven processes are performed through community-level analyses. Progression onto this area of research has so far been limited by the novelty of plastisphere research (28), and thus, the necessity to first characterise its members using 16S rRNA metabarcoding; a low resolution technique used to identify bacteria based on a variable region of the 16S rRNA gene (45, 57, 68, 75). This technique cannot distinguish between active, inactive, or dead cells (159), meaning any assumptions made based on taxonomic markers are skewed by present dead and dormant bacteria (138). However, many plastispheres have now been characterised using 16S rRNA metabarcoding (28, 35, 54, 69), and the use of metagenomic sequencing (31), has also allowed for more in-depth analyses of the plastisphere community. Now two studies have used metaproteomics to characterise the plastisphere's metabolic profile (68, 75).

Metaproteomics enables researchers to identify the proteins expressed by a complex microbial community, revealing the most prevalent microbial processes at the time of sampling (68, 160). When used alone, or in conjunction with other high-throughput methods such as metatranscriptomics and metabolomics (72, 87), these techniques can provide an accurate method for characterising the plastisphere's active bacteria and their metabolism. Comparative metaproteomics could answer the questions: (i) What is the functioning of the plastisphere? (ii) Are the potential pathogens/plastic degraders metabolically active? (iii) How do microorganisms metabolically respond to plastic co-pollutants?

A focus on these questions and the topics highlighted in *Section 1.5.1* would reveal the risks that a dynamic plastisphere may pose (22), and which microorganisms may be utilised to reduce the marine plastic pollution problem (12, 35).

Conclusion

In this review, we have examined studies of plastics, the plastisphere, and bacterial isolates to predict plastisphere activity and the factors that influence it in the ocean. For the first time, the effects of cell wall composition, organism-organism interaction, plastic-bound co-pollutants, plastic type, and location on the formation and function of the marine plastisphere are considered. In doing so, we critically discuss the synergistic interactions between these factors and explore how they likely underpin the key microbial metabolic processes in this niche. Although location is currently considered to be one of the primary driving forces of plastisphere formation, in this review we articulate how the presence of specific microorganisms not only depends on their occurrence in the water column, but also on their cell wall composition and resulting compatibility with the plastic surface at the molecular level. Thus, cell wall composition, and the expression of adhesins, pili, genetic plasticity, and other traits of biofilm-forming bacteria, directly affect the resulting taxonomic diversity of the community, contributing to the metabolic profile of the biofilm. We discuss how cell wall composition also likely plays a role in a microorganism's response to potentially toxic sorbed co-pollutants and exposed additives, impacting their growth and ability to perform characteristic metabolic processes (i.e. biodegradation). The toxicity of organic UV-filters especially is of emerging concern considering their significant impact on microbial metabolism, affinity for plastic, and high concentrations in coastal environments. In this review, we therefore recommend further study of the abiotic factors associated with location (i.e. temperature), the forgotten organisms – beyond plastic-biodegraders and potential pathogens –, and the impact of plastic leachates on the plastisphere and the ocean. Most importantly, we strongly encourage a shift in plastisphere research away from taxonomic descriptions, and towards more advanced molecular techniques to allow for a greater mechanistic understanding of plastisphere development and function.

Credit author statement

Charlotte Lee: Literature review, figure production, Writing – original draft, conceptualization. Lauren Messer: Review & editing. Sophie Holland: Review & editing. Tony Gutierrez: Review & editing. Richard Quilliam: Review & editing. Sabine Matallana-Surget: Review & editing, conceptualization.

2. The use of proteomics to study marine microbial ecotoxicology

2.1 Omics and marine microbial ecotoxicology

Marine pollutants have been shown to elicit positive and negative growth responses in an array of marine microbial taxa (10). By finding these taxa's LC₅₀s for tested chemical pollutants, policy makers are informed of the upper limits of pollutant emission before important producers and other biogeochemical cyclers are lost. However, before growth reduction and death, or the inverse, a number of essential metabolic processes are impacted by pollutant exposure. Indeed, a pollutant's toxicity is more often measured by such metabolic processes in multicellular organisms such as fish, molluscs, and sea urchins before mortality is considered (8, 161, 162). Concerningly, in using such metrics, Downs *et al.*, (2016) found BP3 – investigated in **Chapter 3** – to induce ossification, impaired development, and genotoxicity in corals at 8 µg/L – 340 µg/L; below the maximal observed environmental concentration of 1.395 mg/L (143). Further examples of marine pollutant toxicity in multicellular organisms can be found in the review by Vagi *et al.*, (2021) (162). Pollutant exposure may therefore impact ecosystem function and other microbe-associated regulatory processes before their recorded LC₅₀s are reached. As highlighted *Section 1*, it is therefore important to study microbial metabolism, as well as abundance in response to pollutant exposure.

Microbial metabolism has historically been studied using microscopy and a combination of stains, fluorescence genes, and fixatives (163). These studies have led to the understanding of essential metabolic concepts in microbiology which are now expanded upon using omics. Omics allow for a deeper understanding of microbial metabolism through the characterisation and quantification of biological molecules (164). Analysis of these biological networks are used to determine community structure, as well as the homeostatic and reactive processes which are prevalent within a community or singular organism. Perhaps as the most established form of molecular study (164), genomics is a commonly used molecular tool in marine microbial ecotoxicology studies and surveys (165). Consequentially, genomics, and more specifically metagenomics has now been used to survey a broad range of aquatic environments. Such studies reveal that pollution can alter microbial diversity (166-168), increase the transfer of heavy metal, POP, and antibiotic resistance genes (ARGs), plus associated functional structures (i.e. plasmids, secretion systems) (166, 169-171), and induce expression of pollutant (i.e. alkene) degradation pathway -associated genes (167, 168). The

accessibility of environmental metagenomics has enabled these studies to span global transects (172) and provide an insight into the community response to environmental pollution across continents. Such a tool has therefore also been highly useful in marine plastisphere research, enabling the characterisation of these microniches in a broad array of biogeographical contexts (*Section 1.2.1*). However, the interpretation of these genomic studies is limited by the variability in how a gene might be expressed. Depending on gene regulation, and the selection of exons versus introns, one gene may be associated with the expression of many protein isoforms (173). Each of these isoforms will pertain to distinct functions which will affect the cell in different ways. One method to more accurately investigate cell function is to therefore use transcriptomics or metatranscriptomics to unveil the parts of each gene being transcribed at the time. However, the messenger-RNA (mRNA) used to study this transcription has an average half-life of ~5 minutes in prokaryotes, and is very easily degraded despite careful handling (174). Interpretations of these results can therefore be skewed by fragment shearing, or the general degradation that occurs over time. The production of mRNA also does not guarantee its transcription into a protein used in downstream processes. The direct study of proteins may therefore be the most biologically relevant technique for the study of microbial metabolism (160).

2.2 Proteomics explained

Proteomics can be used to perform top-down, and bottom-up analysis depending on the sample's input format (Figure 1.7). The complete protein data used in top-down studies provides a structural analysis of the proteins themselves, while bottom-up studies investigate peptides which are easier to process (175). The bottom-up studies utilising peptides in **Chapters 2, 3, and 4** therefore yield significantly greater sample coverage than any top-down study, enabling broad-scale analysis. Proteomic studies can also be gel-based or gel-free (Figure 1.7). Gel-based proteomics uses gel electrophoresis to separate proteome proteins before their identification using a mass spectrometer (MS). The gel-free approach also separates the proteins prior to analysis, though uses tandem liquid chromatography-mass spectroscopy (LC-MS/MS) (176-178). Here, the liquid chromatographer is a component part of the mass spectrometer, meaning the entire sample can be fed into the machine, and separation occurs directly before analysis. This can be used to analyse label-based, and label-free samples (Figure 1.7) (175).

In label-free proteomics, as used here, protein digestion occurs directly after protein extraction and precipitation using a consolidated approach. The resulting peptides are then analysed through Data-Dependent Acquisition (DDA) or Data-Independent Acquisition (DIA) (179). DDA selects the most dominant peptides, or Precursor Ions (PI) from LC separation, and fragments them using Collision Induced Fragmentation (CIF) one-at-a-time (Figure 1.7). In the time-of-flight (TOF) MS used in **Chapters 2, 3, and 4**, the intensity, and mass-to-charge (m/z) ratio of each fragment is then determined by the time it takes each ion to pass through the final chamber. The fragment data can then be referenced back to the PI data to produce spectra for each peptide. These spectra are run through specialised protein identification tools such as ProteinPilot™ to identify the peptide sequences and corresponding proteins (180). For axenic culture, a complete proteome can be downloaded from online repositories for this aim. For complex cultures, a database may be created through prior examination of the community structure (using genomics), and the retrieval of its associated proteomic data through automated software such as mPies (MetaProteomics In Environmental Sciences) used in **Chapters 2 and 4** (181). DIA follows a similar protocol to DDA, though here, all PIs are analysed regardless of abundance (Figure 1.7). In the case of Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH) used in **Chapter 3** the PIs are fragmented in ‘windows’ of similar m/z ’s, instead of one-by-one. This means that the resulting spectra cannot be referenced back to the PIs, but instead requires a database from previous DDAs or from in-silico library retrieval for spectral assembly (160, 179, 182). Otherwise, the resulting data is processed in the same way. The data-acquisition methods introduced here each have benefits and drawbacks which are discussed in **Chapter 5**.

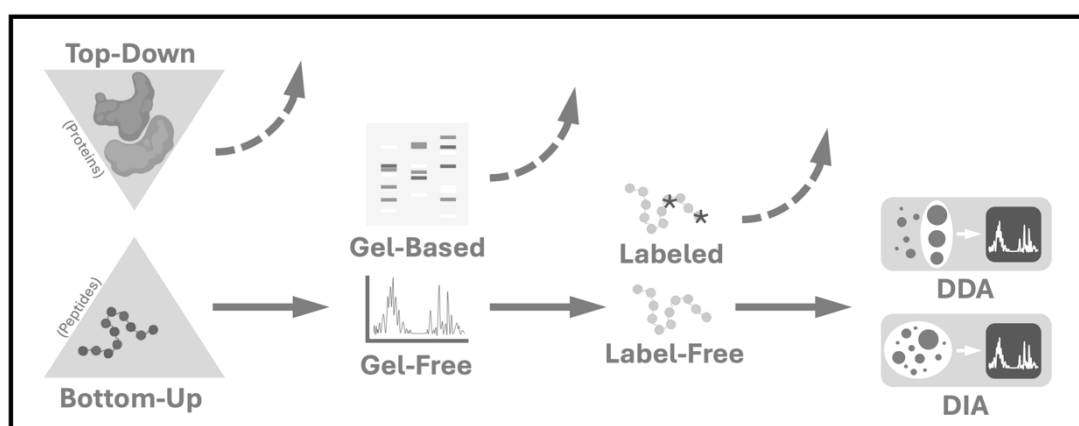


Figure 1.7 Methodologies used to study proteomics.

Solid arrows – methodologies used in this thesis; Dashed arrows – unused methodologies; DDA – data-dependent acquisition; DIA – data-independent acquisition.

2.3 Proteomics in practice

Proteomics has been utilised for axenic culture to reveal the metabolic pathways used by microorganisms to mitigate and facilitate pollutant toxicity and biodegradation (Figure 1.8). Studies of *E.coli*, *Epibacterium mobile*, *Alcanivorax sp. 24*, *Halomonas sp. ATBC28* (and *Mycobacterium sp. DBP42*), and *Marinobacter hydrocarbonoclasticus* have so far revealed respective responses to silver (120), BP3 (183), plastic (184), plasticisers (185), and other hydrocarbons (186). Consequentially, the use of axenic culture with proteomics has begun to reveal how toxicity is induced in receptive species, how they may counteract it, and the mechanisms through which polymeric pollutants can be biodegraded. This approach to proteomics conveys distinct advantages for these researchers wishing to scrutinise intracellular pathways. Specifically, this approach allows researchers to control study parameters (i), collect sufficient protein for downstream analysis (ii), and increase study reliability (iii) (187). For example, by selecting the culture and stressor, researchers can explore specific questions which other forms of study might not accommodate. The use of proteomics to study axenic culture also produces higher spectral coverages than other methods, like metaproteomics (Section 2.4) (188). This allows the investigator to pick apart discrete molecular interactions within the proteome that may otherwise be marginalised through broad-scale analysis. By growing microorganisms in a controlled setting, sample collection can also occur at the time where the most growth is observed. This is essential for proteomic analysis as a technique which is entirely dependent on the amount of sample retrieved during extraction, unlike genomics and transcriptomics where the sample can be amplified. More growth, and initial sample collected therefore increases the likelihood of successful sample detection and analysis during mass spectroscopy (160). This control over study parameters also allows the researcher to use near-identical technical replicates, increasing study reliability.

2.4 Metaproteomics

A complementary use of metagenomics and metaproteomics may also be used to reveal the functional dynamics of an entire microbial community. First, metagenomics is used to elucidate the structural diversity of a microbial community and create a comprehensive database for downstream analysis of the metaproteome (Section 2.2). This metaproteome is then analysed to discover the active members of the microbial community and what they are doing, then targeted searches can be used to search for studied processes of interest (177).

Within marine microbiology, metaproteomics has therefore been used to study functional diversity in North Sea (189) and South Atlantic (190) communities, seasonal algal blooms (191), day/night changes in surface seawater (192), responses to the Deepwater Horizon oil spill (193), and activity of the marine plastisphere (68, 75, 177) in pilot-scale studies (Figure 1.8). As seen here, metaproteomics can be used on mesocosms (75, 193), environmentally sampled communities (68, 177, 189-192), and can also be applied to enriched communities (**Chapters 2 and 4**; Figure 1.8). As such, metaproteomics has a broad range of applications, and is consequentially the leading approach for many advanced ecotoxicological studies. Studies using this approach, particularly for environmentally sampled communities, can reveal a significant amount about microbial activity and responses within the marine environment. Their exposure to community-level interactions and abiotic factors (e.g. seasonal influences) enhances the environmental relevance of this form of study, providing valuable insights into how microbial cells function in natural ecosystems (187, 194). Furthermore, environmental sampling facilitates the exploration of uncultured microbial genera, thereby enhancing the discovery of novel ecological processes (194). Metaproteomics therefore offers a crucial tool for advancing our understanding of marine ecosystems and their response to pollution.

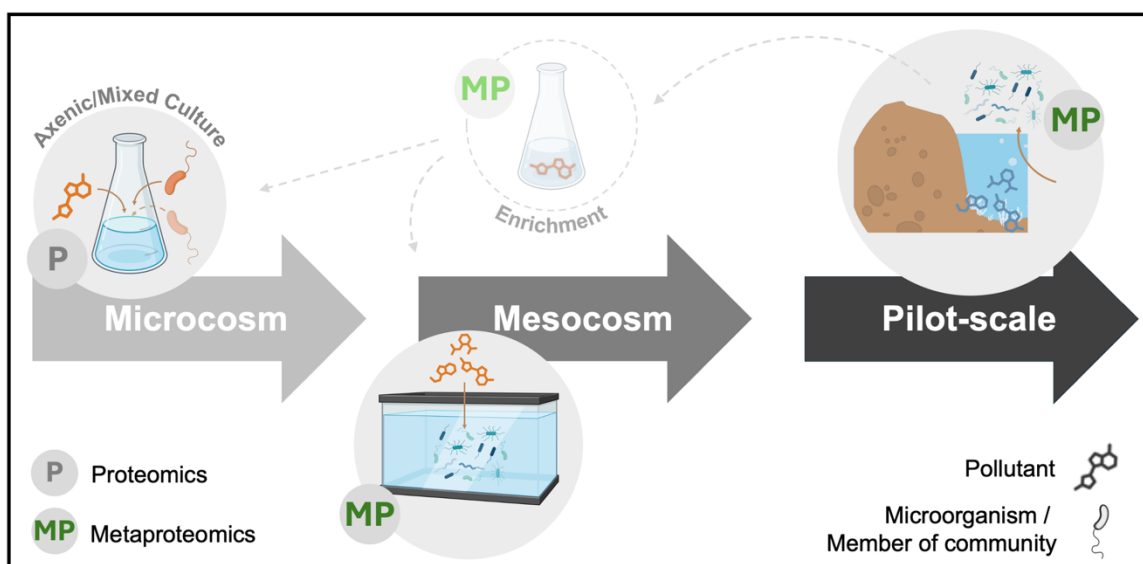


Figure 1.8 Proteomics and metaproteomics applied to different scales of study. Orange icon – researcher-controlled factor; Blue icon (excluding water) – environmentally contingent factor.

In summary, metaproteomics provides a broad scale search of microorganisms or mechanisms of interest, while proteomics used with axenic culture provides more in-depth insights into such microorganisms' mechanisms. As such, both are useful for the study of marine microbial ecotoxicology, and can feasibly be used in tandem. However, when selecting one approach over the other, the researcher's main consideration will be the scale of study. To understand the community-wide response to a stimuli (i.e., Gulf of Mexico communities to dispersant), metaproteomics is required. To understand the precise molecular processes which underpin the functioning of an organism of interest (i.e., plastic biodegradation by *Ideonella sakaiensis*), proteomics and axenic culture is required. The use of both scales of the proteomic approach are essential for our further understanding of the microbial cell, and how it responds to marine pollution. As a result, both are utilised throughout this thesis for their ability to encompass broad- and narrow- scale research questions.

3. Thesis outline

In *Section 1.5.2*, three central questions were posed to guide this research:

- (i) What is the functioning of the plastisphere?
- (ii) Are the potential pathogens/plastic degraders metabolically active?
- (iii) How do microorganisms metabolically respond to plastic co-pollutants?

The studies comprising this thesis were designed to answer these questions and provide greater context for future discoveries within plastisphere research. Accordingly, the functioning of the plastisphere (i) was investigated in both **Chapters 2, and 4**. The significance and activity of potential pathogens and plastic degraders (ii) was investigated in **Chapter 2**. And finally, the metabolic response of microorganisms to plastic co-pollutants (iii) was explored at the individual and community level in **Chapters 3, and 4** respectively.

Plastic was chosen as the primary focus of this research due to its prevalence as a pollutant, its prominent role in contemporary research, and its interaction with other pollutants, resulting in combined pollution. As another central focus of this thesis, any form of co-pollution was controlled, and methodological variations that could introduce inconsistencies (i.e., plastic type) were minimised. In **Chapters 2, and 4** investigating the plastisphere, we therefore used pristine low-density polyethylene plastic, chosen based on its minimal inclusion of additives, environmental abundance, and susceptibility to biodegradation.

To investigate plastic co-pollution – additional pollutants which sorb onto plastics – this thesis used organic UV-filters due to their environmental persistence and continued use in polluting personal care products (*Section 1.4.4*). Benzophenone-3 (BP3), a UV-filter previously studied in our co-authored paper “Unravelling the molecular effects of oxybenzone [BP3] on the proteome of an environmentally relevant marine bacterium” was shown to significantly impact bacterial metabolism (183). However, BP3 usage has drastically declined after the global recognition of this filter as an ecological hazard. Within this thesis, a more frequently used organic UV-filter, Ethylhexyl Methoxycinnamate (EHMC), was therefore selected for ongoing study of co-pollution.

To start, **Chapter 2** explores the metabolic interactions within the nascent plastisphere, and how these change over time by utilising a combination of metagenomic and metaproteomic approaches. Key processes such as plastic adhesion, stress response, cell-cell interactions,

and biogeochemical cycling are investigated. Additionally, the present microbial genera, and their proteomes are analysed to identify potential pathogens and their virulence proteins, as well as pollutant-degrading bacteria and their catalytic enzymes. **Chapter 3** diverges from the plastisphere to investigate EHMC, and contrast its impact on an environmentally marine bacterium, *Epibacterium mobile*, to the established impact of BP3 on the same organism. In this investigation, insufficient data was retrieved to draw firm conclusions of *E. mobile*'s response to UV-filter exposure through the secretome, though indicate that EHMC may impact protein expression without affecting bacterial growth. In **Chapter 4**, EHMC was therefore used to investigate the impact of organic UV-filter co-pollution on the composition and metabolic profile of the nascent plastisphere. Again, all metabolic processes are examined within the plastisphere metaproteome to find the most significantly impacted processes. **Chapter 5** then discusses all findings from the experimental chapters, offering recommendations for future research into plastisphere dynamics and microbial responses to combined pollution. Results from all combined chapters and preliminary investigations are critically reviewed to provide a complete summary of this thesis' key findings and implications for future research and policy.

Chapter 2

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Decoding Microbial Plastic Colonisation

As outlined in Chapter 1, significant gaps remain in our understanding of the marine plastisphere, particularly regarding the functional dynamics that shape its composition. Key processes such as the adhesive mechanisms enabling substrate binding and cell-cell interactions have yet to be fully explored. Additionally, the essential metabolic activities that influence ecosystem functions, public health, and policy including biogeochemical cycling, plastic biodegradation and virulence respectively remain poorly characterised within this community. Metaproteomics offers a powerful approach to uncover and characterize these functions. Since the completion of the review presented in Chapter 1, two recent studies (177, 195) have employed metaproteomics to investigate plastispheres at later stages of development (≥ 1 week growth). However, understanding the initial adhesive mechanisms that govern plastic colonisation, and biodegradation requires the removal of any interference of earlier colonisers. By investigating these early-stage dynamics and unravelling the complexities of two highly annotated metagenome-assembled genomes (MAGs), we therefore aim to provide novel insights into the functional evolution of this microbial ecosystem and bridge the existing gaps in our understanding of plastisphere biology.

Decoding Microbial Plastic Colonisation: Multi-Omic Insights into the Fast-Evolving Dynamics of Early-Stage Biofilms

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For access to supplementary files, see <https://doi.org/10.1002/pmic.202400208>.

This paper serves as the foundation for the information presented here, although the text and content have been revised slightly for inclusion in this thesis.

Abstract

Marine plastispheres represent dynamic microhabitats where microorganisms colonise plastic debris and interact. Metaproteomics has provided novel insights into the metabolic processes within these communities, however the early metabolic interactions driving the plastisphere formation remain unclear. This study utilised metaproteomic and metagenomic approaches to explore early plastisphere formation on low-density polyethylene (LDPE) over three (D3) and seven (D7) days, focusing on microbial diversity, activity, and biofilm development. In total, 2,948 proteins were identified, revealing dominant proteomes from *Pseudomonas* and *Marinomonas*, with near-complete metagenome-assembled genomes. *Pseudomonas* dominated at D3, while at D7, *Marinomonas*, along with *Acinetobacter*, *Vibrio*, and other genera became more prevalent. *Pseudomonas* and *Marinomonas* showed high expression of reactive oxygen species (ROS) suppression proteins, associated with oxidative stress regulation, while granule formation, and alternative carbon utilisation enzymes, also indicated nutrient limitations. Interestingly, 13 alkane and other xenobiotic degradation enzymes were expressed by five genera. The expression of toxins, several type VI secretion system (TVISS) proteins, and biofilm formation proteins by *Pseudomonas* indicated their competitive advantage against other taxa. Upregulated metabolic pathways relating to substrate transport also suggested enhanced nutrient cross-feeding within the more diverse biofilm community. These insights enhance our understanding of plastisphere ecology and its potential for biotechnological applications.

Significance of the study

Given the increasing plastic pollution in marine environments, understanding early plastisphere assembly is essential from both ecological and biotechnological perspectives. This study advances knowledge by identifying 13 pollutant-degrading enzymes in addition to those found through prior metaproteomic research, shedding light on the bioremediative potential of multi-species biofilms. Their expression by constitutive genera, particularly *Marinomonas*, also underscores the recognised association of these genera with environmental bioremediation. Additionally, interspecies competition and oxidative stress responses, shaped by resource limitations, were found to govern biofilm dynamics. The selection for generalist species and potential pathogens is concerning due to plastic's ability to travel through marine ecosystems. However, the cooperative behaviour among plastisphere members supports prior research demonstrating biofilms as resilient microbial solutions. This research not only deepens our understanding of microbial colonisation and interaction but also highlights the utility of metaproteomics in studying complex environmental communities. Insights from this study also contribute to the broader field of plastisphere ecology by offering another alternative to how the marine plastisphere may be structured, and how a marine plastisphere of this structure may function. The enzymes identified may also be utilised in the search for solutions to the plastic pollution problem by developing biotechnological strategies for marine ecosystem resilience.

Key Words

Plastisphere; Metaproteome; Marine biofilms; Biofilm formation; Plastic debris.

1. Introduction

Over 400.3 million metric tonnes of plastic are manufactured, utilised, and discarded annually (196). Despite diverse waste management strategies implemented by national governments and public initiatives aimed at controlling plastic waste, an estimated 19 to 23 million tonnes of plastic pollution enter the marine environment each year (197, 198). Marine plastic debris, along with the resulting microplastics, is now anticipated to enter nearly all marine ecosystems, threatening the health of our oceans (199). Direct studies of marine plastic debris reveal a complex relationship between plastics and the ocean's most abundant biogeochemical cyclers and producers: microorganisms (10, 200). As natural biofilm-forming organisms, and secondary surface colonisers, these microorganisms directly interact with marine plastic debris through the formation of the 'plastisphere' (28).

Marine plastispheres are complex, multi-species biofilms that consist of both eukaryotic and prokaryotic producers, hydrocarbonoclastic organisms, pathogens, and other biogeochemical cyclers (10, 28, 29, 68, 195). The composition of these communities is influenced by several factors, including the type of plastic, its chemical additives, environmental location, seasonal changes, and the time available for colonisation (10). Among these factors, the duration of plastisphere development (i.e., the stage at which the plastisphere is collected) appears to exert the most consistent influence across studies. Microbial abundance and diversity are often low in the initial hours following colonisation, peak within 1-2 weeks, and subsequently decline (29, 201-203). Metaproteomic studies of early biofilm formation, though not specific to the plastisphere, have uncovered both competition and cooperation between species; interactions that are likely to be prevalent within the first days of plastisphere development (204, 205). This early plastisphere is particularly significant, as it may be the only stage of biofilm development where direct microbe-plastic interactions occur. As biofilms continue to form, new layers often cover older ones, shielding the upper biofilm layers from direct contact with the plastic surface (201).

Prior metaproteomic research on multi-species biofilm formation (204, 205) and established marine plastispheres (68, 75, 177) has offered insights into how microorganisms interact with plastic substrates and with each other. A recent study by our research group also highlighted the importance of comparing new versus established plastispheres by studying their meta-genome and proteome after one-, and two-weeks respective growth. This study demonstrated a significant shift in microbial diversity and composition while maintaining a core

assemblage of active *Proteobacteria*, including *Marinomonas*, *Pseudomonas*, and *Pseudoalteromonas* (195). Additionally, this study highlighted the differential regulation of proteins involved in cellular attachment and energy metabolism during the first 2 weeks of colonisation, offering important context for understanding the evolution of biofilm dynamic. However, the molecular mechanisms driving the functioning of marine plastisphere communities in the earliest stages of development remain poorly characterised at the proteome level.

In the present study, our aim was to build on our previous research by providing a deeper understanding of the community structure of the young plastisphere and the metabolic activity of present microbial genera (195). We employ metaproteomics to dissect the molecular basis of plastisphere formation within the first 7 days of colonisation, with a particular focus on the mechanisms associated with stress, biofilm-formation, interspecies interaction, and plastic biodegradation.

2. Materials and Methods

2.1 Recovery of plastisphere stock communities

Beached plastics were collected from Oban Bay (56° 24' 50.4'' N, 5° 28' 19.2'' W; Scotland) on August 6th, 2022 and washed in artificial seawater (206) to remove loosely attached organisms, prior to preservation at -20°C until use. For biofilm extraction, the transparent plastics within this collection were isolated from coloured plastics due to their close resemblance to the low-additive, transparent low-density polyethylene (LDPE) used in *Section 2.2* (207). To increase plastisphere biomass, the transparent plastics were placed into glass containers with 300ml artificial seawater (24 g/L NaCl, 10.8 g/L MgCl₂.H₂O, 4 g/L Na₂SO₄, 1.5 g/L CaCl₂.2H₂O, 0.68 g/L KCl, 0.5 g/L NH₄Cl, 0.2 g/L NaHCO₃, 0.1 g/L KBr, 0.04 g/L NaHPO₄, 0.025 g/L H₃BO₃, 0.024 g/L SrCl₂.6H₂O, 0.002 g/L NaF, PH 7.0) supplemented with vitamins, trace metals, and glucose (ASW+G), and incubated with shaking (65 rpm) at 15°C for three days, equalling Oban Bay's highest seawater temperature. Excess ASW+G was carefully removed, and plastics were vortexed in the remaining media and rinsed with additional ASW+G to ensure the biofilm was fully detached. The plastics were then removed and the detached plastisphere cells were pelleted by centrifugation (14°C, 5000 g, 7mins), resuspended in 15ml ASW+G, and preserved with 30% glycerol at -80°C until further use.

2.2 Plastisphere growth

For this study, LDPE, one of the most widely produced plastics globally (45.7 Mt annually) (196), was selected as the substrate for plastisphere growth. Five pieces of LDPE (2x10 cm folded in/to 8ths; ET31-FM-000101; Goodfellow, England) per replicate were added to 150ml of Bushnell Haas Broth supplemented with vitamins and trace metals (BHB+v) (208), and inoculated with 50µl of plastisphere stock community. Each replicate (n = 4) was then incubated (65 rpm, 15°C) in 250ml glass Erlenmeyer flasks for 3 or 7 days under exposure to natural light (Autumn, Scotland) to monitor early plastisphere development. These incubation times were selected based on our previous findings (209), as they allow for biofilm formation, providing sufficient material for metaproteomic analysis, while also capturing the earliest stages of plastisphere formation (29).

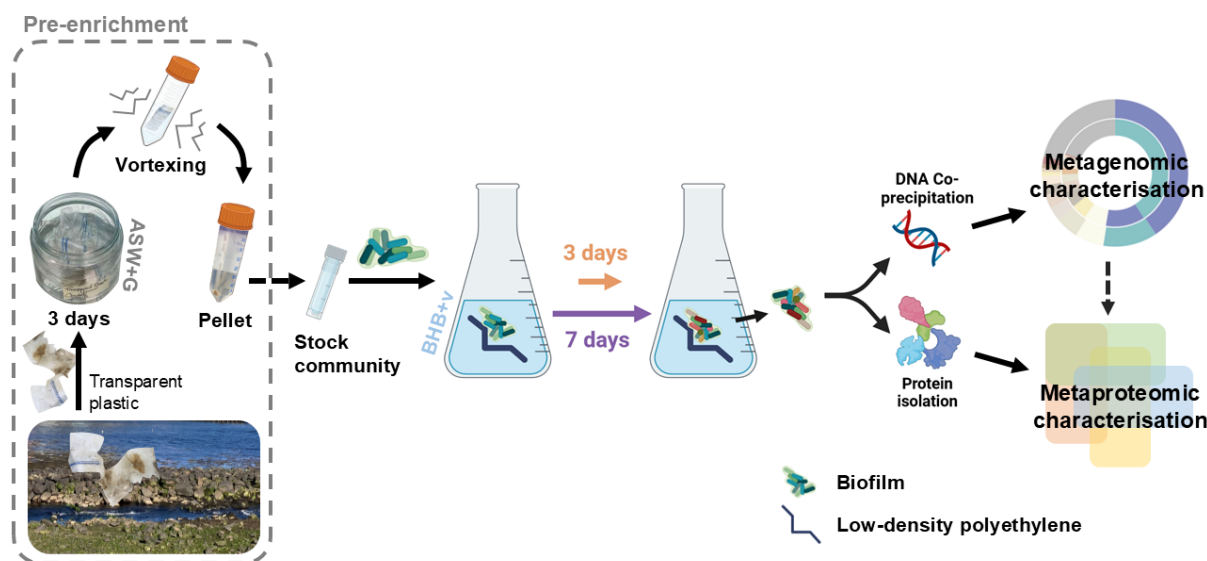


Figure 2.1 Three- and seven-days growth of a marine microbial community on LDPE, and analysis of biofilm structure and functioning.

ASW+G, artificial seawater supplemented with vitamins, trace metals, and glucose; BHB+v, Bushnell Haas Broth supplemented with vitamins and trace metals.

2.3 Protein extraction

After incubation, protein was extracted from plastics and digested according to Messer et al. (2024). Plastics were removed from media using forceps and allowed to dry under laminar flow. Once dry, plastics were bead beaten (1mm glass beads) in a 2% sodium dodecyl sulphate (SDS) solution 3 times in 10-minute intervals. Cell media was then sonicated (1s pulse, 1s gap, 40% Amp.) twice, and centrifuged (8000 g, 10 minutes). The pellet was then sonicated and centrifuged again (as above). A quarter of the supernatant was preserved at -80°C for metagenomic analyses (Section 2.4), and the remaining supernatant (5-6 mL) was transferred to 3K centricons and centrifuged at 7000 g until $\leq 250 \mu\text{L}$ sample remained. Proteins were precipitated with cold acetone overnight at -80°C , with an acetone/aqueous protein solution ratio of 4:1. The protein pellet was resuspended in 6 M Guanidine HCl in dipotassium phosphate buffer, sonicated (1s pulse, 1s gap, 40% Amp.), then centrifuged (13,000 g, 15 minutes). The supernatant was then diluted with LC-MS grade water, with a water/aqueous protein solution ratio of 1:1 and preserved at -80°C . Protein quantification revealed 35.13 mg (± 1.31 ; $n = 4$), and 6.68 mg (± 4.76 ; $n = 4$) of protein within the samples extracted after three (D3) and seven (D7) days incubation respectively (Figure 2.1).

For protein digestion, 100 μg of protein per sample was reduced (10 mM 1,4-Dithioerythritol), alkylated (25 mM iodoacetamide), and precipitated with acetone

(acetone/aqueous protein solution ratio of 4:1) before being digested at 37°C overnight in 20 µL of sequencing grade modified trypsin (EC 232-650-8) as described previously (177).

Protein samples were analysed on an ultra-high-performance liquid chromatography–high-resolution tandem mass spectrometer (UHPLC-HRMS/MS) with a Eksigent NanoLC 400 and AB Sciex TripleTOF 6600 system. Two micrograms of peptides were analysed using acquisition parameters previously reported (210) in data dependent acquisition (DDA) mode. Mass spectrometry (MS/MS) runs were conducted with micro injection (75 min LC separation) modes.

2.4 Shotgun metagenomics

DNA was co-precipitated from the plastisphere samples for metagenomic analysis by adding protein precipitation solution (Promega, A7951) with a sample / protein precipitation solution ratio of 2:1, followed by centrifugation (20,379 g, 15 mins). Filter-sterilised 3M sodium acetate (300 µL) was added to the supernatant, followed by >95% cold ethanol at a sample/ethanol solution ratio of 1:2, and DNA was precipitated overnight (–20°C). The precipitated DNA was pelleted by centrifugation (20,379 g, 15mins), and washed twice using 70% ethanol, centrifuged again (16,000 g, 15mins), then briefly dried under laminar flow. DNA pellets were resuspended in 20µl UltraPure water, heated to 55°C (5 mins) to facilitate dissolution, vortexed, and preserved at –80 °C.

Metagenomic analysis was performed on the MinION (Mk1c; Oxford Nanopore Technologies; ONT), using the NEBNext Oxford Nanopore companion module and associated protocols to prepare the DNA sequencing library. Replicates were pooled to reach 800ng, resulting in one sample per condition (D3 and D7) for input into the Native Barcoding Kit 24 (SQK-NBD112.24). The resulting library was loaded onto the flowcell (FLO-MIN106) according to the manufacturer instructions, and samples were sequenced for 24 hrs. Read lengths below 200 bp, and a quality score of 8 were discarded during subsequent real-time basecalling in MinKnow (v22.10.5). This resulted in 6.72 Gb of estimated bases, and 5.66 million reads which were trimmed (minimum quality score 7) and normalised using BBDuk and BBNorm respectively. These reads were taxonomically annotated using Kaiju (211), assembled (2955 total contigs) using MetaFlye (212), and functionally annotated with DRAM, leveraging KBase, as previously described (177).

2.5 Shotgun metaproteomics

Protein searches were performed within ProteinPilot (v5.0.3.1029, 9521aa4603a; Paragon Algorithm: 5.0.3.1029, 1029; AB SCIEX) using the AB SCIEX OneOmics software package, and databases created from our combined metagenomic data (Section 2.4), and other publicly available databases for plastic metabolism (PlasticDB) (213), virulence factors (VFDB) (214), and antimicrobial resistance (CARD) (215). To create a database of our combined metagenomic data, the genus-level taxonomic (.txt), and functional (.faa) annotations (Section 2.4) were used in the mPies (v1.0) (181) database creation workflow (<https://github.com/johanneswerner/mPies>). Each database was then used to match spectral (DDA) data to taxonomic and proteomic data in ProteinPilot with a global false discovery rate (FDR) of 1%. The resulting files were then reprocessed with mPies using parameters described in Messer *et al.*, (2024) for protein grouping (177). This produced functionally annotated proteins, and corresponding taxonomic annotations based on the available data after protein alignment with Diamond, within the mPies workflow (181). All taxonomic data was subsequently filtered according to confidence score ($\geq 80\%$), and proteins with < 2 associated peptides were removed. Proteins matching these criteria in one or more replicates for each condition were accepted, though proteins found in < 2 replicates are interpreted with caution (Figure 2.4). Before annotation, intensity-based relative quantification between the conditions was also performed in Skyline (v. 22.2) using the following parameters: mass analyser TOF, normalisation method Total Ion Current (TIC), centroided peak integration, MS1 tolerance 0.05Da, MS2 tolerance 0.1Da, maximum missed cleavages 2, and minimum peptides per protein 2. To identify significantly up- or down-regulated proteins between D3 and D7, the MSstats package was leveraged within Skyline. An adjusted P-value of < 0.05 (Welch's t-test) and fold change of 1.5 was considered significant. Missing annotations caused by protein inference issues were manually curated using BLAST (<https://www.uniprot.org/blast>) if the same protein was matched repeatedly ($> 50\%$ matches), the highest-rated peptide score equalled > 100 , there was a match at the species level, or a combination of these criteria. Metagenomic data, database search exports, relative quantification data, peptide and protein data are provided in Supplementary files S1: S6. Subsequent data wrangling, and the production of figures was performed using RStudio (v. 4.2.2).

3. Results

Metaproteomic analysis of early plastisphere communities resulted in the identification of 2037 and 1886 proteins at D3 and D7 respectively, with corresponding spectral coverages of 33.85% and 28.1% (Supplementary File S5). The majority of proteins in each dataset (D3 = 70.2%; D7 = 72.6%) were successfully annotated using the consensus approach employed by mPies, and the remaining 29.8% (D3) and 27.4% (D7) of proteins were annotated manually. Of the identified proteins, 1499 were non-redundant at D3 and 1449 at D7 (2-11 unique peptides per protein), of which 74.5% (1117) and 69% (998) respectively were found in ≥ 2 replicates. Furthermore, only 1.85% ($\pm 0.05\%$) were taxonomically unknown or functionally uncharacterised after manual annotation. Overall, 97.4% ($\pm 1.2\%$) of proteins were taxonomically annotated to the phylum level, 96.5% ($\pm 0.8\%$) were annotated to class level, 93.4% ($\pm 0.5\%$) to the order level, 92.2% ($\pm 0.6\%$) to the family level, 87.5% ($\pm 3.7\%$) were annotated to the genus level, and 27.1% ($\pm 8.1\%$) of proteins were annotated to species level (Supplementary File S1). However, a difference in the coverage of these annotations was observed between D3 and D7 metaproteomes, such that 90.9% of proteins were annotated to genus level at D3, compared to 84.1% at D7, and 35.2% of proteins were annotated to species level at D3, relative to 19% at D7. The relative quantification of identified proteins revealed that 711 non-redundant proteins were shared across D3 and D7, with 387 of these found to be upregulated on D3, and 323 were upregulated on D7 (Supplementary File S4). These results allowed detailed characterisation of the active taxa and their expressed functions during the early colonisation of plastic.

3.1 *A heterotrophic marine plastisphere*

At both time-points, the majority of the bacterial community were annotated as Gammaproteobacteria (>85%), with an increased relative abundance of Alphaproteobacteria (1.1% to 10.3%) and Bacteroidetes (0.05% to 0.35%) between D3 and D7 (Figure 2.2). Within Gammaproteobacteria, two high quality metagenome assembled genomes (MAGs) were recovered for the genera *Pseudomonas* and *Marinomonas*, with a completeness of 98.08% and 96.54% respectively. Within these MAGs, 1668 and 979 proteins (1% FDR) were identified for *Pseudomonas* and *Marinomonas* respectively (Supplementary File S3).

Upon examination of our combined metagenomic and metaproteomic data at the genera level, both the composition and activity of the community exhibited a marked shift between D3 and D7. Initially dominated by active *Pseudomonas* at D3, the plastisphere appears to have transitioned into a more diverse community by D7, where *Marinomonas*, *Pseudomonas*, *Acinetobacter*, *Paracoccus*, *Psychromonas*, *Vibrio*, *Rhodobacter*, *Pseudoalteromonas*, and other minor genera were present in greater abundance (Figure 2.2). All active bacterial genera identified in this study were mainly facultative heterotrophs. Proteins associated with four bacterial autotrophs, photoheterotrophs, and mixotrophs (*Rhodobacter*, *Photobacterium*, *Sinirhodobacter*, and *Sinorhizobium*) were identified on both D3 and D7, though no proteins detected for these minority genera were associated with autotrophic processes.

This heterotrophic nature of the biofilm was evidenced by the expression of key metabolic enzymes, including succinate dehydrogenase, citrate synthase, malate dehydrogenase, and isocitrate dehydrogenase— all integral to the tricarboxylic acid (TCA) cycle— as well as glyceraldehyde-3-phosphate dehydrogenase and enolase, which are involved in carbohydrate metabolism, and ATP synthase, which is involved in energy production (Supplementary File S4). These metabolic proteins were among the most ubiquitously expressed across both time points, encompassing 10 of the 20 active organisms in this study.

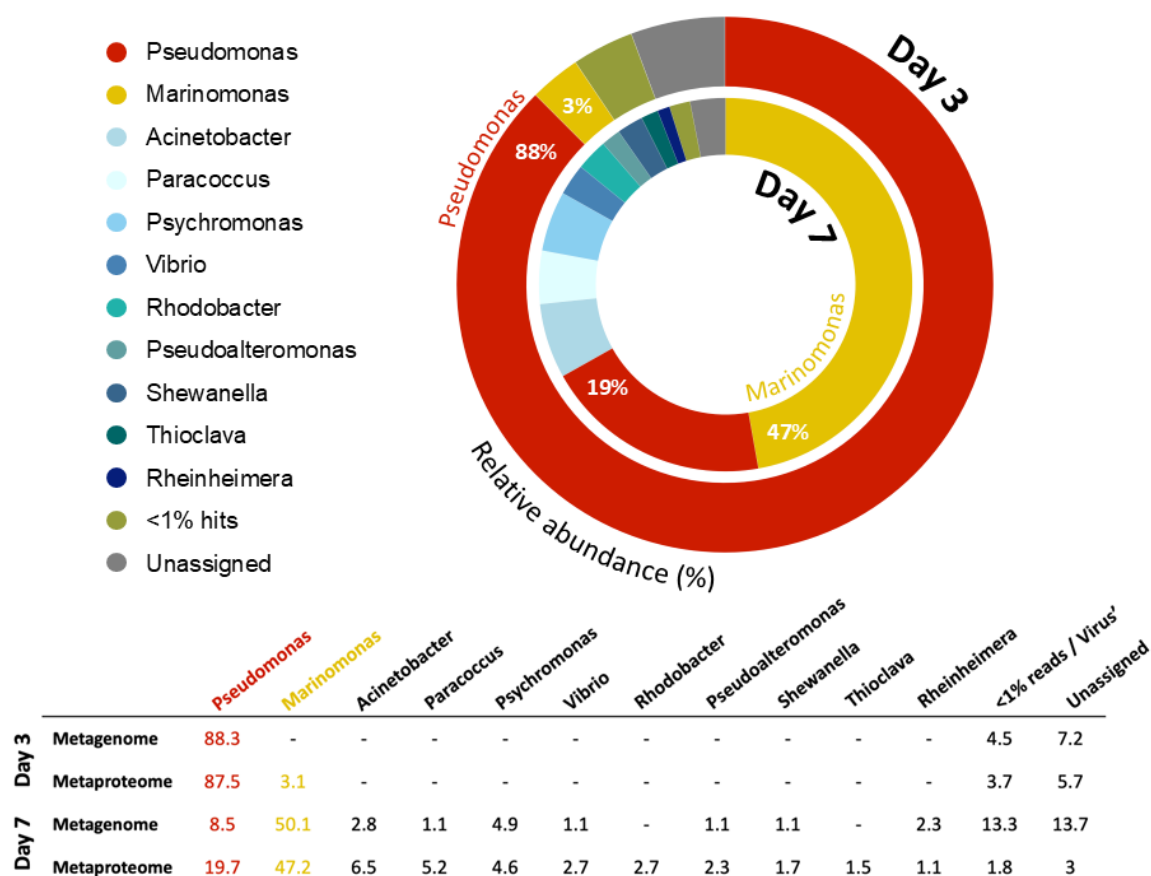


Figure 2.2 Relative abundance of microbial genera found within the metaproteome (table, doughnut plot) and metagenome (table) at each time point. Calculated using the number of proteins associated with each genus relative to all the proteins found in that condition. Values used for doughnut plot shown in table below, as well as corresponding metagenomic data.

3.2 Key functional categories driving early plastisphere development

The proteins expressed on D3 and D7 were classified into functional categories, with 'translation, ribosomal structure and biogenesis,' 'energy production and conversion,' and 'amino acid transport and metabolism' being the most abundant across most genera (Figure 2.3a). These categories were then followed by functions associated with 'cell wall/membrane/envelope biogenesis', 'cell motility', and biofilm formation.

Within 'energy production and conversion,' proteins linked to the TCA cycle were prominent and differentially expressed between time points. These include succinate dehydrogenase, which, based on current taxonomic and proteomic data, was assigned to species of *Pseudomonas* (>D3; FC 2.83), *Marinomonas* (>D7; FC 4.53), *Paracoccus* (>D7; FC 2.72), *Acinetobacter* (>D7; FC 4.27), and *Vibrio* on both days, as well as *Rhodobacter* and *Shewanella* on D7. Additionally, citrate synthase was assigned to the genera *Pseudomonas* (D3; D7), *Marinomonas* (D7), *Acinetobacter* (D7), and *Psychromonas* (D7), while malate dehydrogenase was putatively expressed by *Pseudomonas*, *Marinomonas* (>D7; FC 3.6), *Paracoccus* (>D7; FC 2.92), and *Pseudoalteromonas* (>D7; FC 4.13) on both days. Isocitrate dehydrogenase was assigned to the genera *Pseudomonas* (>D7; FC 3.01 ± 0.79), *Marinomonas* (>D7; FC 3.21 ± 0.52), *Psychromonas* (>D7; FC 3.85), and *Flavobacterium* (>D7; FC 4.07) (Supplementary File S4).

Interestingly, proteins associated with 'amino acid transport and metabolism' were differentially regulated across nine genera (Figure 2.3a), accounting for 11.5% (± 1.5) of the total differentially regulated proteins. These include enzymes involved in glutamate cycling (e.g. glutamate methylesterase, >D7 *Marinomonas*, FC 3.03; glutamine synthetase, >D7 *Marinomonas*, FC 2.7; >D7 *Paracoccus*, FC 2.1), arginine cycling (e.g. acetylornithine aminotransferase, >D7 *Marinomonas*, FC 3.79), and the regulation and transport of branched-chain amino acids (e.g. acetohydroxy-acid isomeroreductase, >D7 *Flavobacterium*, FC 4.95).

Within the categories of 'cell wall/membrane/envelope biogenesis', and 'cell motility', several membrane proteins facilitating substrate binding and biofilm formation were expressed. Key adhesive structures include lipoprotein (assigned to *Pseudomonas* on D3 and *Marinomonas* on D7), large adhesive protein (LAD; assigned to *Pseudomonas* on D3), and outer membrane protein A (OmpA; assigned to *Thioclava*, *Shewanella*, *Pseudomonas*, *Rheinheimera* on D7), with *Acinetobacter* showing the highest expression on D7 (FC 4.4 $\pm$

0.14; Figure 2.3). Additionally, Type V secretory adhesin AidA ($FC\ 2.92 \pm 0.76$) and elements of the Type II secretion system (TISS) were annotated as belonging to *Pseudomonas* on D3 (Figure 2.4). Pili may have also been expressed by *Pseudomonas* on both D3 and D7, with pili assembly proteins PilZ (*Pseudomonas*, D3) and FimV (*Marinomonas*, D7) similarly expressed. In parallel, the curli assembly/transport component CsgG was annotated for *Marinomonas* on D7 (Figure 2.4), indicating potential involvement in biofilm formation. (Figure 2.4) Additionally, flagellin, which may facilitate substrate adhesion, exhibited higher expression on D7 compared to D3. This increase was particularly noted in the genera currently annotated as *Marinomonas* ($FC\ 4.86 \pm 0.79$), *Pseudoalteromonas* ($FC\ 4.5 \pm 0.13$), *Rheinheimera* ($FC\ 4.02$), and *Pseudomonas* ($FC\ 2.96 \pm 0.32$). However, microbes currently taxonomically linked to the *Pseudomonas* genus also demonstrated greater flagellin expression on D3 ($FC\ 3.35 \pm 0.31$), suggesting variation in flagellar expression between genera over time.

Proteins associated with biofilm arrangement including components of calcium-gated (EF-hand domain-containing protein, dCache_2 domain-containing protein, *Pseudomonas*), and potassium channels (potassium uptake protein TrkA, *Marinomonas*) were expressed on D3 and D7, respectively. One instance of quorum sensing, via the expression of the autoinducer 2-binding periplasmic protein LuxP, was found on D7, associated with an uncharacterised species of *Vibrio*. Further examples of cell-cell interactions were identified through the expression of competence protein ComEA (*Pseudomonas*, D3), involved in horizontal gene transfer (HGT), the OAA-family lectin-sugar binding domain-containing protein, (>D3 *Pseudomonas*, $FC\ 4.72$), and TIVSS protein (*Marinomonas*, D7). Proteins involved in purine nucleotide biosynthesis (e.g. bifunctional purine biosynthesis protein PurH), which are also thought to play a role in early biofilm formation, were expressed on both days (Figure 2.3; Supplementary file S4).

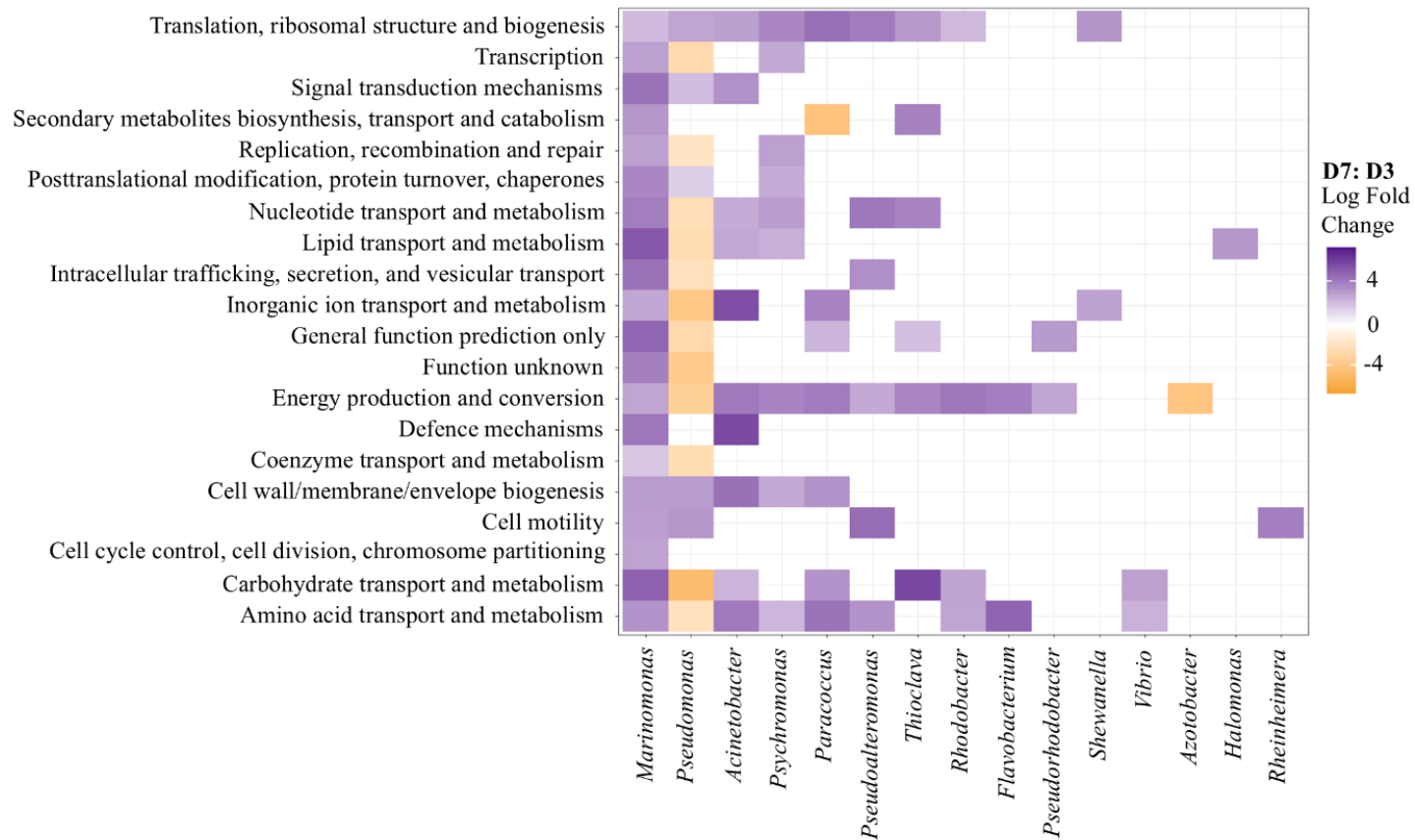


Figure 2.3 Total differentially regulated proteins (FC>1.5, P-adj. <0.05) according to function and taxonomic classification. Positive fold change (purple) indicates greater abundance of proteins on D7 (purple), and negative fold change (orange) indicates greater expression on D3.

3.3 Stress-driven resilience and competition in a nascent marine plastisphere

Proteins associated with the regulation of oxidative stress, nutrient limitation, and interspecies competition were abundantly expressed, reflecting this community's resilience to environmental stress and intense competitive dynamics (Figure 2.4). Evidence of nutrient starvation, particularly of carbon, was observed through the expression of cobalamin biosynthesis protein, CobW (*Marinomonas*), and stringent starvation protein (*Marinomonas*, *Psychromonas*) on D7. Additionally, poly(3-hydroxyalkanoate) granule-associated proteins PhaF, PhaI and poly(R)-hydroxyalkanoic acid (Pha) synthase taxonomically associated with *Pseudomonas* on D3 (Figure 2.4), provided evidence for starvation responses due to the limitation of other nutrients (viz. nitrogen, phosphorus). Moreover, evidence for alternative carbon source utilisation was observed, including three enzymes associated with the alkane degradation pathway. Including, catalase-peroxidase (Kat) associated with *Marinomonas* (>D7; FC 2.96), *Psychromonas* (D7), and *Pseudomonas* (D3), aldehyde dehydrogenase (ALDH) associated with *Pseudomonas* (D3; D7), *Thioclava* (D7), *Rhodobacter* (D7), and *Marinomonas* (D7), and alcohol dehydrogenase (AD) associated with *Marinomonas* (D7; Figure 2.4). Protein identification using PlasticDB - a database containing proteins specifically mediating plastic biodegradation – identified the polyethylene-degrading enzyme laccase was taxonomically linked to *Psychromonas*, on D7 (Figure 2.2; Supplementary file S2).

Proteins associated with carbon metabolism, under the functional classification 'carbohydrate transport and metabolism' (Figure 2.3), were consistently upregulated by the genus annotated as *Pseudomonas* on D3, and by *Marinomonas* on D7. CreA family proteins and response regulatory domain-containing proteins were also associated with *Pseudomonas* on D3. Tricarboxylic transporters associated with *Pseudomonas* on D3, and *Pseudomonas*, *Pseudorhodobacter*, *Rhodobacter* and *Marinomonas* on D7 were also found (Supplementary file S4). Interestingly, enzymes involved in aromatic hydrocarbon biodegradation were characterised, including dienelactone hydrolase, mostly associated with *Marinomonas* on D7 (FC 2.03; Figure 2.3), and *Pseudomonas* on D3. Nine other enzymes associated with this process were discovered on both days, including 3-oxoadipate-CoA transferase, 3-hydroxyacyl-CoA dehydrogenase, acetyl-CoA carboxylase, acetyl-CoA acetyltransferase, acetyl-CoA dehydrogenase, pyruvate aldolase, p-cresol methylhydroxylase (PCMH)-type protein, thiolase I, and thiopurine s-methyltransferase. Of these, acetyl-CoA acetyltransferase

and acetyl-CoA dehydrogenase were most expressed on D7 (FC 4.51 and 2.48, respectively). Evidence for nitrogen cycling was found through the constitutive expression of nitrate reductase (*Pseudomonas*, D3; *Pseudomonas*, *Marinomonas*, D7), nitrous-oxide reductase (*Pseudomonas*, D7), and nitrogen regulatory protein PII (*Pseudomonas*, D3; *Pseudomonas*, *Acinetobacter*, D7) on D3 and D7. Nitrogen and urea transporters were also taxonomically annotated to *Pseudomonas*, *Marinomonas*, and *Paracoccus* on both days.

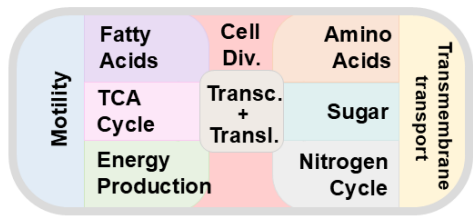
In response to intracellular ROS, the plastisphere communities expressed a variety of stress-response proteins, including alkyl hydroperoxide reductase (Ahp), catalase (CAT), catalase peroxidase (Kat), glutathione peroxidase (Gpx), thiol peroxidase (TP), and thioredoxin peroxidase (TPx) in response to hydrogen peroxide (H₂O₂), flavohemoprotein (Fhp) to respond to nitric oxide (NO), and superoxide dismutase (SOD) to neutralise superoxide radicals. The most frequently expressed stress-response proteins were Ahp (*Pseudomonas*, D3; *Pseudomonas*, *Paracoccus*, D7; >D7 *Acinetobacter*, FC 5.83; >D7 *Marinomonas*, FC 4.32), Fhp (*Pseudomonas*, D3; *Acinetobacter*, D7; >D7 *Marinomonas*, FC 3.5), Kat (*Pseudomonas*, D3; *Psychromonas*, D7; >D7 *Marinomonas*, FC 2.96), and SOD (*Paracoccus*, D3; *Acinetobacter*, *Psychromonas*, *Rhodobacter*, D7; >D3 *Pseudomonas*, FC 3.92; >D7 *Marinomonas*, FC 2.74) due to their consistent annotation across multiple genera (Figure 2.4; Supplementary file S4). In response to metals and anions, *Marinomonas* (D7), and *Paracoccus* (D7) were associated with the expression of copper resistance protein A (CopA), and toxic anion resistance protein (TelA), respectively, and *Pseudomonas* (D3) was associated with the expression of tellurium resistance protein (TerD) (Figure 2.4). Osmotic stress proteins OsmC (*Pseudomonas*, *Marinomonas*, D7), and glucans biosynthesis protein C (*Pseudomonas*, D3) were also found. The downstream effects of stress were apparent in the expression of membrane repair proteins ATP-dependent zinc metalloprotease (FtsH), α -2 macroglobulin (α 2m), and phage shock protein (Psp). Additionally, transcriptional response proteins such as HU, RecA, RdgC, cold shock-domain (CSD) proteins, and DNA repair proteins such as GrpE, Lon protease, and methionine sulfoxide reductase were also abundant. Further stress mitigation was supported by thioredoxin/thioredoxin reductase systems, along with a suite of chaperones and chaperonins (e.g., ClpA, ClpB, DnaJ, DnaK, GroEL, GroES, HtpG, SurA), and the redox enzyme glucose-6-phosphate dehydrogenase (Figure 2.4). Among these, the cold shock-domain protein was the most abundantly expressed protein, and with taxonomic annotation was found to be putatively expressed by *Pseudomonas* (>D3 FC 3.8), *Marinomonas* (D7), *Acinetobacter* (D7), *Paracoccus* (D7), and *Shewanella* (D7).

Further protective mechanisms were also identified, such as dipicolinate synthase expression by the prevalently annotated genus *Pseudomonas* on D3 and capsular biosynthesis protein on D7. Interestingly, proteins linked to reactive oxygen species (ROS) generation, including sarcosine oxidase (*Pseudomonas*, D3) and Na(+)-translocating NADH-quinone reductase (*Marinomonas*; > D7 FC 7.01), were also expressed.

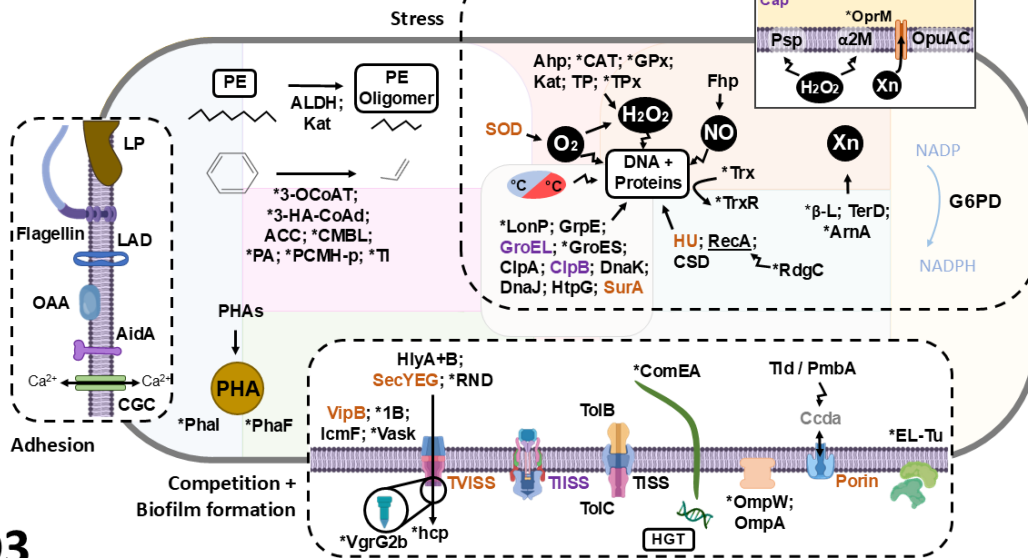
Competition for resources was evidenced by the expression of proteins associated with competitive stress. This included the type VI secretion system (TVISS) proteins, such as TssM, contractile sheath large subunit, and secretion proteins Evp, IcmF, and VasK, associated with the genera *Pseudomonas* (D3; FC 1.74 ± 0.12), *Pseudoalteromonas* (D7; FC 3.54), and *Acinetobacter* (D7) (Figure 2.4; Supplementary file S4). Competitive advantage was also indicated by the expression of the actin cross-linking toxin VgrG2, which may have been expressed by *Pseudomonas* on D3. Hemolysin-related proteins, which may have been similarly involved in this competitive response, were also associated with *Pseudomonas* at this time-point (Figure 2.4). In potential response to antibiotic production, resistance mechanisms such as polymyxin resistance protein and outer membrane efflux system protein detected on D3 were taxonomically assigned to *Pseudomonas* on D3, while β -lactamase was also assigned to *Pseudomonas* and colicin-I receptor proteins were assigned to *Shewanella* on D7 based on current taxonomic and proteomic annotations.

Interestingly, the potential pathogens *Pseudomonas syringae* and *Pseudomonas aeruginosa* were taxonomically annotated on D3, both of which were associated with the expression of two TVISS proteins, while *P. syringae* may have also expressed the identified tol-pal system proteins and FeADH domain-containing protein (Figure 2.4). These results indicate a complex competition network within the biofilm, where both resource limitation and interspecies antagonism shape the microbial community structure and resilience.

Key:



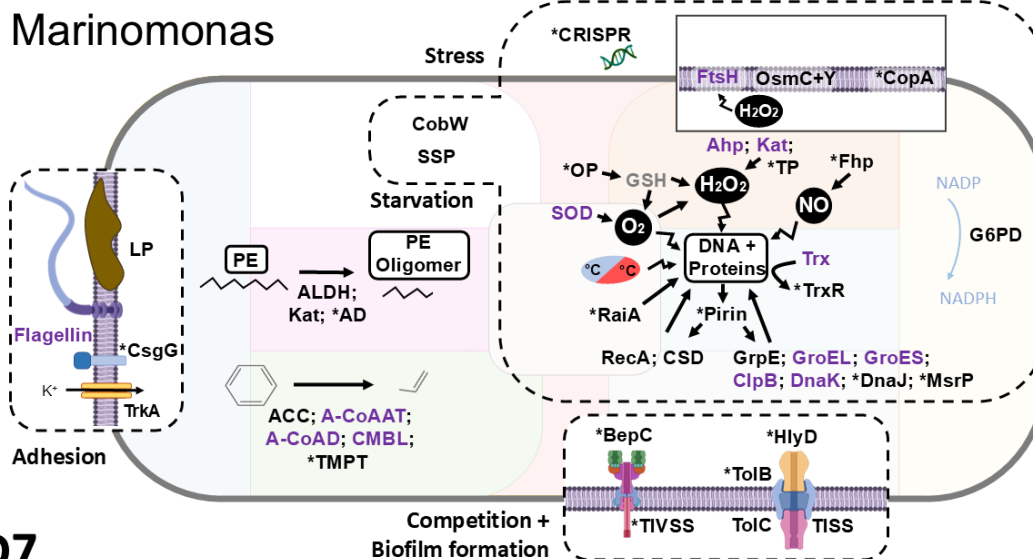
Pseudomonas



D3



Marinomonas



D7

Figure 2.4 Graphic representation of biologically relevant processes found within this study's most frequently annotated bacterial genera from relative quantification and qualitative analysis (manual functional annotation) of the metaproteomic dataset for each condition. Purple = more expressed on day 7, orange = more expressed on day 3, black = qualitative results, * = qualitative results with 1 entry, grey = not found, but can be assumed. Colour code indicates all basal processes active within the genus at that time (>1 protein each). All basal processes active in the largest, annotated microorganisms. **Acronyms:** **Figure elements:** H₂O₂, hydrogen peroxide; HGT, horizontal gene transfer; NO, nitric oxide; O₂, superoxide; PE, polyethylene; PHA, polyhydroxyalkanoate granule; Xn, xenobiotic. **Stress** (osmotic, oxidative, heat/cold, interspecies interaction, xenobiotic): Ahp, alkyl hydroperoxide reductase; ArnA, polymyxin resistance protein; α 2M, alpha-2-macroglobulin; β -L, β -lactamase; Cap, capsular biosynthesis protein; CAT, catalase; CopA, copper resistance protein A; CSD, cold shock domain containing protein; Fhp, flavohemoprotein; FtsH, ATP-dependent zinc metalloprotease; G6PD, glucose-6-phosphate dehydrogenase; Gpx, glutathione peroxidase; GrpE, DnaK nucleotide exchange factor; GSH, glutathione; HU, transcriptional regulator HU; Kat, catalase peroxidase (also PE degradation); LonP, lon protease; MsrP, methionine sulfoxide reductase; OP, 5-oxoprolinase; OprM, outer membrane efflux system protein; Osm, osmotically inducible protein; Psp, phage shock protein; RdcC, recombination-associated protein; RecA, SOS response protein; SOD, superoxide dismutase; OpuAC, substrate binding domain of ABC-type glycine betaine transport system; TerD, tellurium resistance protein; TP, thiol peroxidase; TPx, thioredoxin peroxidase; Trx, thioredoxin; TrxR, thioredoxin reductase; Chaperones- ClpA, ClpB, DnaJ, DnaK, HtpG, SurA; Chaperonins- GroEL, GroES. **Virulence and biofilm formation:** 1B, hemolysin co-regulated protein; BepC, outer membrane efflux protein; CcdA, control of cell death protein; ComEA, competence protein; EL-Tu, elongation factor Tu; hcp, hemolysin-coregulated protein; Hly, hemolysin activation/secretion protein; Omp, outer membrane protein; RND, efflux resistance-nodulation-division (RND) transporter; SecYEG, translocon; TISS, type-I secretion system; TIISS, type-II secretion system; TIVSS, type-IV secretion system; TVISS, type-VI secretion system; VgrG2b, actin cross-linking spike protein; Translocons- IcmF, Tld/PmbA, Vask, VipB. **Starvation:** CobW, cobalamin biosynthesis protein; PhaF, polyhydroxyalkanoate (Pha) granule-associated protein F; Phal, Pha granule-associated protein L; PHAs, PHA synthase; SSP, stringent starvation protein. **Adhesion:** AidA, adhesin; CGC, calcium-gated channel; CsgG, curli assembly protein G; LAD, large adhesive protein; LP, lipoprotein; OAA, OAA-family lectin sugar binding domain containing protein; TrkA, Potassium uptake protein. **Aromatic hydrocarbon degradation:** 3-OCOAT, 3-oxoadipate-CoA transferase; 3-HA-CoAd, 3-hydroxyacyl-CoA dehydrogenase; ACC, acetyl-CoA carboxylase; A-CoAAT, acetyl-CoA acetyltransferase; A-CoAD, acetyl-CoA dehydrogenase; CMBL, dienelactone hydrolase; PA, pyruvate adolase; PCMH-p, p-crestol methylhydroxylase (PCMH) type protein; TI, thiolase I; TMPT, thiopurine s-methyltransferase. **PE degradation:** AD, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase.

4. Discussion

In this study, we identified 2,948 non-redundant proteins from the LDPE plastisphere, providing near-complete proteomes for *Pseudomonas* and *Marinomonas* – the two genera which proteins were most frequently taxonomically assigned to – within the first days of colonisation. Importantly, while metagenomic analysis reveals the genetic potential of microbial communities, the use of metaproteomics provided distinct advantages by enabling direct observation of enzymes expressed by bacteria at the plastic-biofilm interface. This approach allowed us not only to confirm gene expression but also to precisely link specific proteins to their active roles, bridging the gap between genetic potential and functional protein production (216). This extensive dataset offered valuable insights into the possible metabolic activity of such key taxa, as well as the broader functioning of the young plastisphere community. Through this approach, we revealed the intense competition between taxa within the rapidly developing community and identified the molecular mechanisms which may support plastisphere resilience to nutrient limitation and oxidative stress. Our results also revealed molecular interactions between the early plastic colonisers and the plastic itself, with important implications for the modulation of biofilm formation and the exploration of plastic biodegradation. These results provide new insights into the dynamics of the nascent plastisphere and their biotechnological potential. However, it should be noted that genus-level annotations of detected proteins are tentative, as many functional proteins are conserved across taxa and assignment is limited by database resolution (181, 217).

4.1 Dominance of key taxa in the young marine plastisphere

Based on current data availability for taxonomic annotation, the activity of the young marine plastisphere appears to have been dominated by heterotrophic bacteria, particularly Gammaproteobacteria, consistent with previous studies examining plastisphere diversity, formation, and function (10, 28, 35, 69, 177, 195). In the present study, plastisphere communities sampled from the Scottish coast were enriched in a controlled laboratory setting, perhaps accounting for the reduced plastisphere diversity (≤ 15 genera) compared to other direct studies (≤ 30 genera) of the marine plastisphere (35, 59, 218). It is not known whether this was directly reflective of the sampled community as the stock community composition was not tested. Nevertheless, community development in the present study mirrored previous observations demonstrating that plastisphere diversity increases over time (29, 201-203). Indeed, we observed a plastisphere community whose activity was dominated

by *Pseudomonas* after three days of colonisation, shifting to an active community comprised of >8 active genera, including Alphaproteobacteria and Bacteroidetes after seven days. *Pseudomonas*, the pioneering genus in this study and one of two high-quality MAGs recovered from the marine plastisphere, is known for its metabolic versatility and biotechnological potential. Its species span biogeochemical cyclers, symbionts, decomposers, denitrifying bacteria, and pathogens (219). This genus is particularly known for its biofilm-forming capabilities, facilitated by the secretion of specialised membrane proteins and secretion systems which likely facilitate interactions with plastic (Section 4.3) (220). We have previously observed *Pseudomonas* within the marine plastisphere, but at <25% of the active community (177, 195), indicating that the functional role of *Pseudomonas* may be most significant within the initial stages of plastisphere development. As such, the putatively annotated proteome of this organism provides important insights into microbe-plastic interactions at the time of most significance, where colonisers specifically attracted to the plastic surface may utilise it as a substrate (218). Nevertheless, the most abundant and active genera annotated for D7, *Marinomonas*, was the second high-quality MAG recovered and is also a marine-adapted genus typically associated with pollutant bioremediation (193, 221). Its enrichment in this study suggests that the LDPE, and this study's restrictive growth conditions facilitated its growth and biofilm formation as a secondary coloniser. Consistent with this, in our previous work, *Marinomonas* was also the dominant and active taxa following 1-2 weeks LDPE colonisation, but it was less important within the mature marine plastisphere (195). Additionally, further bioremediative genera such as *Thioclava* (72) are thought to be active on D7 (relative abundance 1.5%), alongside the presence of potential pathogens *P. aeruginosa*, and *P. syringae* which represented 4.6% and 17.5% of all annotated species, equalling 285 non-redundant proteins (222, 223). Indeed, *P. syringae* was the most annotated species in the D7 plastisphere, indicating a concerning dominance of potential pathogens within the portion of the plastisphere (19%) that could be annotated to this level. The observed diversity in this early-stage plastisphere highlights the biofilm's dynamic nature, while the enrichment of two taxa as high-quality MAGs underscores the selectivity of the plastisphere and this study's conditions, likely shaped by competitive interactions.

4.2 Biofilm formation

Biofilm formation is a key survival strategy for microbial proliferation in challenging, oligotrophic marine environments (224, 225). Several proteins involved in surface adhesion were found to be repetitively identified across different taxa known for their propensity to form biofilms. In this way, biofilm formation might have been promoted in *Pseudomonas*, *Marinomonas*, *Acinetobacter*, and *Vibrio* through the expression of secretion systems I, II, and IV, as well as flagellin, adhesin, curli, Omps, lipoprotein, and large adhesive protein (226-228). These surface adhesion mechanisms were predominantly associated with *Pseudomonas*, potentially aiding their dominance and rapid colonisation of plastic at D3. The succession of *Marinomonas* after the initial colonisers further supports their biofilm-forming ability, which is well-documented in marine environments (221). The detection of quorum sensing molecules for the *Vibrio* genus on D7 also indicates that this genus was involved in processes potentially related to surface adhesion, such as the regulation of membrane sorting proteins, which are often associated with coordinated biofilm development (229).

Biofilm formation may have also been supported by the upregulation of purine biosynthesis, the TCA cycle, arginine, and glutamate cycling proteins, which are crucial for cross-feeding within the biofilm community (224, 225, 230). Moreover, our previous findings highlighted the critical role of glutamine in biofilm proliferation, likely due to this cross-feeding (177, 205, 231). Its regulation was found to be closely linked with ammonium availability and oxidative stress within carbon-deficient environments, with *Marinomonas* showing distinct glutamine metabolism within the plastsphere (195). This suggested a differential nutrient-utilisation strategy between biofilm-associated and planktonic communities, further supporting the notion of a resilient and metabolically adaptive plastsphere biofilm (177). The observed increase in microbial diversity over time implies that the resources produced by pioneering species (e.g., carbon, amino acids) may have facilitated the growth of subsequent colonisers, creating a more complex and resilient biofilm (205, 231).

Importantly, plastsphere biofilms offer distinct advantages to microorganisms in these environments. For instance, the expression of TIVSS and a competence protein suggests the potential for HGT within the plastsphere (226), allowing microbes to share adaptive traits. Moreover, the expression of calcium-gated channels (e.g., EF-hand and dCache_2 domain-containing proteins) and potassium uptake proteins (e.g., TrkA) further indicates that

nutrients may have been exchanged between neighbouring cells, further enhancing cross-feeding, and the biofilm's capacity to thrive under nutrient-limited conditions (232). Moreover, nitrogen sufficiency is suggested by the expression of nitrogen cycling proteins like nitrate reductase, nitrogen regulatory protein PII, and glutamine. On the other hand, carbohydrates and amino acids were metabolised and transported within the community, further suggesting possible resource sharing through symbiosis or cross-feeding, contributing to the overall resilience and stability of the biofilm (205, 231).

However, it remains unclear to what extent biofilm formation on plastic confers unique advantages over microbial communities in the surrounding seawater. Our previous research has suggested that the proteins associated with biofilm-formation and cross-feeding are enriched in plastsphere communities (195). Though in this study of the nascent plastsphere, it is possible that the observed shifts in community composition (*Section 4.1*) may also reflect similar dynamics in seawater communities, where these same proteins could also be induced. Furthermore, in this study, significantly less protein was retrieved from the more developed plastsphere community (D7) compared to the plastsphere only grown for three days (*Section 2.3*). This reduced protein yield may be a result of natural biofilm maturation and the increased cell death, dormancy, and EPS production which accompanies this progression (233, 234). Alternatively, this decline may be attributed to a loss in biomass after biofilm shearing and detachment. While such detachment is also a hallmark of biofilm progression (234), it would suggest that the plastsphere community was less structurally stable than the proteomic data indicates. Consequently, while the proteins expressed here are associated with biofilm formation and nutrient cross-feeding in other studies, their specific roles in supporting biofilm structure and interspecies interactions on plastic are not fully resolved in this nascent community. Moreover, it is important to note that biofilm communities are not only cooperative but also competitive, as microorganisms compete for dominance and resources within these complex ecosystems.

4.3 Resilience and competition

Proteins associated with the regulation of metabolic stressors were among the most abundant expressed non-housekeeping proteins found in our datasets. Enzymes responsible for mitigating oxidative stress were frequently associated with multiple genera (e.g., *Pseudomonas*, *Marinomonas*, *Acinetobacter*, *Paracoccus*, *Psychromonas*, and *Rhodobacter*), with nearly the entire process of reactive ROS suppression (antioxidant production → DNA

regulation → DNA repair) being characterised (235). Oxidative stress in multi-species biofilms may be triggered by spatial constraints, forced oxygen gradients, competition, nutrient limitation, and the accumulation of abiotic stressors such as metals (e.g., CopA, TelA, TerD) (10, 236). The plastic itself could also contribute to oxidative stress through the accumulation of ROS under UV-light exposure (237, 238). The multifunctionality of proteins identified here, such as the frequently expressed CSD protein (*Pseudomonas*, *Marinomonas*, *Acinetobacter*, *Paracoccus*, *Shewanella*), suggests that they may have been induced by a combination of these stressors. Indeed, in addition to their role in cold adaptation, cold shock proteins are crucial for maintaining protein stability and proper folding under stress conditions, extending their protective function against oxidative and metabolic stress (239).

Granule storage, competition, and the expression of carbon-limitation-related proteins highlight nutrient stress during both sampling days. Interestingly, granule formation is often triggered by the scarcity of nitrogen and phosphorous, and an excess of carbon (240). This nutrient limitation, particularly of carbon and energy sources, is known to cause dysregulation of core metabolic processes, leading to the accumulation of ROS, as well as an increased competition for resources (241, 242). In support of this, the expression of VgrG2, which has roles in virulence, and in inducing morphological defects in competing bacteria, may suggest competitive interactions within the plastisphere biofilm (243). The production of this toxin, alongside other virulence factors (i.e., hemolysin), may have therefore induced membrane damage in plastisphere microorganisms adjacent to the organism assigned as *Pseudomonas* through the action of the TVISS (243, 244). Interestingly, we identified a near-complete annotation of the TVISS machinery (i.e. TVISS proteins IcmF, VasK, VipB, membrane subunit TssM, contractile sheath small subunit, and large subunit, Hcp1 family TVISS effector, EvpB family TVISS protein) expressed by active members of the marine plastisphere (68, 75, 177). These findings suggest that interspecies competition within the biofilm could be a significant factor in shaping planktonic community structure and dynamics. The expression of these virulence factors (i.e., hemolysin-related proteins) within the nascent marine plastisphere also emphasises the proposed hazards of plastic debris as a carrier of potential pathogens (244, 245).

Using the TVISS as an indication of virulence (246), the organism annotated as *Pseudomonas* was most competitive on D3 within a predominantly single genera biofilm. Previously, *Pseudomonas*, and *Pseudoalteromonas* have also been found to outcompete other microbial

species through the secretion of toxins (80, 247, 248), perhaps actively antagonising other members of the present microbial community. The expression of proteins used to convey antibiotic resistance (i.e. β -lactamase, efflux RND transporters, capsule protein) (80, 226, 249) by the genera assigned as *Pseudomonas* and *Marinomonas* on D7, highlight the intense competition within diverse microbial communities, which may shape the plastisphere. These mechanisms are important for maintaining cell integrity in a stressful environment, but their expression puts great demand on the cell's intracellular resources (226, 241, 246, 247), including those which may be used in key metabolic processes. The deregulation of these processes can further precipitate oxidative stress – similar to nutrient deprivation (241, 242) – and limit the growth of impacted bacteria (235). Strategies used for interspecies competition by plastisphere, and also by planktonic microorganisms may have therefore contributed to this plastisphere's function and composition, though the influence of the surrounding planktonic community was not investigated here.

4.4 Metabolic versatility for plastic degradation

In addition to the proteins which may have increased carbon utilisation efficiency in these plastispheres (*Section 4.3*), we identified proteins linked to the utilisation of alternative carbon sources, which is particularly relevant for plastic biodegradation. We do not find this to be conclusive evidence of pollutant biodegradation within these plastispheres. However, it is interesting to note that the two enzymes expressed here which align with Delacuvellerie's (2022) depiction of the alkene degradation pathway (AD, ALDH) are expected to be involved in the initial depolymerisation process of polyethylene degradation by Tao (2023) (68, 250). Catalase peroxidase, also found here, but not in the alkane degradation pathway, is expected to play a role in the first steps of polyethylene biodegradation. Notably, this enzyme was taxonomically assigned as being expressed by *Pseudomonas*, a genus frequently associated with polyethylene biodegradation (93, 251, 252). Our recent paper on the late-stage plastisphere also revealed the expression of acyl-CoA dehydrogenase (177), another element of the alkene degradation pathway, which was also expressed in Delacuvellerie's (2022) study alongside ferredoxin and ferredoxin reductase (68). In combination, five enzymes associated with the alkene degradation pathway have now been annotated from within the marine plastisphere, plus an additional enzyme which may directly depolymerise polyethylene.

In alignment with our previous investigation, 10 proteins involved in fatty acid beta-oxidation (i.e. acetyl-CoA carboxylase, acetyltransferase, and dehydrogenase) were found in these plastispheres (177). This process, like the alkene degradation pathway, has been linked to both plastic biodegradation and the biodegradation of other xenobiotic hydrocarbons due to the need to dismantle their similarly structured hydrocarbon base (253). Metabolites from xenobiotic degradation are also likely funnelled directly into the fatty acid beta-oxidation pathway once initially depolymerised (250). The dienelactone hydrolase enzyme expressed here is not linked to this pathway but is associated with the final stages of aromatic hydrocarbon (chlorophenol) degradation (254, 255). The discovery of such enzymes is interesting because no such xenobiotics were present in these samples, and the plastics used were purportedly free of additives. Another trend of note is the probable upregulation of these proteins by *Marinomonas*. Even if these organisms did not actively biodegrade aromatic hydrocarbons in these samples, this may reveal a little of their adaptability to other xenobiotics such as crude oil in the marine environment (193).

The combined expression of enzymes which may be used by bacteria to biodegrade plastics, and other noted xenobiotics here may not indicate that plastic biodegradation is occurring in this scenario. However, it may suggest that organisms found here and in other marine plastispheres have the capacity to perform this biodegradation. Interestingly, the incomplete annotation of these processes can rarely be attributed to a singular genus. Instead, several genera express these enzymes in tandem. Encouraging a community-level approach to bioremediation may therefore lead to more resilient and efficient strategies for managing plastic pollution in marine environments (256). Additionally, by promoting research on microbial plastic degradation, policymakers can address critical gaps in understanding the ecological roles of these organisms, which could inform environmental risk assessments and the development of new, biodegradable plastic alternatives (69, 209). Together, these findings underscore the potential of diverse microbial communities within marine plastispheres to contribute to plastic biodegradation, highlighting the importance of these natural processes as a foundation for sustainable plastic waste management solutions.

Conclusion

To date, the molecular mechanisms underpinning the initial days of microbial colonisation of marine plastic debris have remained uncharacterised. To resolve the interactions between the earliest colonisers and plastic surfaces, we utilised multi-omics to investigate the activity of the marine plastisphere community grown on LDPE for 3 and 7 days. Our findings reveal a complex relationship between pioneering and secondary colonisers, shaped by stressors such as reactive oxygen species (ROS) and nutrient limitation, while also highlighting the interplay of cooperation and competition among microorganisms. Consistent with previous research, the majority of these nascent plastisphere microorganisms were primarily composed of heterotrophic bacteria, indicative of marine plastisphere communities found in colder environments. The near-complete assembly of metagenome-assembled genomes (MAGs) for *Pseudomonas* and *Marinomonas* significantly enhanced our ability to characterise the associated proteomes and elucidate their precise functions within the dynamic young plastisphere. In this study, *Pseudomonas*, the pioneering genera, was likely aided through its known ability to form biofilms, while the genera detected in the later plastisphere diversified over time, potentially fuelled by metabolites produced within these biofilms. Proteins related to the biodegradation of plastic and other xenobiotics were taxonomically assigned as being expressed by *Pseudomonas*, *Marinomonas*, and three additional genera, highlighting the potential for these microorganisms to contribute to plastic biodegradation processes. Further studies exploring the functioning of the nascent plastisphere are required to determine their role in those processes. For example, targeted proteomic approaches focusing on the most prominent species of these key genera would yield a more complete proteome of these genera, uncover new enzymes of interest, and refine our knowledge of their functional roles. Combining these strategies with weight-loss measurements, CO₂ loss assays, and advanced techniques such as stable isotope probing (SIP) could directly track carbon flow from the plastic into the plastisphere community (see General Discussion). Overall, this study significantly establishes an understanding of the early formation of the plastisphere and its interactions with plastic, providing valuable insights essential for addressing the growing environmental challenge of plastic pollution.

Associated data

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (<https://proteomecentral.proteomexchange.org/>) via the MassIVE partner repository (257, 258) with the dataset identifier PXD056358.

Chapter 3

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Organic UV-filters in Marine Ecosystems

Chapter 2 revealed that microorganisms in the plastisphere can express a range of functions which may be directly related to their proximity to the plastic (i.e., stress, xenobiotic biodegradation) or a natural part of biofilm activity (i.e., cross-feeding, competition). These are key findings which contribute to the understanding of how microorganisms interact with plastic in the ocean. However, we acknowledge that this study lacked any influence of the extraneous variables often found in the ocean (i.e. chemical pollutants) so may not be representative of the plastisphere in the marine environment. To study this early plastisphere we therefore planned to introduce another pollutant and study its effect on the plastisphere community. Organic UV-filters were selected as the co-pollutant we would use to investigate this interaction due to their prevalence in the ocean and known impact on other marine organisms.

Chapter 3 was therefore designed to investigate which prevalent organic UV-filter might have the greatest impact on bacterial metabolism – the most prevalent microorganism in our study's plastisphere community – and to use exoproteomics to investigate how marine bacteria might be first interacting with pollutants and other external stimuli.

Secreted Proteins as a Key Interface in Microbial Response to Organic UV Filters

Abstract

Organic UV-filters are prevalent pollutants in coastal environments due to their widespread use in personal care products (PCPs) and subsequent accumulation near coastal touristic hubs. Ethylhexyl Methoxycinnamate (EHMC) and Benzophenone-3 (BP3) especially are organic UV-filters found at highest concentrations in sunscreens and marine environments respectively, and both are found to delay the growth of marine microorganisms. While the toxicity thresholds of such compounds in marine organisms are well established, their impact on bacterial metabolic processes – critical for maintaining oceanic biogeochemical cycles – remains underexplored. Investigating the secretome – the collection of proteins secreted by a cell, and the first interface with its environment – can provide valuable insights into how bacteria respond to and interact with these pollutants. In this study, we therefore investigated the secretome of *Epibacterium mobile*, an environmentally relevant marine bacterium, following exposure to the UV-filters EHMC and BP3. Growth response assays revealed that *E. mobile* tolerated higher concentrations of EHMC than BP3. Proteomic analysis then identified a significant upregulation of 27 and 33 proteins with EHMC compared to the control and BP3, respectively. Many of these proteins, particularly those detected uniquely in response to EHMC, were associated with transmembrane transport, suggesting an impact of protein contamination within the data pool. This study also faced many additional challenges common in secretome research, including low protein yields, and limited database coverage, which may have skewed the results, and influenced their interpretation. We therefore highlight the need for methodological improvements and expanded databases to enhance the accuracy and depth of secretome studies. This research represents a pioneering effort to investigate bacterial secretome responses to UV-filters, offering insights into microbial adaptations and providing a foundation for further exploration of marine pollution impacts on microbial communities.

1. Introduction

Marine microorganisms represent over 70% of all marine biomass, thereby supporting much of the marine food-chain, and controlling many trophic interactions both in and out of the water-column (259, 260). Of these marine microorganisms, bacterial metabolic processes govern a significant portion of the influx and efflux of essential gasses and solutes (e.g., O₂, NO₃⁻) through biogeochemical cycling, contributing to the maintenance of marine ecosystems (190). A proteomic approach to the study of these organisms has revealed their dynamic adaptations to environmental conditions, linking an abundance of proteins to their nutrient acquisition, stress responses, and metabolic versatility (183, 189, 261-264). Additionally, the study of their extracellular proteome, or 'exoproteome', has highlighted the contributions of bacteria to marine biogeochemical cycling, the full complexity of bacterial pathogenesis, and their individual sensitivity to external pressures (e.g., temperature, PH) (265-268). These extracellular proteins represent up to 30% of a cell's total proteome, and are of essential importance for the complete understanding of a bacterium's entire metabolic profile (267). Up to 69% of the ocean's total metaexoproteome is also represented by bacterial proteins, emphasising the bacterial community's unrivalled influence over the marine ecosystem (265). Importantly, the exoproteome also contains the proteins which are exclusively secreted by these bacteria, termed the 'secretome' (269, 270). This is the first interface that bacteria have with their environment, containing proteins which mediate their response to stimuli including cells, hosts, toxins, and biodegradable substrates (266, 269-271).

In the marine environment, one of the most significant emerging stimuli facing bacterial communities is pollution (272). Persistent organic pollutants (POPs) especially can remain in the water column for years and continue to impact marine life for that time (30, 273). Many of these POPs will become diluted in the ocean, thereby diluting the impact they will have on the bacterial community (273). However, organic UV-filters are consistently used in personal care products (PCPs) including toothpastes, moisturisers, and sunscreens for UV-protection, resulting in their accumulation in coastal regions near municipal outlets and touristic areas (48, 140). Organic UV-filters especially are also highly lipophilic, leading to their accumulation in the sea surface microlayer (SML) specifically, and the additional formation of slicks in these coastal regions (142, 145, 146). Concerningly, these coastal regions also host diverse bacterial communities, with many of those found in the SML specifically being

instrumental in regulation essential biogeochemical processes for the communities below (32, 274). Organic UV-filters therefore have the potential to impact hundreds to thousands of important bacterial genera. Many of these UV-filters cause defects in fish, coral, and other marine biota (275). And in marine microorganisms, the organic UV-filter EHMC (EthylHexyl MethoxyCinnamate) has demonstrated toxicity in five of 27 tested marine bacteria; more than any other tested UV-filter (48, 276). This is one of the most used, and is now identified as the most concentrated (4.3 ng/L to 4.043 µg/L) organic UV-filter in otherwise distinct marine regions (277-281). Furthermore, our previous study of the marine plastisphere – the marine biofilms which form on plastic – revealed that EHMC significantly impacts microbial community composition and dynamics (282). Similarly, BP3 (benzophenone-3), or oxybenzone, a UV-filter now widely regulated due to its detrimental effects on coral, was found to induce toxicity in *Epibacterium mobile* at 364 µg/L (48, 283). Concerningly, BP3 concentrations in marine environments can sometimes reach these levels, and this toxicity threshold for *E. mobile*, as well as likely other bacteria (Chapter 1).

Epibacterium mobile, previously known as *Ruegeria mobilis* (284), is a marine bacterium of the *Roseobacter* genus, one of the most abundant bacterial genera ($\leq 20\%$ total cells) in coastal locations (285, 286). In continuation of the aforementioned study, we revealed that *E. mobile* regulates intracellular protein expression in response to the coastal pollutant BP3, including those related to oxidative stress, DNA damage responses, motility, protein synthesis inhibition, and export mechanisms (183). The exoproteome of *Roseobacter* also contains proteins related to motility, adhesion, transporter, toxin- resistance, and other adaptive strategies (267). However, due to the dilution of proteins which occurs post-secretion, study of the secretome is more challenging than study of the proteome, meaning it remains much less studied (269, 287). As a result, the role of these secreted proteins in responding to stimuli like organic UV-filters or other marine stressors remains poorly understood. Expanding research into the stimulus-response of the bacterial secretome is therefore essential for understanding how these key organisms adapt to the increasing threat of anthropogenic change. In this study, we therefore build upon previous research of *E. mobile*, and attempt to characterise its complete early-stage response to BP3, and EHMC exposure through analysis of the secretome.

2. Materials and Methods

2.1 Strains and reagents

For secretome analysis, stock solutions of Benzophenone-3 (BP3; CAS – No. 131-57-7) and EthylHexyl MethoxyCinnamate (EHMC; CAS – No. 5466-77-3) were prepared in dimethyl sulfoxide (DMSO, Sigma-Aldrich, purity >99%) at 1240 mg/L, and 870 mg/L respectively; stored in the dark at room temperature. For growth curve analysis, EHMC (CAS – No. 5466-77-3) was prepared in DMSO (Sigma-Aldrich, purity >99%) at 2100 mg/L. *Epibacterium mobile* (BBC367) stock was kept at -80 °C in marine broth 2216 (DIFCO, United States) with 30% glycerol. *E. mobile* was grown on marine agar plates. After 24–48 h of incubation, colonies were suspended and grown aerobically on a rotary shaker (95 rpm) at 25 °C in artificial seawater with 3 mM D-glucose, vitamins, and trace elements (ASW-G) (206).

2.2 Bacterial exposure to organic UV-filters

For growth curve analysis, two EHMC exposure conditions of 500 µg/L + 0.024% DMSO, and 1500 µg/L + 0.071% DMSO were used. ASW-G, and DMSO (0.143%) control conditions were also tested. Growth curves were monitored in triplicate, with all conditions grown at 25 °C, 80 rpm, in 100 mL ASW-G. During this period 200 µL samples were taken at 1.5 hr samples during the day, and measured at a wavelength of 620 nm. The growth of BP3-exposed *E. mobile* was not monitored at this time as this was completed in our previous study (183) using the same parameters apart from rotary speed (110 rpm).

For the core experiment (secretome analysis), bacterial cultures of 450 mL were prepared in Erlenmeyer flasks under four conditions: (1) seawater control of only ASW-G (ASW; 09/07/21) (2) the solvent control including 0.028% DMSO (09/07/21) (3) 350 µg/L BP3 + 0.028% DMSO (09/07/21) and (4) 1 mg/L EHMC + 0.115% DMSO (13/06/22). Four replicates per condition were used and grown anaerobically (80 rpm) at 25 °C.

2.3 Secretome extraction

To record *E. mobile*'s early response to pollution, and align with our previous study of *E. mobile*'s proteome, after two (ASW, DMSO), and three (BP3, EHMC) days growth, and once an optical density (620 nm) of 0.1 was reached (Figure 3.1a), cultures were centrifuged at 4000 g for 10 min. Supernatants were then filtered (0.2 µm syringe filter) and transferred to 3K centricons® (Merck; c3043) and centrifuged at 3000 g until ≤5 mL sample remained. Samples were centrifuged at 10,000g for 15min, removed from the remaining pellet, and

further concentrated until ≤ 400 μL remained. Proteins were precipitated with cold acetone overnight at -80 $^{\circ}\text{C}$, with an acetone/aqueous protein solution ratio of 4:1. The protein pellet was resuspended in 50 μL of 1 M Urea, 200 mM Thiourea, 1.67 mM HEPES, 167 μM dithiothreitol (DTT) (ASW, DMSO, BP3), or 40 μL of 15 mM di-potassium hydrogen phosphate, 6 mM guanidine hydrochloride (EHMC) for protein quantification using the Bradford method (288). The protein profiles of each resuspended sample (approx. ≤ 30 μg) were visualised using a 4-12% NuPAGE[®] Bis-Tris gel, and the Pierce Silver Stain Kit (CAT – No. 24612) according to manufacturer's instructions, then proteins within the remaining sample were precipitated as above.

Protein extraction was performed as described in Matallana-Surget and colleagues (2018) at the same time (14/07/22) for all conditions regardless of original sampling time (160). Pellets were resuspended in 50mM ammonium bicarbonate, and reduction was performed with 100mM 1,4-Dithioerythritol (DTE) at 4 $^{\circ}\text{C}$ for 1 hr. Alkylation was performed with 250mM iodoacetamide in the dark 4°C for 1 hr, and tryptic digestion (sequencing grade modified trypsin, Promega) was performed overnight at 37°C , with an enzyme/substrate ratio of 1:25.

2.4 LC-MS analysis and data processing

Protein samples were analysed on an ultra-high-performance liquid chromatography–high-resolution tandem mass spectroscopy (UHPLC-HRMS/MS) Eksigent nanoLC 400 and AB Sciex TripleTOF 6600 system (20/07/22). Two micrograms of peptides were analysed using acquisition parameters previously reported (210). In order to generate the spectral library, analyses were conducted with the instrument operating in data-dependent acquisition (DDA). Mass spectrometry (MS/MS) runs were conducted with micro injection (75 min LC separation) modes.

Protein searches were performed with ProteinPilot (ProteinPilotSoftware 5.0.1; Revision: 4895; Paragon Algorithm: 5.0.1.0.4874; AB SCIEX, Framingham, MA, United States) (Matrix Science, London, United Kingdom; v. 2.2). Paragon searches were conducted using LC-MS/MS Triple TOF 6600 System instrument settings. Other parameters used for the search were as follows: Sample Type: Identification, Cys alkylation: Iodoacetamide, Digestion: Trypsin, ID Focus: Biological Modifications, Search effort: Thorough, Detected Protein Threshold [Unused ProtScore (Conf)] > 0.05 . Proteins identified within the above data-dependent acquisition (DDA) mode at a false-discovery rate of below 1% were used as

the reference spectral library for subsequent data-independent acquisition (DIA). Resulting .wiff files were processed using the AB Sciex PeakView software and the SWATH™ Acquisition MicroApp considering up to 6 peptides (with at least 99% confidence) and 6 transitions per peptide. The extracted ion intensity chromatogram (XIC) peak area was extracted and exported in AB Sciex MarkerView™ 1.3.1 software. The significance of the relative abundance (fold change) observed between our experimental conditions was determined through Student's t-test with a statistical threshold set at 5% (p-value <0.05). The cut-off for significant differential regulation (fold change) was set to 1 for upregulated and downregulated proteins. The initial DDA outputs were also exported and used as a baseline for data-subsetting within an online system for venn-diagram generation (www.pangloss.com/seidel/Protocols/venn.cgi).

Subsequently, protein functions were manually annotated, and the secretion of all proteins was verified using SignalP-6.0 (289). DDA and MarkerView outputs can be found in Appendices A: E.

2.5 In-silico analysis of *E. mobile*'s theoretical secretome

The complete proteome data used for an in-silico analysis of *E. mobile*'s exoproteome – found under the name *Tritonibacter mobilis* F1926 (aka. *Ruegeria mobilis*) – was retrieved from UniProt (290) on 06/02/25 in .FASTA format. Online sources were checked to find accessible resources for in-silico grouping of a full proteome's proteins according to their subcellular location, and likelihood of secretion. Two online platforms were identified and subsequently used: SignalP-6.0, to identify proteins containing signal peptides, and LiSC by Galaxy Community Hub, to identify proteins which may be secreted through the Type-III secretion system (289, 291). The data export from SignalP-6.0 contained the complete list of proteins expressed by *E. mobile* (n= 4518), and their associated signal peptides. Any proteins without a probable signal peptide were assigned 'other'. The LiSC outputs also contained all proteins, and secreted proteins were identified on a 'true'/'false' basis. All proteins with a prediction of 'other' were therefore excluded from SignalP's theoretical exoproteome, as were all proteins assigned 'false' by LiSC. Theoretical exoproteome lists can be found in Appendix F and G.

3. Results and discussion

3.1 Confirmation of growth, and secretome production of *E. mobile* with UV-filter exposure

Growth curve analysis was conducted to perform an initial assessment of *E. mobile*'s response to (DMSO+) EHMC in addition to its response to DMSO and (DMSO+) BP3, which had been examined in our previous study (183). This analysis revealed that *E. mobile* exhibits a resilience to EHMC, reaching a plateau ($OD_{620} 0.143 \pm 0.004$) after 36 hrs; a comparable growth rate to control conditions (Figure 3.1a). This is in contrast with *E. mobile*'s previously reported response to BP3, where growth was significantly delayed with an EC_{50} of 364 $\mu\text{g/L}$ (48). These results suggest that *E. mobile* may possess adaptive mechanisms enabling it to tolerate EHMC, but not BP3.

Protein gel electrophoresis revealed distinct banding patterns in the extracellular fraction obtained from *E. mobile* in response to DMSO, BP3, and EHMC exposure (Figure 3.1c), consistent with – but not conclusive for – the presence of secreted proteins. Notably, protein profiles of the extracellular proteins was significantly different to the proteins extracted from the cell pellet (Figure 3.1b; 3.1c), meaning the proteome and secretome of *E. mobile* may convey distinct strategies for maintaining cellular homeostasis (270). The distinct profiles observed between the extracellular fraction and the whole-cell proteome indicate that the applied methods (Section 2.3) were effective in separating extracellular material from cellular proteins. However, it cannot be confirmed from these results alone whether the extracellular proteins originated solely from secretion. At this stage, no significant difference in proposed secretomes was observed between conditions aside from with EHMC, which was likely due to a faulty gel (Figure 3.1c).

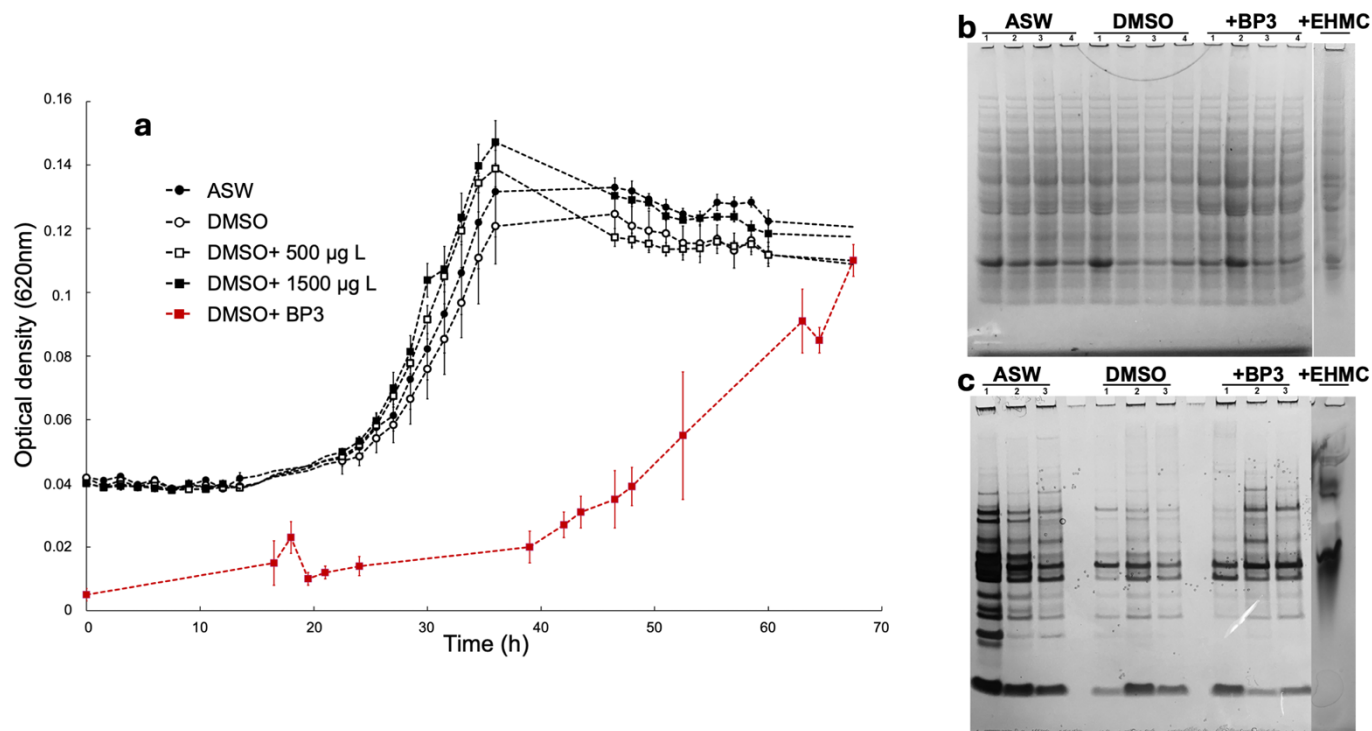


Figure 3.1 Pilot data for the study of *Epibacterium mobile* and its response to two organic UV-filters using secretome analyses. Growth curves of *E. mobile* (a) in artificial seawater only (dark circle), with 0.046% DMSO (white circle), plus 500 µg/L (white square), or 1500 µg/L (dark square) of (DMSO+) EHMC (average $\pm$ standard deviation, $n = 4$). Overlaid onto this plot is also growth curve *E. mobile* in artificial seawater with 364 µg/L of (DMSO+) BP3 (red square) from DOI: 10.1016/j.scitotenv.2021.148431. In addition, SDS-PAGE gels of *E. mobile* proteomes stained with Coomassie Blue (b), and secretomes stained with Pierce Silver Stain (CAT – No. 24612) (b) after growth in artificial seawater alone, with 0.046% DMSO, plus 350 µg/L BP3, or 1500 µg/L EHMC.

3.2 Protein yields following secretome extraction

Following protein extraction and concentration, and using the Bradford method, *E. mobile*'s secretome was estimated to contain 396.4 µg (± 126.5), 301.5 µg (± 106.6), 483.8 µg (± 81.5), and 32.8 µg (± 9.86) of protein after ASW, DMSO, BP3, and EHMC exposure respectively (Figure 3.2). In alignment with previous study of *Roseobacter* (267), the secretome of *E. mobile* therefore contained less protein (304 µg ± 81) compared to the intracellular proteome (9098 µg ± 499 ; Figure 3.1b); equalling 3.2% of the total proteome. Furthermore, this study's DDA analysis (Section 2.4) identified 33, 45, 17, and 44 proteins expressed by *E. mobile* in the seawater control (ASW), solvent control (DMSO), BP3, and EHMC conditions respectively (Figure 3.2). The secretome identified here therefore represented <6% of the total identified proteome of 901 proteins as characterised in our previous study (183), and <1% of *E. mobile*'s total theoretical proteome of 4,518 proteins (290). These numbers are

significantly lower than the theoretical coverage of *E. mobile*'s secretome predicted using SignalP- 6.0 and LiSC (Section 2.5), which contain 507 and 594 proteins respectively (Appendices F, G), equalling to a total coverage of 11.22% and 13.15%. This is also lower than the predicted proteome coverages for any other *Roseobacter* species (~30%) (267).

It was expected that protein yields from *E. mobile*'s secretome would be lower than protein yields from *E. mobile*'s intracellular proteome. Indeed, following preliminary testing, the size of the secretome was estimated to be even lower than calculated using the Bradford method (Figure 3.2) due to the necessary use of silver-staining to visualise protein bands (Figure 3.1c), which reveals nanograms, not micrograms of protein (292). It is also common to detect less than 200 extracellular proteins per bacterial species (268, 271), while studies reporting >40% coverage usually culture strains under multiple conditions to increase coverage (286, 293). This approach could not be used in this study due to our interest in a specific response to stimuli, which is why the proteins found in our previous study – testing the full bacterial pellet for *E. mobile*'s response to a UV-filter – still only represented <20% of the theoretical proteome (183). Indeed, no bacterium or organism will express all proteins at all times, so limiting the number of proteins which may be detected. It was in response to this predicted low yield that 450 mL of culture per replicate was used as opposed to the 70 mL used in our previous study (183). It is therefore remarkable that still only <50 proteins were identified from 1.8 L of supernatant per condition. And furthermore, that the ASW, DMSO, BP3 and EHMC samples represented only 6.05% (± 0.45), 8.25% (± 0.35), 3.15% (± 0.25), and 8.05% (± 0.35) of *E. mobile*'s total theoretical secretomes.

It is possible that the theoretical secretomes produced by SignalP- 6.0 and LiSC overestimated the number of proteins secreted by *E. mobile*. And when examined, these protein lists (Appendices F, G) do include proteins which may be secreted for the degradation of aromatic compounds (e.g., 3,4-dihydroxyphenylacetate 2,3-dioxygenase), competition (e.g., hemolysin), and detoxification (e.g., organic hydroperoxide resistance protein) (244, 294, 295). However, there are many more proteins within these theoretical secretomes which are known intracellular or membrane-bound proteins (e.g., transcriptional regulators, transmembrane transport proteins, cell division proteins, tricarboxylic acid cycle proteins, flagellins) and are rarely secreted (227, 296-298). These theoretical secretomes are therefore likely to have been overestimated, meaning *E. mobile*'s secretome could still be very small,

and the secretome proteins identified here would represent a greater portion of the total exoproteome than calculated above.

An alternative explanation or an additional factor for the low protein yields in this study – besides a small, or even non-existent secretomes – is that protein recovery was limited by factors such as low *E. mobile* biomass, reduced centrifugation speed during protein concentration, and protein size. As stated in *Section 2.3*, this study's methods were designed to mirror our previous investigation of *E. mobile*'s proteome for comparison purposes (183). The *E. mobile* studied here were therefore grown for two and three days for study of *E. mobile*'s early response to pollution, and secretomes were collected after cultures reached an optical density of 0.1. These time points equated to a collection time at the point of greatest growth ($OD \leq 0.15$) within our growth assays (Figure 3.1a). However, this highest recorded optical density still equated to very little biomass (299), which may have impacted protein yields. This study's increased sample volumes to 450 mL/ replicate may have further impacted protein capture by necessitating the use of 70 mL centricons (*Section 2.3*) for protein concentration, limiting the maximal centrifugation speed to 3000g for this step. Crucially, lower centrifugation speeds ($<10,000$ g) can result in less capture of proteins smaller than 30 kDa (300), meaning almost half ($45.3\% \pm 3.8$) of proteins identified in *E. mobile*'s theoretical secretomes may have been lost due to this limitation (Appendices F, G; <https://www.uniprot.org/uniprotkb?query=Ruegeria+mobilis>). The exclusive use of trypsin as the digestion enzyme (*Section 2.3*) may have also limited protein recovery, as some proteins are resistant to tryptic cleavage, which can skew the population of proteins analysed through LC-MS/MS (301). This skewing of the protein population due to low biomass and analytical methods may pose a critical issue for this study. All results presented here have therefore been interpreted with caution.

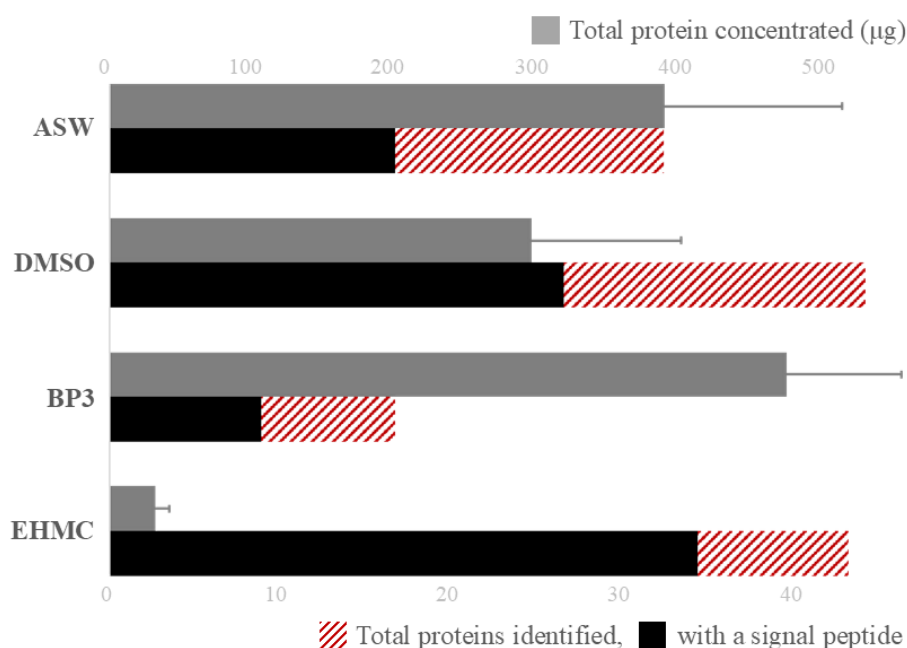


Figure 3.2 Proteins quantified (μg) using the Bradford Assay and identified (≥ 2 peptides) after mass spectroscopy (aka. DDA) analysis.

3.3 Challenges involved in capturing a bacterial exoproteome

Quality control is an essential precaution for all forms of proteomics. For mass spectrometry-based proteomics, it is typically used to control false discovery rates (FDR), increase confidence in detected trends, and reduce the incidence of contaminating proteins (302, 303). Contamination is a particular issue in secretomics, as here it may come from extraneous sources as well as the intracellular fraction which pollutes the exoproteome through handling errors or cell lysis before and during sample collection (287, 304-306). To account for this, protein lists were filtered based on the presence or absence of signal peptides (*Section 2.4*). Of the identified proteins, 19, 29, 10, and 36 contained signal peptides, equating to 58%, 64%, 59%, and 82% of secretome proteins identified for ASW, DMSO, BP3, and EHMC respectively (Figure 3.2), and 3.47% (± 0.27), 5.3% (± 0.42), 1.83% (± 0.15), and 6.58% (± 0.52) of the theoretical secretome. Not all proteins secreted into the exoproteome require a signal peptide, however the excluded proteins here namely included flagellin, ribosomal proteins, and energy metabolism proteins (Figure 3.2) which are common contaminants in studies of the secretome (307, 308). In this study, proteins lacking a signal peptide were therefore assumed to represent contamination, resulting in a level of proteome contamination ($34.35\% \pm 11.15$; StDev) which is consistent with the level reported in most secretome studies (305, 306). Interestingly, the samples containing the most contamination were the seawater, and solvent controls (Figure 3.2) collected after two days growth, suggesting that cell lysis –

a common cause of this contamination – may have been more pronounced at this earlier time point (287, 305, 306).

However, as discussed in *Section 3.2*, even proteins containing signal peptides including transcriptional regulators, transmembrane transport proteins, TCA cycle proteins, and flagellins can represent contamination (227, 296-298). Concerningly, this represents a significant portion of the proteins remaining in each condition's pool of characterised proteins (Figure 3.3; *Section 3.4*). This also represents a significant portion of the theoretical exoproteomes from SignalP- 6.0 and LiSC (Appendices F, G). Taken together, this may indeed indicate that *E. mobile* does not produce a secretome under these conditions. This would also mean the protein bands found in Figure 3.1c represent a contamination which is consistently retrieved through the sampling process used. Alternatively, the growth and sampling method which also caused issues for protein yields (*Section 3.3*), may have resulted in the retrieval of protein samples which did not accurately represent the secretome. This highlights the potential influence of handling errors and experimental refinement on contamination levels, and the difficulties associated with secretome-focussed research. Further highlighting this issue, is the incidence of uncharacterised proteins within the pooled samples. Within the secreted portion of this study's exoproteome, 14% of the detected proteins were classified as 'uncharacterised' (i.e., DUF3576 domain-containing proteins; Figure 3.3), aligning with the 13.8% (± 0.08) of the total *E. mobile* proteome that remains uncharacterised. If characterised, these proteins could provide valuable insights into the metabolic response of *E. mobile* to organic UV-filters. However, uncharacterised proteins is a recurring issue in secretome and exoproteome research due to the novelty of the field and the limited characterisation of proteins specific to secretome-related processes (265, 309). Until further exploratory studies of protein function reveal the metabolic roles of these proteins (310), the number of proteins available for researcher interpretation in this study has therefore been further restricted. Consequently, the extent to which this study has captured *E. mobile*'s secretome is unclear.

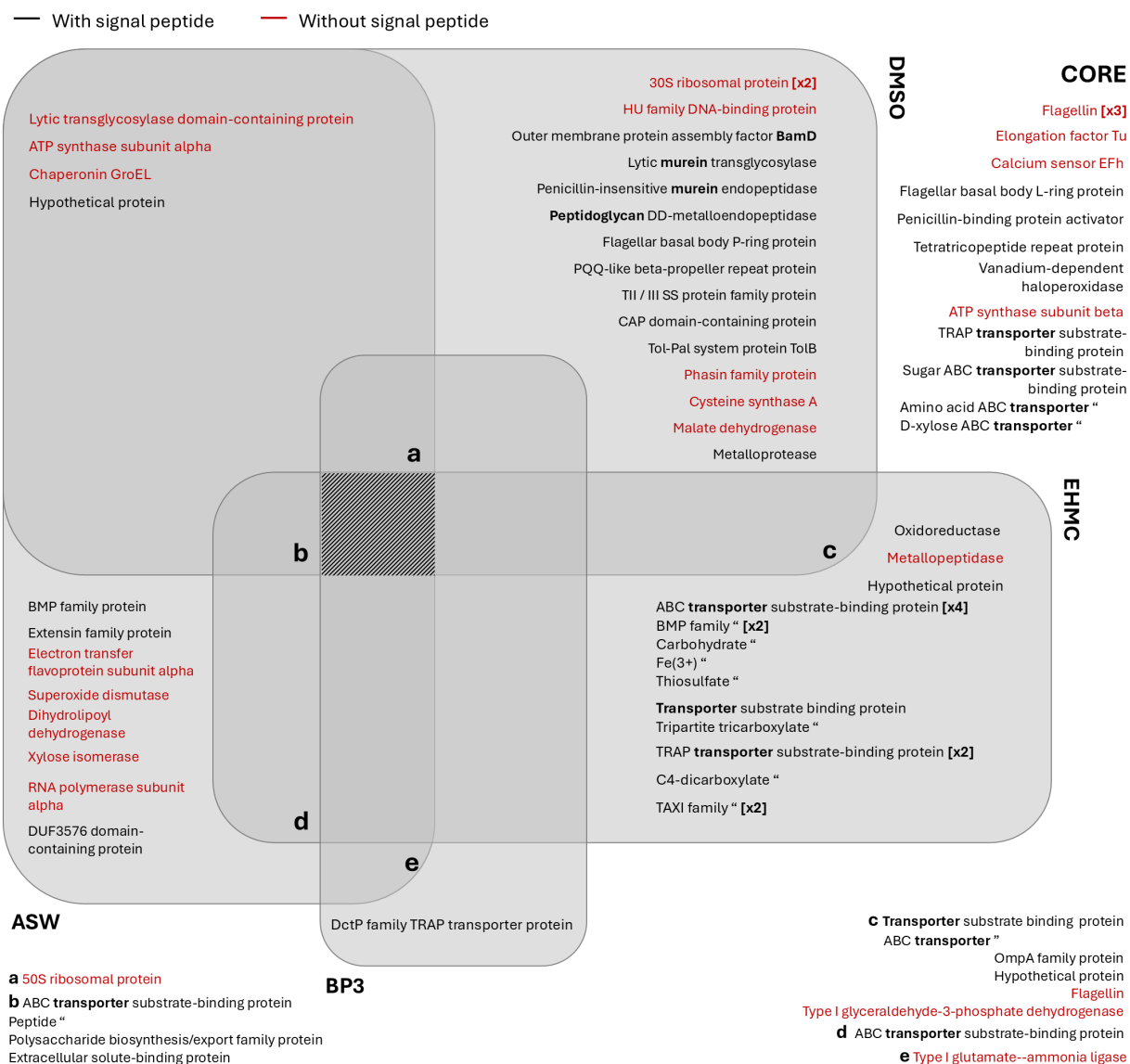


Figure 3.3 Qualitative data for each protein unique to and shared by *E. mobile* exposed to each condition. Proteins retrieved from all replicates (n=4) and all proteins were above the required cutoffs for DDA outputs (≥ 2 peptides).

3.4 The expression of a unique exoproteome by *E. mobile* with EHMC

While most of this study's data appear to result from contamination (Section 3.3), some interesting trends are still present, either arising from the extracellular fraction or intracellular proteins expressed at levels high enough to contaminate it. To distinguish these trends, DIA outputs were used to perform fold-change analysis, and the differential expression of 118 proteins (>1 peptide) between each condition (e.g., BP3 v DMSO, EHMC v BP3) was analysed. Of those compared proteins, none were significantly regulated in response to DMSO compared to ASW, or to BP3 compared to DMSO (Figure 3.4). However, in response to EHMC, *E. mobile* significantly upregulated (p-value <0.05) 27 and 33 proteins, and downregulated 1 and 4 proteins compared to DMSO and BP3 respectively (Figure 3.4). Interestingly, most downregulated proteins were of unknown function, further indicating a need for database development within the field of secretome research (Section 3.3). Though even with a portion of the secretome represented by unknown proteins, TT proteins represented 77.8 % and 57.6 % of all proteins upregulated with EHMC exposure compared to DMSO and BP3 respectively (Figure 3.4). Their configuration here may therefore reveal how *E. mobile* better responds to EHMC compared to BP3.

Following EHMC exposure, the most expressed (Figure 3.3), and upregulated (Figure 3.4) transmembrane transport (TT) proteins were ABC transporters, which facilitate the movement of key compounds across the cell membrane (311). Notably, half (n = 9) of all ABC transport proteins (n = 18) found in this study were expressed only after EHMC exposure (Figure 3.3), suggesting how transmembrane transport may have contributed to *E. mobile*'s resistance to EHMC toxicity (Figure 3.1a). Among these ABC transporters was the iron (Fe³⁺) ABC transporter substrate-binding protein (312) which may indicate that EHMC interacts with intracellular Fe³⁺ complexes, causing iron starvation and cell damage, as predicted for DMSO in our previous study (183, 312, 313). The expression of ABC transporters and other multi-solute exporters (i.e., TolB; Figure 3.3) also play a well-established role in bacterial resistance to xenobiotics (314, 315). This may explain why *E. mobile* tolerates higher concentrations of EHMC compared to BP3 (Figure 3.1), particularly as BP3 exposure only uniquely induced expression of one DctP family TRAP transporter (Figure 3.3), which primarily imports solutes, so is unlikely to confer xenobiotic resistance (311, 316). Furthermore, *E. mobile* secreted significantly less protein when exposed to BP3 compared to any other condition (Figure 3.2). This suggests that BP3 may inhibit protein

secretion as part of its toxic effect on *E. mobile*, or that the majority of *E. mobile*'s response to BP3 is mediated through the proteins expressed within the cell (183, 262). In contrast, *E. mobile* secreted more proteins when exposed to EHMC than with any other condition (Figure 3.2). This increased protein secretion may have therefore contributed to *E. mobile*'s apparent resistance to EHMC toxicity (Figure 3.1a), and is demonstrative of the variability in bacterial response to chemically similar compounds (48).

In this study, the greater detection of proteins after EHMC exposure may otherwise have been caused, in-part, by the time difference between protein collection and analysis (01/08/22), for each sample (*Section 2.2*). While all samples had a period in cold storage, the samples resulting from EHMC exposure were collected 11 months after the BP3 and control conditions. This delay for these conditions, combined with repeated freeze-thaw cycles and an absence of protease inhibitors, likely exacerbated the protein degradation known to occur during storage (307, 317). This would have decreased the number of proteins which may be detected for these conditions, so increasing the number of proteins detected after EHMC exposure by comparison. By sampling this condition at a different time, variations in researcher experience and protocol (i.e., Bradford method; *Section 2.3*) may have also been introduced, impacting the proteomic result (Figure 3.2). Furthermore, it is also important to note that while the *E. mobile* exposed to EHMC and BP3 were both sampled after three days, the *E. mobile* from the former condition would have been in late stationary phase, while the latter would have been in early stationary or late exponential (Figure 3.1a), along with the control conditions collected after two days growth. This difference in growth phases is known to produce significantly different protein profiles, and likely impacted this study's results (293, 307).

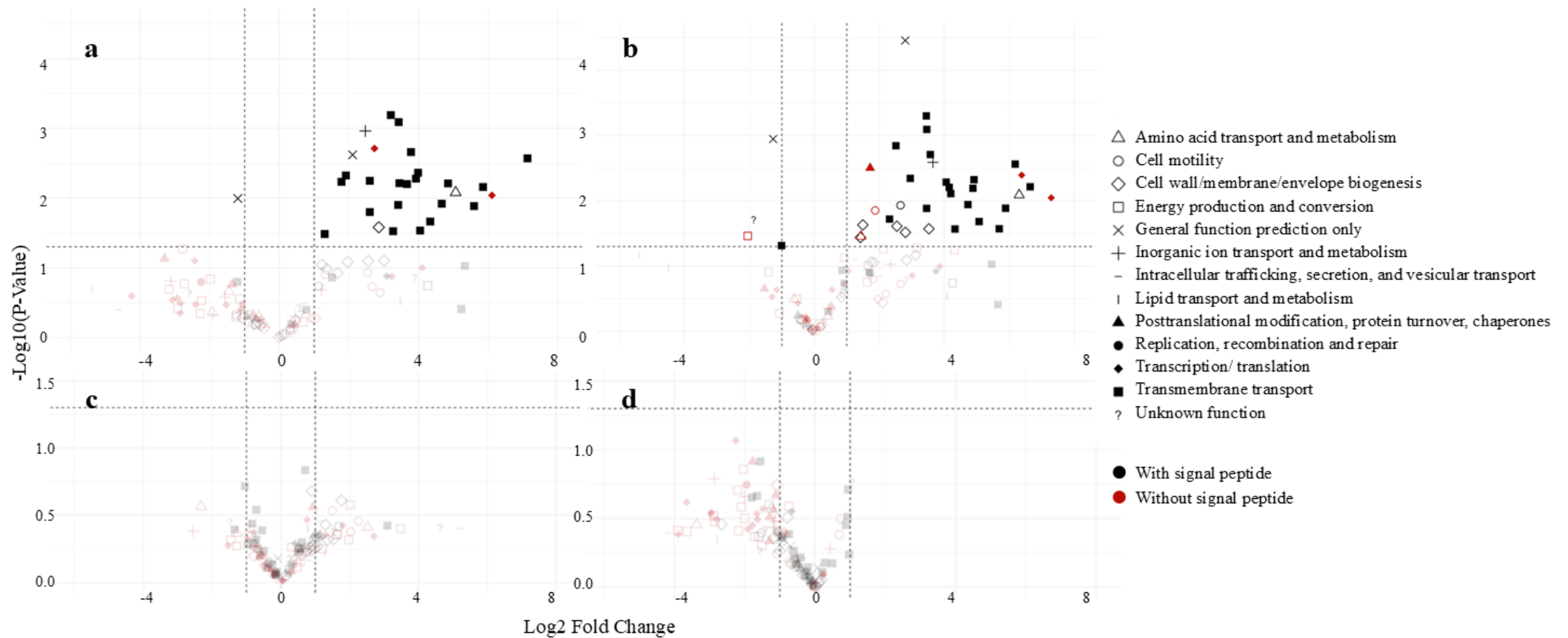


Figure 3.4 Differential regulation of proteins between the solvent control and EPMC (a), BP3 and EPMC (b), seawater control and solvent control (c), and solvent control and BP3 (d) conditions. Positive fold changes correspond with upregulated proteins for the EPMC (a, b), solvent control (c), and BP3 (d) conditions respectively. Horizontal dashed line: p-value 0.05; Vertical dashed lines: Log2 Fold change -1 and 1. Unsaturated points represent differentially regulated proteins with a p-value of >0.05.

The discussed variation in collection timing may also explain why the DMSO treatment induced the expression of a unique set of proteins compared with the BP3 and EHMC treatments, despite the inclusion of four times more DMSO in the EHMC condition than any other (*Section 2.2*). Indeed, DDA analysis revealed ten proteins which were estimated to be secreted only after DMSO exposure, four of which – outer membrane protein assembly factor BamD, lytic murein transglycosylase, penicillin-insensitive murein endopeptidase, and peptidoglycan DD-metalloendopeptidase – control cell remodelling during cell -growth, and -stress events (Figure 3.3) (318, 319). This would support the evidence found in our previous study for the induction of stress and membrane damage in *E. mobile* through exposure to DMSO (183). However, it remains inconsistent that *E. mobile* exhibited such pronounced responses to EHMC and DMSO, yet showed no detectable molecular response to BP3; a UV-filter previously reported to cause membrane stress in this species. The variation in protein expression observed here with EHMC therefore likely reflects a combination of experimental variation and a limited but genuine biological response. Consequently, the results here will not contribute to our broader understanding of *Epibacterium mobile* and UV filter toxicity, but rather highlight the precautions and challenges researchers must navigate when conducting secretomic investigations within environmental microbiology.

Conclusion

The secretomes of marine bacteria remain largely unexplored, particularly in response to environmental stressors like organic UV-filters. By investigating the secretome of *Epibacterium mobile* after exposure to EHMC, BP3, solvent-, and seawater- controls, this study therefore provides insight into how bacteria may respond to such pollutants, as well as the hurdles that researchers face when attempting to address this gap. The use of secretomics in this study especially introduced issues of low protein yield, and contamination from intracellular proteins. Future secretomic research, may mitigate such issues through trials in study scale, sampling protocol, adjustments in sample preparation, and the use of additional representative controls during experimentation. With such improvements, and a consolidated approach, it may be possible to determine which marine bacteria produce a functional secretome, and how they are used to adapt to environmental stressors. Here we may conclude that EHMC induces a greater extracellular response from *E. mobile* compared to BP3. And contrasted to *E. mobile*'s growth response to these UV-filters, which reveals a vulnerability to BP3, the increased production of extracellular proteins would plausibly protect the bacterium from UV-filter toxicity. Nonetheless, inconsistent secretome coverage across samples limits confidence in these observations. These results therefore underscore the uses of secretome analysis as a potential tool for detecting early molecular responses to stimuli, though we must also stress the methodological complexity inherent to this emerging field.

Chapter 4

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EHMC in the Nascent Marine Plastisphere

Ethylhexyl Methoxycinnamate (EHMC) is the most commonly used organic UV-filter in cosmetics (276), and one of the most commonly found UV-filters in marine environments (Chapter 1). In this study, EHMC was therefore selected as an appropriate organic UV-filter to study the impact of co-pollution on the metabolism of a marine plastisphere community dominated by bacterial genera (Chapter 2).

The following study therefore exposed EHMC to a plastisphere community grown on pristine low-density polyethylene (LDPE) to remove the influence of further contaminants. This exposure was performed after seven days in accordance with the time required for the highest nascent plastisphere diversity in Chapter 2.

The Invisible Threats of Sunscreen as a Plastic Co-Pollutant: How Organic UV Filters Impact Biofilm Formation and Metabolic Function in the Nascent Marine Plastisphere

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Charlotte E Lee: Project Administration, Conceptualisation, Experimental Design, Methodology, Investigation, Analysis, Visualisation, Writing- Original Draft. **Lauren F Messer:** Methodology, Data curation, Writing- Review and Editing. **Ruddy Wattiez:** Resources. **Sabine Matallana-Surget:** Supervision, Resources, Writing- Review and Editing.

This paper serves as the foundation for the information presented here, although the text and content have been revised slightly for inclusion in this thesis.

Pages 98 to 124 have been extracted from this version of the thesis during the embargo period required by the Elsevier publisher. After 29th June 2027 the full version of the thesis will be available for download from <http://hdl.handle.net/1893/37546>

Chapter 5

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General Discussion

1. Summary of Findings

Marine microbial communities face significant challenges from anthropogenic pollutants, including persistent pollutants, which accumulate and cause widespread damage to marine life. As foundational components of marine ecosystems, these microorganisms are crucial for ecosystem services and trophic interactions (3-5). Exposure to pollutants elicits a range of responses in these microbial communities, from stress to altered growth (10). This unpredictable response of microorganisms to marine pollutants, coupled with their ecological importance may therefore have important consequences for broader ecosystem processes and functioning. Until now, studies have primarily investigated these effects by assessing each pollutant in isolation, overlooking the combined effects of multiple pollutants in complex, heterogeneously contaminated environments (**Chapter 1**). This thesis addresses this gap by employing molecular techniques to explore the intricate metabolic interactions between marine microorganisms and a combination of the ocean's most abundant persistent pollutants.

The first pollutant examined in our series was plastic (**Chapter 2**). As highlighted in the review (**Chapter 1**), plastic is the most prominent persistent marine pollutant and interacts with microorganisms through the formation of the 'plastisphere,' the central focus of the article. To investigate this plastic-microorganism interaction, metagenomics and metaproteomics were used to characterise the metabolic profiles of microorganisms present in the nascent plastisphere (**Chapter 2**). Organic UV-filters, as common co-pollutants for plastic, were then studied using axenic culture and exoproteomics to investigate a marine bacterium's first line of response to chemical pollutants (**Chapter 3**). These studies revealed that the nascent plastisphere was most diverse after seven days growth, rather than three, and that Ethylhexyl Methoxycinnamate (EHMC) may be an organic UV-filter of interest for ongoing study. Our final study (**Chapter 4**) combined these insights, and used meta-omics to examine the response of a seven-day-old plastisphere to EHMC to understand the impact of these co-pollutants on marine microbial communities.

Our results from the use of these combined approaches emphasised the heterogeneity of marine microbial responses to pollutants. *Epibacterium mobile*, selected for its environmental relevance, tolerated higher concentrations of EHMC compared to Benzophenone-3 (BP3) (**Chapter 3**). Additionally, heterotrophic bacteria of the genus *Pseudomonas* and *Marinomonas* were found to most colonise low-density polyethylene (LDPE) in the nascent plastisphere (**Chapters 2 and 4**). In the plastisphere, stress, particularly oxidative stress, was a consistent response of the bacteria to the pollutant or their surroundings (**Chapters 2 and 4**). Proteins associated with this stress, substrate adhesion, biofilm formation, competition, and cross-feeding highlighted the metabolically demanding nature of the plastisphere (**Chapters 2 and 4**). Furthermore, consistent upregulation of transmembrane transport proteins in axenic and mixed cultures indicated a universal response by bacteria to export harmful pollutant biproducts, or the pollutant itself during exposure (**Chapters 3 and 4**). Concerningly, within this collection of microorganisms, potential-pathogens were frequently annotated in the marine plastisphere, further highlighting this as a hazardous niche in need of further investigation (**Chapter 2**). In addition, pollutant-degrading genera (i.e., *Marinomonas*) were enriched, and the expression of enzymes associated with plastic, and aromatic hydrocarbon degradation was found (**Chapters 2 and 4**). However, aerobic respiration, and the activity of these associated genera were reduced with EHMC, suggesting how plastic biodegradation may be inhibited by co-pollution in the marine environment (**Chapter 4**). Overall, this work examined the diverse responses of microbial communities to persistent marine pollutants, emphasising broader implications for marine ecosystem health and pollutant bioremediation.

2. Unravelling the Complexity of Marine Plastisphere Metabolism

This thesis presents the metabolic outcomes of the first ever recorded interaction between plastics, microorganisms, and organic UV-filters. This metaproteomic investigation of combined pollution forms the basis of many novel findings presented in **Chapter 4**.

Crucially, this use of metaproteomics has also further contributed to current understanding of the marine plastisphere community and its functioning.

Aside from investigating the impact of time, **Chapter 2** provided a unique opportunity to contrast a largely monocultural plastisphere of *Pseudomonas* to a plastisphere with more diversity. In this study, a significant coverage of the proteomic database (>25%) was achieved, as well as a high protein yield (>2000), and the novel retrieval of two high-quality metagenome assembled genomes (MAGs) from an environmental sample. This investigation revealed, for the first time, the importance of attachment strategy during plastisphere formation through the noted regulation of membrane and quorum sensing protein expression in the plastisphere's most abundant bacteria. In addition, in **Chapter 4**, strategies associated with biofilm formation and maintenance (i.e., EPS production) were found to be strongly associated with the resistance of associated genera to the negative effects of EHMC exposure. Here, all members of the community exhibited metabolic shifts in response to EHMC exposure, the most notable of which being the never-before recorded switch from aerobic to anaerobic respiration in a co-pollutant exposed plastisphere community.

This study contributes to our understanding of the marine plastisphere's functioning and has highlighted what future study might further improve our understanding of these communities. First, it has been found that the early plastisphere is a fast-changing niche with many opportunities for metabolic discovery. Consequently, an additional time-series of this early plastisphere using metagenomics, or 16S rRNA sequencing might therefore identify further intervals of interest (159, 165). Metaproteomics could then be focused on these time-points to provide deeper metabolic insights and reveal more intricate patterns of community progression and maintenance. A novel time-series of up-to six months may also distinguish the activity of early- and late- plastisphere communities by direct comparison. To maintain a plastisphere community for this length of time, the plastic would need to be suspended in a medium with constant access to all resources necessary for microbial growth. Emerging methodologies, such as the use of dialysis bags to submerge plastics in marine environments,

may allow this by enabling the free movement of microorganisms and nutrients without polluting the environment (371). A combined use of genomics, metaproteomics, and LIVE/DEAD staining might then be used to investigate biofilm layering, which we estimate will increase as the plastisphere matures (372). In addition, secretome analysis in post-processing using online platforms such as LiSC (**Chapter 3**) may further resolve which discovered proteins are involved in cell-cell, or -pollutant interaction, and which are involved in cell maintenance (291). To increase the validity of these studies, the above investigations might be further complemented by using 18s rRNA sequencing to provide greater insight into the role of the currently underrepresented eukaryotic microorganisms of the marine plastisphere (10, 54, 158, 373). Furthermore, investigation of the increasingly produced ‘bioplastics’ (e.g., poly hydroxy alkanoates; PHAs), and microplastics, which are now found in a rising number of environmental and tissue samples, would further increase the scope of this future research (374-377).

3. The ‘Good’ and the ‘Bad’ of the Marine Plastisphere

In addition to the previously discussed discoveries found by studying the nascent marine plastisphere (*Section 2*), **Chapter 2** revealed that microorganisms may indeed be virulent within the marine plastisphere through the expression of secretion systems and microbial toxins. Notably, the observed expression of multiple secretion system proteins, linked to both competition and virulence, may represent the first recorded instance of active virulence in any marine plastisphere. Furthermore, our subsequent study on EHMC co-pollution (**Chapter 4**) uncovered an apparent selection for genera that include potential pathogens (e.g., *Pseudomonas*), while simultaneously selecting against genera known for their pathogen-antagonistic (e.g., *Pseudoalteromonas*), and significantly, pollutant-degrading properties (e.g., *Marinomonas*). Marine plastispheres, particularly those impacted by co-pollution may therefore exhibit greater virulence than the average marine microbial community (51, 378). Moreover, these communities may also be transported over vast distances by the marine plastic debris, plausibly introducing recognised biohazards to human interfaces (e.g., aquaculture farms), and vulnerable niches of the marine ecosystem (209, 379-381).

Given the implications of this research for public health and ecosystem stability, further investigation of this enriched metabolic activity is essential. First, it must be established whether the marine bacteria on plastic are more virulent than their planktonic counterparts. To achieve this, further studies which contrast the composition and functioning of the early marine plastisphere to the planktonic community (195) using microcosms, mesocosms (*Section 2*), and meta-omics may be conducted. In addition, the use of infection models such as *Galleria mellonella* (382) would help assess the actual pathogenic potential of these organisms to human and marine life. A search for, and examination of specific mechanisms associated with virulence, such as type VI secretion systems (TVISS), may also provide critical insights into how this virulence may result in pathogenicity.

Beyond concerns of virulence, the apparent depletion of pollutant-degrading organisms in a co-polluted plastisphere also indicates a secondary impact of co-pollution on plastic persistence. Investigating the interaction between co-pollution and plastic biodegradation may therefore be crucial to understanding the true relationship between known pollutant-degraders and plastics in the ocean. Currently, it is not confirmed whether microorganisms

within a marine plastisphere community perform plastic biodegradation here. First, the activity of plastic degrading organisms within the marine plastisphere must therefore be determined. To study this, proteomics might remain a central analytical tool, though could be complemented by scanning electron microscopy (SEM) to monitor microbial growth and plastic surface interactions (28). Additionally, the development an antibody specific to enzymes such as PETase, an enzyme used for PET biodegradation, could enable precise tracking of their expression under varying conditions (383-385). The use of DNA stable isotope probing (SIP) has also been successfully used to track the consumption of pollutant-sourced molecules through a system (386-388). The use of DNA-SIP here may therefore also provide insights into the movement of plastic-derived products through a marine microbial community. To ensure validity, this would be performed using a representative, rather than enriched microbial community. Such techniques would allow the researcher to confirm that any observed weathering – recorded through weight-loss or Fourier transform infrared spectroscopy (FTIR) (71, 72) – is a result of microbial activity, rather than a consequence of physical weathering or photodegradation.

If confirmed, the impact of co-pollution on plastic biodegradation may then be studied. To begin to address this, we designed a study to investigate the proteomic response of two PET-degrading bacteria – *Ideonella sakaiensis* and *Rossallomorea aquimaris* (**Chapter 1; Section 2.2**) – to two co-pollutants – BP3, and the dye, Sudan IV (389, 390) – when grown with PET (Figure 5.1). These cultures were grown in isolation for two months to allow degradation to occur, and we expect that because of this isolation, or nutrient deprivation, no growth was observed, and no protein was retrieved. However, future studies utilising the methods described above (i.e., PETase antibody development) may discover the inhibitory effects of co-pollution on plastic biodegradation within the plastisphere. Such study could then be expanded to encompass further plastics, co-pollutants, and regions.

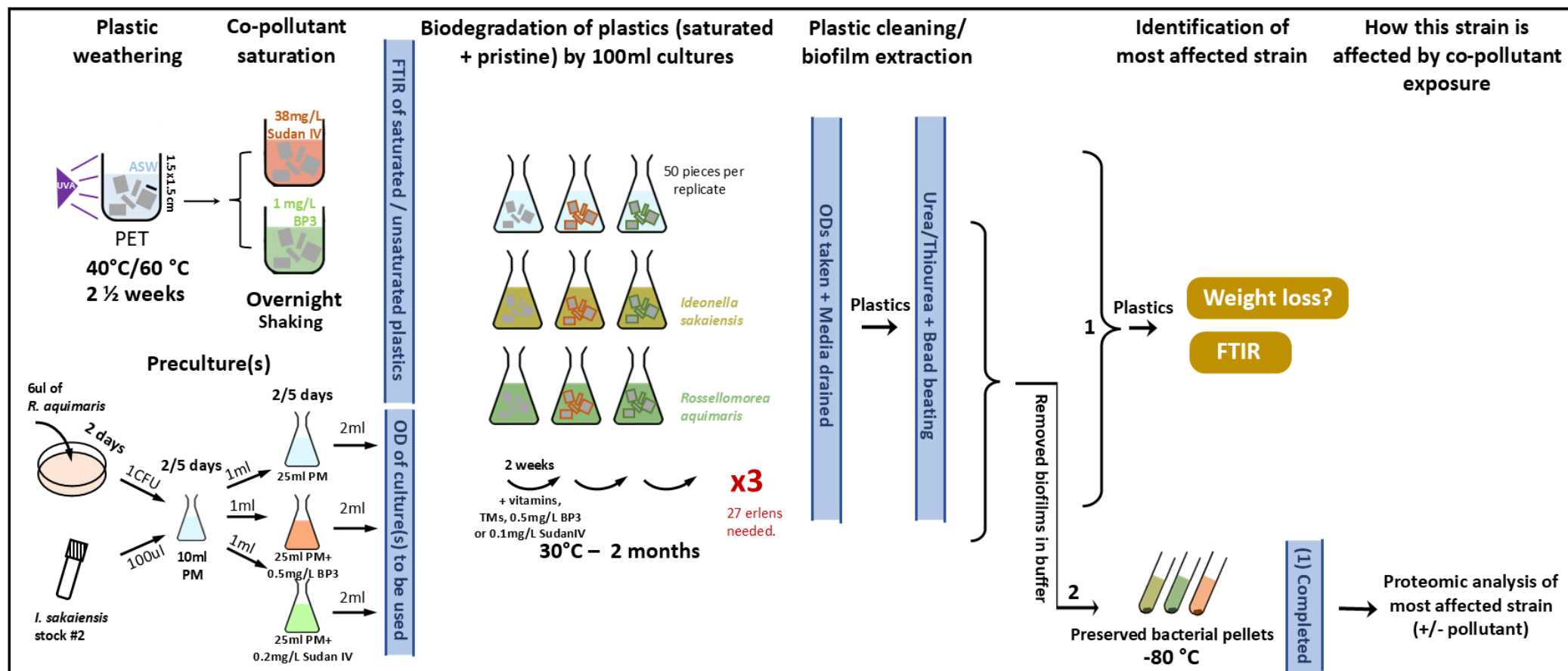


Figure 5.1 Experimental design for study investigating the impact of co-pollutants on PET biodegradation by *Ideonella sakaiensis* and *Rossellomorea aquimaris*. Acronyms: PET, polyethylene terephthalate; ASW, artificial seawater; BP3, benzophenone-3; PM, preferred media; OD, optical density (600nm), FTIR, fourier transform infrared spectroscopy

4. The Impact of Plastic Co-Pollutants of Marine Microbial Metabolism

In this thesis, two complementary approaches were used to improve our understanding of microbe-(co-)pollutant interaction in the marine environment. Exoproteomics was used to investigate the initial molecular interface between one co-pollutant and one planktonic bacterium (**Chapter 3**). Additionally, metaproteomics was used to examine, for the first time, the interaction between a co-pollutant and an entire plastisphere biofilm community (**Chapter 4**).

The exoproteomic research presented here represents the first ever analysis of *Epibacterium mobile*'s hypothetical secretome, and the proteins that it may contain. Examination of the resulting data after organic UV-filter exposure also revealed some of the complexities that a researcher must navigate when investigating microbe-pollutant interactions. Beyond these insights, EHMC was found to induce significant shifts in plastisphere composition and metabolism, as outlined in *Sections 2* and *3*.

In both studies, significant variation was observed in microbial response to pollutants in pollutant-dependent (**Chapter 3**), and microbe-dependent (**Chapter 4**) manners. In **Chapter 3**, this is primarily observed in *E. mobile*'s growth response to the two pollutants, with little evidence to currently support any reason for this varied response with the current data. To study this effect of UV-filters further, a first step may therefore be to confirm the presence of *E. mobile*'s secretome in a study designed to achieve maximum coverage. This would include the use of multiple collection times, growth conditions, and protocols to ensure that all variables discussed in **Chapter 3** have been accounted for. Afterwards, all tests of the bacterium's metabolic response may examine both the intracellular and extracellular fraction (if applicable) to provide a comprehensive assessment of its metabolic activity. To investigate this further, gas chromatography-mass spectrometry (GC-MS) may be used to analyse pellet, secretome, and media samples for pollutant content to track the pollutant's location (391). Pellet samples could then be fractionated into membrane and intracellular proteins and investigated with GC-MS again to discover its subcellular location. Alternatively, assuming that all pollutants cause oxidative damage (10), a pollutant's subcellular location might also be tracked using redox proteomics. This would use 2,4-Dinitrophenylhydrazine (DNPH) to tag, and identify oxidised proteins, therefore highlighting which proteins are most negatively impacted by pollutant exposure (392). Using this technique, the subcellular locations of the

damaged proteins could reveal which parts of the cell (i.e., the membrane) the pollutant interacts with. Further pollutants pertaining to different characteristics (e.g., size, structure) may also be tested to find trends in microbial responses to chemical contamination.

In this thesis, organic UV-filters were selected as each study's co-pollutant due to their unique prevalence, and persistence in areas of high plastic pollution as introduced in **Chapter 1**. This has revealed an interesting interaction between co-pollutants and bacteria which may be expanded on by using full sunscreens as the co-pollutant. Indeed, UV-filters are rarely leached into the ocean in isolation, and personal care products (PCP) – including sunscreens – alone contain many more ingredients than just UV-filters (161, 393). However, it is often the filter alone, and no other component of the product which becomes regulated after discoveries of marine toxicity are made (394, 395). It may therefore be useful to contrast findings of marine biota's response to filters alone, and their response to entire PCPs (161, 393). Furthermore, the additives found in most commercially used, and polluting plastics, may also cause such a variation. Crucially, environmental weathering, and photodegradation, causes plastics to release these chemical additives, or leachates, such as dyes and pigments (Figure 5.2), which may further impact the marine microbial community.

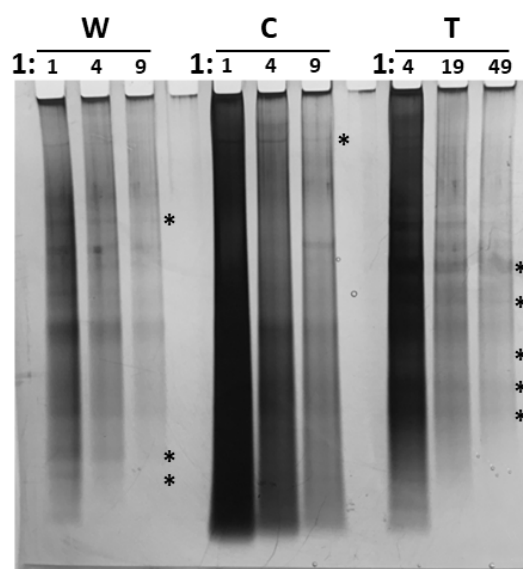


Figure 5.2 SDS-PAGE gel of microbial metaproteomes extracted from white (W), coloured (C), and transparent (T) plastic. Samples diluted 1:1, 1:4, 1:9, 1:19, and 1:49 using ultrapure water after previous overstaining when concentrations estimated from Bradford analysis were used. Gels stained with Pierce Silver Stain (CAT – No. 24612). * - unique protein bands.

A preliminary analysis of plastisphere community composition using protein gel electrophoresis revealed that microbial communities attached to differently coloured plastics expressed distinct protein profiles (Figure 5.2). This confirms that plastic additives, including those used solely for colour, can significantly influence microbial activity (136). To explore this further, we therefore performed a pilot study to assess how plastic-polluted environments might select for different microbiota. First, beached plastics identified as polyethylene terephthalate (PET), ethylene vinyl acetate (EVA) and a styrene-butadiene rubber (Figure 5.3) were irradiated using a reptile lamp in artificial seawater (ASW) to induce photodegradation, and produce leachate-contaminated ASW. Growth assays of *Escherichia coli* (a model pathogen) and *Rosellomorea aquimaris* (a benign, pollutant-degrading bacterium) were then conducted in ASW, and leachate-contaminated ASW in 24-well plates (72, 396). Interestingly, our preliminary results demonstrated that leachate exposure significantly inhibited the growth of *E. coli* while promoting growth of *R. aquimaris* (Figure 5.4), revealing that leachates may indeed inhibit pathogenicity in some circumstances (Section 3). Further studies of different plastic leachates on additional microbial genera may therefore provide a more comprehensive understanding of how plastics interact with the marine environment. The use of molecular analyses could also reveal which metabolic pathways are inhibited or activated by this exposure, resulting in the observed growth response (Figure 5.4).

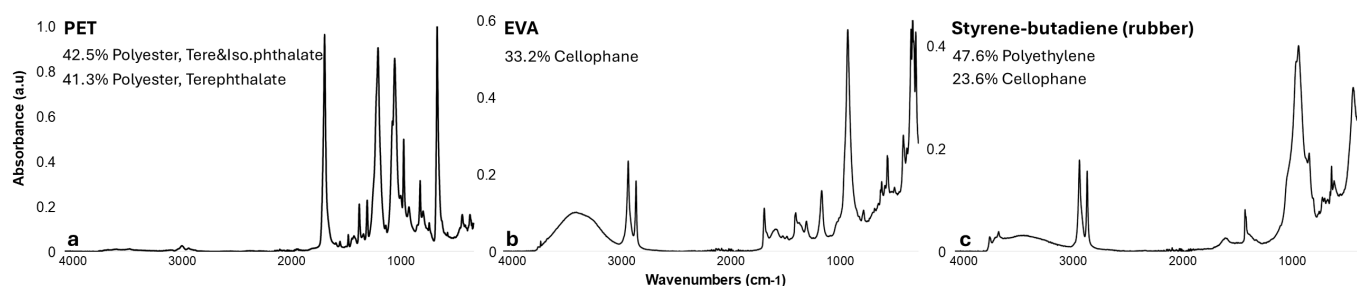


Figure 5.3 ATR-FTIR spectra of plastic bottle (a), flip flop (b), and plastic packaging (c) collected from beaches throughout SE Asia, and used to produce leachate-contaminated ASW after UVA and UVB irradiation. Predicted dominant polymer presented in bold. Percentage matches to polymers in Hummel Polymer Sample library presented below. PET, polyethylene terephthalate; EVA, ethylene vinyl acetate.

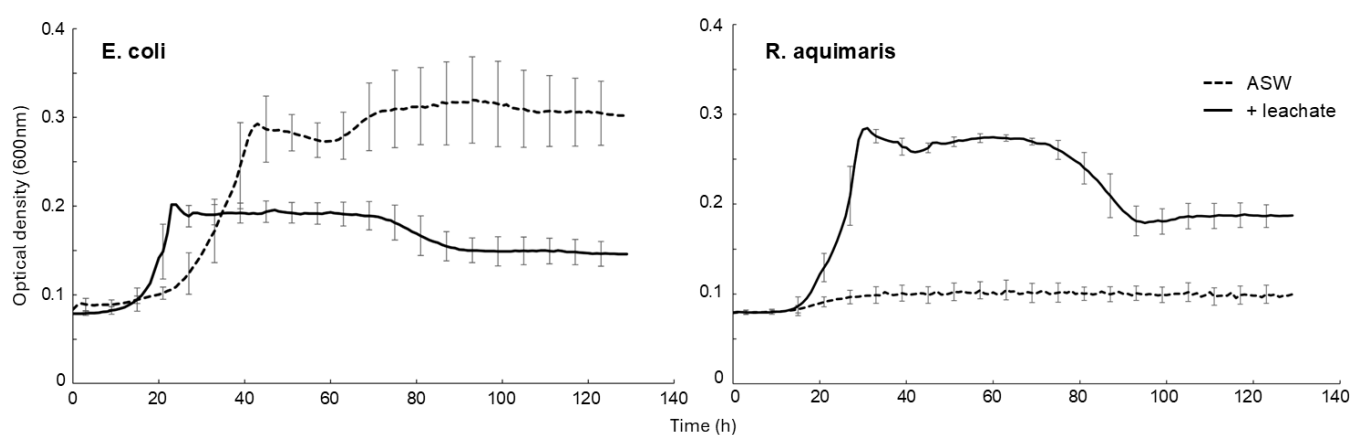


Figure 5.4 Growth curves of *Escherichia coli* and *Rossellomorea aquimaris* in artificial seawater only (dashed line), or with plastic leachates (solid line). Data collected hourly. Error bars for standard deviation presented every seven hrs.

In **Chapter 4**, the heterogeneity of the plastisphere's response to EHMC verifies the previously discovered taxa-specific responses of marine microorganisms to pollutants (39, 98). Here, we suggest that this variation in microbial response may be a result of the pollutants impact on respiratory pathways, which could be tested through dedicated study of aerobic and anaerobic bacteria, and their response to pollutants. Such testing in preliminary experiments for **Chapter 3** revealed which selected marine bacteria may exhibit a metabolic response to organic UV-filters, and still produce a distinct secretome after exposure (Figure 5.5). Additional traits of microbial taxa may also be tested by studying a wide range of model marine organisms – including eukaryotes – with diverse metabolic functions, gram-stains, and phylogenetic groups. This may also reveal how cell features including cell size, membrane composition, or indeed secretome size impacts a microorganisms response to pollutants. Furthermore, further examination of the transmembrane transport proteins identified in **Chapter 3** through the use of knockout studies may also clarify the role of these membrane-bound mechanisms in pollutant response (397).

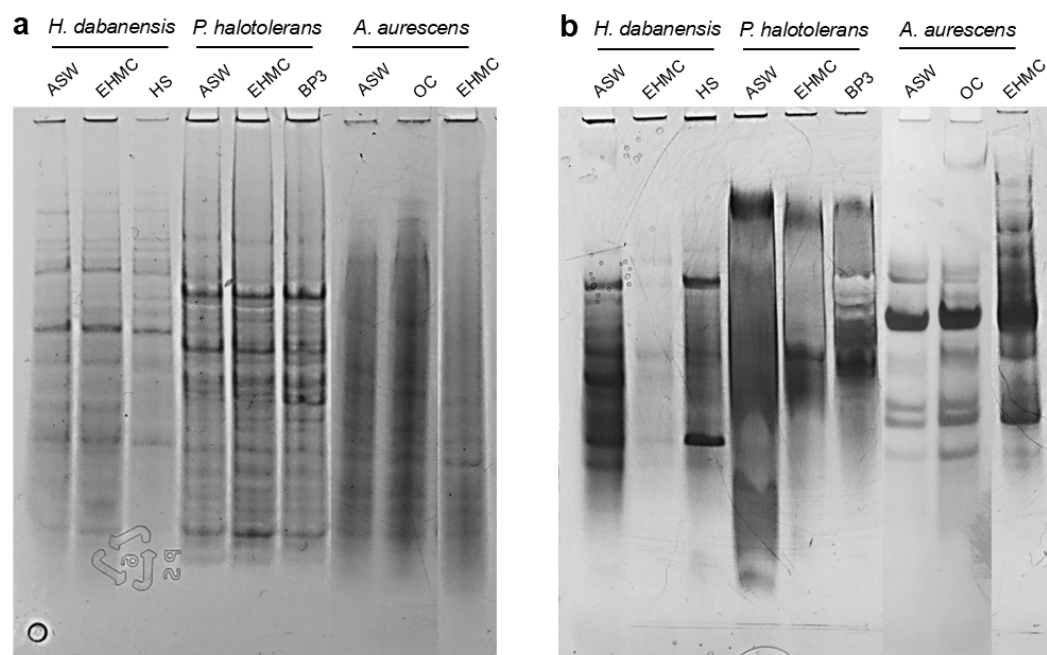


Figure 5.5 SDS-PAGE gels of marine microbial proteomes (a) and secretomes (b) after bacterial exposure to organic UV-filters. Bacterial strains: *Halobacillus dabanensis*, *Pelagibacterium halotolerans*, *Arthrobacter aurescens*. Acronyms: ASW, artificial seawater; EHMC, ethylhexyl methoxycinnamate; HS, homosalate; BP3, benzophenone-3; OC, octocrylene. Proteome gels stained with Coomassie Blue. Secretome gels stained with Pierce Silver Stain (CAT – No. 24612). Photographs adjusted for band clarity.

5. The Use of Metaproteomics to Study Marine Microbial Ecology

At the outset of this four-year investigation, only two research articles had used metaproteomics to investigate the marine plastisphere's metabolic profile (68, 75). However, in this time, the work produced in this thesis alone has doubled the number of studies using metaproteomics to study the plastisphere, using this advanced molecular tool to provide novel insights into marine microbial metabolism, and how it responds to pollutant exposure. In addition to all results presented above, the metaproteomic findings presented here reveal a level of dissimilarity between metagenomic and metaproteomic findings. This means that, this approach likely reveals novel metabolic insights that cannot always be predicted through other forms of molecular study. As such, an increasing number of studies is using environmental metaproteomics to gain insights which would have taken many years of dedicated study, or even been impossible before (177, 193, 195, 398). In this thesis, protein-level functional assignments were, where possible, linked to genus-level taxonomic identities. This dual functional-taxonomic perspective provided insight into both the potential activities of the plastisphere community and its likely microbial contributors. These assignments may be influenced by conserved protein sequences and gaps in marine reference databases, particularly for core metabolic or stress-response proteins (181, 217). However, the genus-level annotations still offered a valid comparative framework for exploring functional shifts in cornerstone taxa over time. Framing results in this way ensures that interpretations remain indicative rather than definitive, while contributing to the broader functional narrative of the plastisphere. In future studies, this approach may also be optimised, and used to provide additional insights into niche functioning. As stated in **Chapter 3**, a key limitation currently facing proteomic research is the proportion of proteins which remain uncharacterised. However, as exploratory knock-down and heterologous expression studies continue to discover protein functions, the yields from proteomic analysis will continue to improve (399, 400). Furthermore, suggested solutions to low protein coverages including the more practiced use of protease inhibitors, the combined use of digestive enzymes (e.g., trypsin), and pre-fractionation of samples before DDA analysis continue to yield improved results (301, 307, 317, 401, 402). These adapted approaches will therefore further improve the analytical yields of many future environmental metaproteomic studies, increasing the relevance of this approach for the continued study of marine microbial ecology.

6. Implications for Environmental Ecology and Policy

Marine microbial communities are integral to maintaining the ocean's ecological balance and biogeochemical functions (403). Crucially, the findings discussed in this thesis underscores the significant risks posed by marine pollution to these communities. **Chapter 4** highlights the ecological disturbance, and increased plastic persistence potentially caused by combined pollution. Moreover, plastic's ability to transport invasive species between ecosystems may introduce taxa that directly, or indirectly lead to a loss in biodiversity (381, 404). Notably, co-pollution is also greatest at the sea surface microlayer (SML), where microorganisms known to regulate the air-ocean interface, and thus global biogeochemistry are most concentrated (30, 32). A failure to address these threats could therefore accelerate detrimental shifts in marine ecosystems, compounding the already adverse effects of climate change. For instance, the ocean's declining capacity to absorb carbon dioxide – a consequence of climate change –, may be further undermined by pollution-, and invasive species- induced disruptions to microbial ecosystem services (405).

As a result of these concerns, marine pollution was formally recognised as a threat to biodiversity with the 2012 adoption of the United Nations Sustainable Development Goals (SDGs) (406). Within this framework, plastic is highlighted as a target for the ocean cleanup initiative within SDG.14 which includes large-scale removal of legacy plastics from the ocean, and initiatives created specifically to reduce plastic pollution. Many policymakers have adopted these initiatives, advocating for the promotion and introduction of systems to create a circular economy (407). Greater accessibility, and incentive to use recycling facilities, the increased use of recycled materials in industry, and the replacement of non-biodegradable plastics (i.e., PET) with bioplastics (e.g., polyhydroxyalkanoate) are all proposed methods to meet this goal (408). If these systems successfully mitigate plastic pollution, a key site of marine pollutant aggregation will be removed, reducing the prevalence of combined pollution, and its associated hazards (**Chapter 1**).

Policies are also now being written to protect the marine environment from pollutants like organic UV-filters (409). Indeed, BP3, an organic UV-filter studied in **Chapter 3**, has now been banned in Hawaii due to its role in coral bleaching, and mainstream PCP brands in the EU have begun formulating their sunscreens without this component (143, 410). Globally,

consumers are also encouraged to use sunscreens which only contain mineral filters (e.g., titanium dioxide) as a more ‘reef-safe’ alternative (411). However, the true safety of this alternative UV-filter has not yet been confirmed by the scientific community (161, 410, 412, 413). As discussed in **Chapter 1**, many combinations of chemicals can accumulate on the surface of plastic, which can also exacerbate the toxicity of any pollutant, even where a singular pollutant’s toxicity is mild. Consequently, until the combined effects of all PCP ingredients entering marine environments are understood, no chemical can be deemed entirely ‘safe.’

While not a primary focus of this work, outcomes of the research presented in this thesis (**Chapters 2 and 4**) align with further studies which find that plastic pollution, beyond affecting marine ecosystems, may also have implications for human health by enabling the growth of potential pathogens on a substrate commonly found in municipal areas. By transporting potential pathogens large distances, and from sewerage outlets to ‘clean’ regions, this plastic pollution places individuals at increasing risk of contracting life-threatening infections after ocean bathing (414). Moreover, fish and crops exposed to these pollutants risk accumulating hazardous plastics and chemicals, threatening global food security and exposing humans to harmful substances through consumption (379, 380). If these plastic-associated hazards are confirmed, stringent regulatory measures may be required to minimise human exposure. These could include revising food safety standards to account for waterborne pollutants and pathogens, restricting access to heavily polluted areas, and further promoting pollution reduction efforts at local and global scales.

Failing to curtail the current rate of marine pollution risks severe damage to global ecological, economical, and humanitarian interests. Addressing this requires a multifaceted strategy which may include: limiting pollutant inputs, adopting safer alternatives, enhancing recycling, and actively removing existing pollutants. Simultaneously, long-term monitoring of marine microbial communities is critical to assess the effectiveness of these mitigation measures and understand their effects on oceanic biogeochemical cycles. Such measures are vital not only for preserving the health of marine ecosystems but also for safeguarding the planet’s essential resources.

7. Concluding Remarks

This project explores the metabolic interactions between marine microorganisms and persistent pollutants, revealing how plastic pollution and co-contaminants like organic UV-filters impact marine microbial communities. By employing molecular and meta-omic approaches, novel insights into microbial adaptation, biofilm formation, and pollutant-induced metabolic shifts have been uncovered. Importantly, the findings presented here raise concerns about the potential for enhanced virulence and reduced plastic biodegradation in co-polluted environments. Future studies integrating additional molecular methodologies could further illuminate these interactions, establishing clear links between combined pollution impacts and global development goals. Ultimately, this work underscores the importance of environmental metaproteomics in marine microbial ecology and sets the foundation for continued exploration of plastisphere functionality in highly polluted locations.

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Appendices

Appendix A Protein summary exports from DDA analysis generated by Protein Pilot (>1peptide), and associated metadata for Chapter 3.

Category	Accession	Protein	%Cov (95)	Peptides (95%)
ASW	WP_065322993.1	amino acid ABC transporter substrate-binding protein	36.3900006	18
ASW	WP_074712646.1	elongation factor Tu	43.4799999	11
ASW	WP_065331464.1	extracellular solute-binding protein	15.7600001	9
ASW	WP_126136331.1	penicillin-binding protein activator	18.2699993	9
ASW	WP_065324298.1	tetratricopeptide repeat protein	15.8999994	8
ASW	WP_065325842.1	flagellin	19.1499993	7
ASW	WP_126138124.1	flagellin	21.3499993	6
ASW	WP_009179192.1	TRAP transporter substrate-binding protein	21.3300005	6
ASW	WP_065321627.1	sugar ABC transporter substrate-binding protein	13.0199999	5
ASW	WP_126136030.1	flagellin	15.2500004	5
ASW	WP_005615637.1	ABC transporter substrate-binding protein	21.9099998	4
ASW	WP_039983277.1	flagellar basal body L-ring protein FlgH	15.9199998	4
ASW	WP_005620552.1	F0F1 ATP synthase subunit beta	9.70500037	4
ASW	WP_126137436.1	chaperonin GroEL	7.67799988	4
ASW	WP_126138954.1	vanadium-dependent haloperoxidase	9.7450003	4
ASW	WP_126136477.1	calcium sensor EFh	18.3400005	4
ASW	WP_009176373.1	50S ribosomal protein L7/L12	25	3
ASW	WP_126137349.1	peptide ABC transporter substrate-binding protein	6.54899999	3
ASW	WP_005620166.1	superoxide dismutase	13.0700007	3
ASW	WP_126136091.1	xylose isomerase	6.65099993	3
ASW	WP_005635258.1	type I glutamate--ammonia ligase	4.91500013	2
ASW	WP_065330136.1	DUF3576 domain-containing protein	11.7299996	2
ASW	WP_005657698.1	polysaccharide biosynthesis/export family protein	4.80000004	2
ASW	WP_126136655.1	hypothetical protein	10.1199999	2
ASW	WP_126137969.1	extensin family protein	5.81800006	2
ASW	WP_126136099.1	ABC transporter substrate-binding protein	7.51900002	2
ASW	WP_126138950.1	BMP family protein	10.3299998	2
ASW	WP_005616945.1	electron transfer flavoprotein subunit alpha	16.5000007	2
ASW	WP_005621805.1	MULTISPECIES: DNA-directed RNA polymerase subunit alpha [Epibacterium]	9.46699977	2
ASW	WP_009178727.1	lytic transglycosylase domain-containing protein	19.2300007	2
ASW	WP_126136534.1	F0F1 ATP synthase subunit alpha	4.28799987	2

ASW	WP_126138606.1	dihydrolipoyl dehydrogenase	4.94600013	2
DMSO	WP_126138084.1	D-xylose ABC transporter substrate-binding protein	49.4199991	19
DMSO	WP_065322993.1	amino acid ABC transporter substrate-binding protein	39.6400005	18
DMSO	WP_074712646.1	elongation factor Tu	26.6000003	10
DMSO	WP_065324298.1	tetratricopeptide repeat protein	16.9599995	10
DMSO	WP_126136331.1	penicillin-binding protein activator	22.3399997	10
DMSO	WP_065325842.1	flagellin	23.0499998	9
DMSO	WP_126138124.1	flagellin	16.0099998	8
DMSO	WP_126136030.1	flagellin	18.7900007	8
DMSO	WP_009179192.1	TRAP transporter substrate-binding protein	16.3399994	7
DMSO	WP_039983277.1	flagellar basal body L-ring protein FlgH	26.9400001	6
DMSO	WP_009179062.1	flagellin	17.4999997	6
DMSO	WP_065321627.1	sugar ABC transporter substrate-binding protein	17.7499995	4
DMSO	WP_005620552.1	F0F1 ATP synthase subunit beta	13.0799994	4
DMSO	WP_126136767.1	peptidoglycan DD-metalloendopeptidase family protein	8.60000029	4
DMSO	WP_005607891.1	flagellar basal body P-ring protein FlgI	15.2600005	4
DMSO	WP_126137349.1	peptide ABC transporter substrate-binding protein	7.61099979	4
DMSO	WP_126136477.1	calcium sensor EFh	18.3400005	4
DMSO	WP_126137436.1	chaperonin GroEL	7.8610003	3
DMSO	WP_009176373.1	50S ribosomal protein L7/L12	25	3
DMSO	WP_009176087.1	lytic murein transglycosylase	7.632	3
DMSO	WP_009178137.1	CAP domain-containing protein	19.5099995	3
DMSO	WP_126138954.1	vanadium-dependent haloperoxidase	7.65699968	3
DMSO	WP_039983616.1	M48 family metalloprotease	11.1900002	3
DMSO	WP_126137747.1	penicillin-insensitive murein endopeptidase	11.4	3
DMSO	WP_005615637.1	ABC transporter substrate-binding protein	10.83	2
DMSO	WP_065329575.1	type I glyceraldehyde-3-phosphate dehydrogenase	6.58700019	2
DMSO	WP_126136534.1	F0F1 ATP synthase subunit alpha	4.67800014	2
DMSO	WP_005657698.1	polysaccharide biosynthesis/export family protein	4.80000004	2
DMSO	WP_040641221.1	type II and III secretion system protein family protein	4.04299982	2
DMSO	WP_126136866.1	ABC transporter substrate-binding protein	5.18300012	2
DMSO	WP_005628000.1	Tol-Pal system protein TolB	4.08199988	2
DMSO	WP_005621904.1	MULTISPECIES: 30S ribosomal protein S3 [Rhodobacteraceae]	12.5499994	2
DMSO	WP_126137147.1	PQQ-like beta-propeller repeat protein	3.62799987	2
DMSO	WP_126136655.1	hypothetical protein	10.1199999	2
DMSO	WP_009178727.1	lytic transglycosylase domain-containing protein	19.2300007	2
DMSO	WP_005621809.1	30S ribosomal protein S13	16.3900003	2
DMSO	WP_126136345.1	transporter substrate-binding domain-containing protein	7.22400025	2

DMSO	WP_009178973.1	outer membrane protein assembly factor BamD	5.65400012	2
DMSO	WP_005618395.1	MULTISPECIES: HU family DNA-binding protein [Rhodobacteraceae]	26.8000007	2
DMSO	WP_126138014.1	hypothetical protein	10.3600003	2
DMSO	WP_005638856.1	cysteine synthase A	6.10500015	2
DMSO	WP_009175577.1	malate dehydrogenase	9.06300023	2
DMSO	WP_126137594.1	OmpA family protein	14.0300006	2
DMSO	WP_005638392.1	phasin family protein	12.8399998	2
DMSO	WP_065331464.1	extracellular solute-binding protein	7.33700022	2
BP3	WP_126138084.1	D-xylose ABC transporter substrate-binding protein	41.5199995	18
BP3	WP_039983277.1	flagellar basal body L-ring protein FlgH	27.759999	9
BP3	WP_126136331.1	penicillin-binding protein activator	10.4099996	6
BP3	WP_065322993.1	amino acid ABC transporter substrate-binding protein	17.1599999	5
BP3	WP_065325842.1	flagellin	19.4999993	5
BP3	WP_009179192.1	TRAP transporter substrate-binding protein	18.0099994	5
BP3	WP_074712646.1	elongation factor Tu	8.1840001	4
BP3	WP_126136030.1	flagellin	8.15600008	4
BP3	WP_065321627.1	sugar ABC transporter substrate-binding protein	13.3100003	3
BP3	WP_065324298.1	tetratricopeptide repeat protein	9.54099968	3
BP3	WP_126138124.1	flagellin	14.2299995	3
BP3	WP_126138954.1	vanadium-dependent haloperoxidase	8.58500004	3
BP3	WP_126136477.1	calcium sensor EFh	18.3400005	2
BP3	WP_005635258.1	type I glutamate--ammonia ligase	4.91500013	2
BP3	WP_005620552.1	F0F1 ATP synthase subunit beta	6.96199983	2
BP3	WP_009176373.1	50S ribosomal protein L7/L12	14.5199999	2
BP3	WP_126136759.1	DctP family TRAP transporter solute-binding subunit	5.68899997	2
EHMC	WP_126138084.1	D-xylose ABC transporter substrate-binding protein	66.960001	92
EHMC	WP_065322993.1	amino acid ABC transporter substrate-binding protein	47.3399997	40
EHMC	WP_065325842.1	flagellin	29.429999	20
EHMC	WP_009179062.1	flagellin	23.9299998	18
EHMC	WP_126136997.1	TAXI family TRAP transporter solute-binding subunit	41.8000013	15
EHMC	WP_126138124.1	flagellin	35.589999	15
EHMC	WP_126136030.1	flagellin	30.8499992	15
EHMC	WP_126137349.1	peptide ABC transporter substrate-binding protein	20.7100004	14
EHMC	WP_009179192.1	TRAP transporter substrate-binding protein	35.4600012	14
EHMC	WP_065331464.1	extracellular solute-binding protein	31.7900002	14
EHMC	WP_126138115.1	ABC transporter substrate-binding protein	15.2099997	11
EHMC	WP_009175631.1	BMP family ABC transporter substrate-binding protein	28.0999988	9
EHMC	WP_126136099.1	ABC transporter substrate-binding protein	15.6000003	7

EHMC	WP_126138668.1	C4-dicarboxylate TRAP transporter substrate-binding protein	15.6800002	7
EHMC	WP_005615637.1	ABC transporter substrate-binding protein	19.1400006	7
EHMC	WP_005629282.1	thiosulfate ABC transporter substrate-binding protein CysP	11.8799999	6
EHMC	WP_126138200.1	ABC transporter substrate-binding protein	11.9900003	6
EHMC	WP_126138954.1	vanadium-dependent haloperoxidase	14.8499995	6
EHMC	WP_065321627.1	sugar ABC transporter substrate-binding protein	19.8200002	5
EHMC	WP_126136364.1	BMP family ABC transporter substrate-binding protein	13.1300002	4
EHMC	WP_126138956.1	M23 family metalloproteinase	11.71	4
EHMC	WP_074712646.1	elongation factor Tu	10.4900002	4
EHMC	WP_005613674.1	transporter substrate-binding domain-containing protein	20.4300001	4
EHMC	WP_126136477.1	calcium sensor EFh	18.3400005	4
EHMC	WP_126136331.1	penicillin-binding protein activator	7.36000016	3
EHMC	WP_005620552.1	F0F1 ATP synthase subunit beta	10.1300001	3
EHMC	WP_126136345.1	transporter substrate-binding domain-containing protein	13.3100003	3
EHMC	WP_005657698.1	polysaccharide biosynthesis/export family protein	7.99999982	3
EHMC	WP_039983277.1	flagellar basal body L-ring protein FlgH	12.2400001	3
EHMC	WP_065324298.1	tetratricopeptide repeat protein	9.89399999	2
EHMC	WP_126136263.1	TRAP transporter substrate-binding protein	7.38499984	2
EHMC	WP_005650227.1	carbohydrate ABC transporter substrate-binding protein	4.31700014	2
EHMC	WP_126137798.1	tripartite tricarboxylate transporter substrate binding protein	6.44199997	2
EHMC	WP_065329575.1	type I glyceraldehyde-3-phosphate dehydrogenase	6.58700019	2
EHMC	WP_009177819.1	Fe(3+) ABC transporter substrate-binding protein	5.65499999	2
EHMC	WP_126136866.1	ABC transporter substrate-binding protein	6.40200004	2
EHMC	WP_065323497.1	TAXI family TRAP transporter solute-binding subunit	9.06300023	2
EHMC	WP_005625522.1	ABC transporter substrate-binding protein	5.15200011	2
EHMC	WP_005609928.1	ABC transporter substrate-binding protein	4.91099991	2
EHMC	WP_126137188.1	oxidoreductase	9.03199986	2
EHMC	WP_126138014.1	hypothetical protein	12.4399997	2
EHMC	WP_005614465.1	hypothetical protein	15.7000005	2
EHMC	WP_126137594.1	OmpA family protein	11.31	2
EHMC	WP_009175915.1	TRAP transporter substrate-binding protein	4.02299985	2

Appendix B Fold-change analysis summary using MarkerView when comparing protein abundance ratio for conditions DMSO and ASW (DMSO:ASW) within Chapter 3.

Protein	p-value	- log10 p-value	Fold Change (DMSO:ASW)	Log2 Fold Change (DMSO:ASW)	Sign alP	Function
ABC transporter substrate-binding protein	0.191824	0.717096	0.484592	-1.04516	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.408353	0.388964	0.684349	-0.5472	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.500741	0.300387	1.527699	0.61136	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.628396	0.201767	0.655469	-0.6094	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.720795	0.142188	0.781976	-0.3548	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.736615	0.13276	0.839259	-0.25281	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.775533	0.110399	0.755719	-0.40408	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.857253	0.066891	0.88103	-0.18274	Y	Transmembrane transport
amino acid ABC transporter substrate-binding protein	0.55694	0.254192	1.433001	0.51904	Y	Amino acid transport and metabolism
bifunctional 2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase	0.513305	0.289625	1.327095	0.408272	Y	Transcription/translation
BMP family ABC transporter substrate-binding protein	0.245145	0.610577	3.392072	1.762167	Y	Cell wall/membrane/envelope biogenesis
BMP family ABC transporter substrate-binding protein	0.559126	0.25249	1.990456	0.993099	Y	Cell wall/membrane/envelope biogenesis
BMP family protein	0.714319	0.146108	0.796671	-0.32794	Y	Cell wall/membrane/envelope biogenesis
C4-dicarboxylate TRAP transporter substrate-binding protein	0.587124	0.23127	1.409599	0.495285	Y	General function prediction only
CAP domain-containing protein	0.39265	0.405995	37.21293	5.217732	Y	Intracellular trafficking, secretion, and vesicular transport
carbohydrate ABC transporter substrate-binding protein	0.287854	0.540828	0.611362	-0.7099	Y	Transmembrane transport
carbohydrate ABC transporter substrate-binding protein	0.364663	0.438108	0.561741	-0.83202	Y	Transmembrane transport
D-xylose ABC transporter substrate-binding protein	0.595908	0.224821	1.422615	0.508545	Y	Transmembrane transport
DctP family TRAP transporter solute-binding subunit	0.478176	0.320412	0.608729	-0.71613	Y	Transmembrane transport

DUF3035 domain-containing protein	0.453663	0.343267	2.184822	1.127516	Y	Unknown function
DUF3576 domain-containing protein	0.663979	0.177846	1.197578	0.26012	Y	Unknown function
extensin family protein	0.208326	0.681257	1.864106	0.898484	Y	Cell wall/membrane/envelope biogenesis
extracellular solute-binding protein	0.829962	0.080942	0.918979	-0.1219	Y	Transmembrane transport
extracellular solute-binding protein	0.882391	0.054339	0.901011	-0.15038	Y	Transmembrane transport
Fe(3+) ABC transporter substrate-binding protein	0.95617	0.019465	1.037281	0.052807	Y	Inorganic ion transport and metabolism
flagellar basal body L-ring protein FlgH	0.380705	0.419411	3.202443	1.679173	Y	Cell motility
flagellar basal body P-ring protein FlgI	0.592952	0.226981	1.67619	0.745185	Y	Cell motility
hypothetical protein	0.390199	0.408714	24.81488	4.633133	Y	Unknown function
hypothetical protein	0.416454	0.380433	2.895125	1.533625	Y	Unknown function
hypothetical protein	0.463629	0.333829	1.895436	0.92253	Y	Unknown function
hypothetical protein	0.583378	0.23405	1.670497	0.740277	Y	Unknown function
hypothetical protein	0.763251	0.117333	1.306309	0.385497	Y	Unknown function
L,D-transpeptidase	0.475247	0.323081	2.89394	1.533035	Y	Cell wall/membrane/envelope biogenesis
lytic murein transglycosylase	0.5474	0.261695	1.815464	0.860338	Y	Cell wall/membrane/envelope biogenesis
M48 family metalloprotease	0.451498	0.345344	2.182712	1.126121	Y	Posttranslational modification, protein turnover, chaperones
OmpA family protein	0.451683	0.345166	2.198724	1.136667	Y	Cell wall/membrane/envelope biogenesis
outer membrane protein assembly factor BamA	0.538627	0.268712	2.095603	1.067365	Y	Cell wall/membrane/envelope biogenesis
outer membrane protein assembly factor BamD	0.482498	0.316504	2.563864	1.358319	Y	Cell wall/membrane/envelope biogenesis
oxidoreductase	0.794953	0.099658	1.189415	0.250252	Y	Energy production and conversion
penicillin-binding protein activator	0.532681	0.273533	1.888718	0.917408	Y	Cell wall/membrane/envelope biogenesis
penicillin-insensitive murein endopeptidase	0.460133	0.337117	2.019258	1.013825	Y	Cell wall/membrane/envelope biogenesis
peptide ABC transporter substrate-binding protein	0.579902	0.236645	0.710425	-0.49324	Y	Transmembrane transport
peptidoglycan DD-metalloendopeptidase family protein	0.57768	0.238312	1.860579	0.895751	Y	Cell wall/membrane/envelope biogenesis

polyisoprenoid-binding protein	0.392085	0.40662	1.735181	0.795086	Y	Lipid transport and metabolism
polysaccharide biosynthesis/export family protein	0.889674	0.050769	1.097184	0.133805	Y	Cell wall/membrane/envelope biogenesis
PQQ-like beta-propeller repeat protein	0.660129	0.180371	0.942827	-0.08494	Y	General function prediction only
sn-glycerol-3-phosphate ABC transporter substrate-binding protein UgpB	0.376731	0.423969	8.553201	3.096464	Y	Transmembrane transport
substrate-binding domain-containing protein	0.402918	0.394783	0.393999	-1.34374	Y	Transmembrane transport
sugar ABC transporter substrate-binding protein	0.561475	0.250669	0.622588	-0.68365	Y	Transmembrane transport
TAXI family TRAP transporter solute-binding subunit	0.849649	0.07076	1.161123	0.21552	Y	Transmembrane transport
TAXI family TRAP transporter solute-binding subunit	0.854495	0.06829	0.897058	-0.15673	Y	Transmembrane transport
tetratricopeptide repeat protein	0.445117	0.351526	1.992409	0.994514	Y	Posttranslational modification, protein turnover, chaperones
thiosulfate ABC transporter substrate-binding protein CysP	0.555905	0.254999	1.451054	0.537101	Y	Transmembrane transport
Tol-Pal system protein TolB	0.940758	0.026522	1.05538	0.077763	Y	Intracellular trafficking, secretion, and vesicular transport
transporter substrate-binding domain-containing protein	0.521222	0.282977	0.51142	-0.96742	Y	Transmembrane transport
transporter substrate-binding domain-containing protein	0.735076	0.133667	1.230824	0.299625	Y	Transmembrane transport
TRAP transporter substrate-binding protein	0.146006	0.83563	1.631492	0.706192	Y	Transmembrane transport
TRAP transporter substrate-binding protein	0.508656	0.293575	0.580551	-0.7845	Y	Transmembrane transport
TRAP transporter substrate-binding protein	0.873966	0.058506	0.908269	-0.13881	Y	Transmembrane transport
TRAP transporter substrate-binding protein DctP	0.562519	0.249863	1.508063	0.592697	Y	Transmembrane transport
tripartite tricarboxylate transporter substrate binding protein	0.452788	0.344105	0.555426	-0.84833	Y	Energy production and conversion
twin-arginine translocation pathway signal	0.346504	0.460292	2.23	1.157044	Y	Intracellular trafficking, secretion, and vesicular transport
type II and III secretion system protein family	0.372207	0.429215	2.469185	1.304035	Y	Cell wall/membrane/envelope biogenesis
vanadium-dependent haloperoxidase	0.512564	0.290252	1.775052	0.827861	Y	Inorganic ion transport and metabolism
30S ribosomal protein S1	0.814507	0.089105	0.82411	-0.27909	N	Transcription/translation
30S ribosomal protein S13	0.452439	0.34444	6.537976	2.708844	N	Transcription/translation

50S ribosomal protein L1	0.422019	0.374668	0.582793	-0.77894	N	Transcription/translation
50S ribosomal protein L3	0.529586	0.276064	0.346369	-1.52962	N	Transcription/translation
50S ribosomal protein L5	0.44315	0.353449	2.47907	1.309799	N	Transcription/translation
50S ribosomal protein L7/L12	0.47547	0.322877	0.514404	-0.95902	N	Transcription/translation
acetyl/propionyl/methylcrotonyl-CoA carboxylase subunit alpha	0.677905	0.168831	0.753754	-0.40783	N	Lipid transport and metabolism
argininosuccinate synthase	0.429396	0.367142	0.412826	-1.2764	N	Amino acid transport and metabolism
calcium sensor EFh	0.679112	0.168059	1.737535	0.797042	N	Intracellular trafficking, secretion, and vesicular transport
carbohydrate kinase	0.396953	0.401261	11.17149	3.48175	N	Energy production and conversion
catalase/peroxidase HPI	0.376122	0.424671	0.534694	-0.90321	N	Inorganic ion transport and metabolism
chaperonin GroEL	0.603353	0.219428	0.680163	-0.55605	N	Posttranslational modification, protein turnover, chaperones
cysteine synthase A	0.388059	0.411102	5.764134	2.527104	N	Amino acid transport and metabolism
dihydrolipoyl dehydrogenase	0.264474	0.577617	4.040327	2.014472	N	Energy production and conversion
DNA primase	0.964793	0.015566	1.033933	0.048143	N	Transcription/translation
DNA-directed RNA polymerase subunit beta'	0.529011	0.276535	0.624283	-0.67973	N	Transcription/translation
Do family serine endopeptidase	0.27403	0.562202	1.921032	0.941882	N	Posttranslational modification, protein turnover, chaperones
electron transfer flavoprotein subunit alpha	0.415163	0.381781	0.169961	-2.55672	N	Inorganic ion transport and metabolism
elongation factor Ts	0.653554	0.184718	0.641395	-0.64071	N	Transcription/translation
elongation factor Tu	0.544272	0.264184	0.559634	-0.83744	N	Transcription/translation
F0F1 ATP synthase subunit alpha	0.54522	0.263428	1.241889	0.312537	N	Energy production and conversion
F0F1 ATP synthase subunit beta	0.841799	0.074792	0.852776	-0.22976	N	Energy production and conversion
flagellar basal-body rod protein FlgG	0.348955	0.457231	4.782394	2.257733	N	Cell motility
flagellar hook-basal body complex protein	0.418428	0.378379	4.078269	2.027957	N	Cell motility
flagellin	0.291557	0.535277	2.826415	1.498973	N	Cell motility
flagellin	0.444136	0.352484	0.515716	-0.95535	N	Cell motility

flagellin	0.597152	0.2239 15	1.668182	0.738277	N	Cell motility
flagellin	0.877041	0.0569 8	0.915952	-0.12666	N	Cell motility
fructose-6-phosphate aldolase	0.560187	0.2516 67	2.309906	1.207834	N	Energy production and conversion
Glu/Leu/Phe/Val dehydrogenase	0.726866	0.1385 46	1.357341	0.440783	N	Inorganic ion transport and metabolism
leucyl aminopeptidase	0.271684	0.5659 35	0.199938	-2.32237	N	Amino acid transport and metabolism
lytic transglycosylase domain-containing protein	0.629693	0.2008 71	1.512455	0.596893	N	Cell wall/membrane/env elope biogenesis
M23 family metallopeptidase	0.632452	0.1989 72	0.583322	-0.77764	N	Cell wall/membrane/env elope biogenesis
malate dehydrogenase	0.766546	0.1154 62	1.389512	0.474578	N	Energy production and conversion
MULTISPECIES: 30S ribosomal protein S3 [Rhodobacteraceae]	0.376807	0.4238 81	3.470468	1.79513	N	Transcription/ translation
MULTISPECIES: DNA- directed RNA polymerase subunit alpha [Epibacterium]	0.877142	0.0569 3	0.861589	-0.21493	N	Transcription/ translation
MULTISPECIES: HU family DNA-binding protein [Rhodobacteraceae]	0.745129	0.1277 68	0.709886	-0.49434	N	Replication, recombination and repair
NADH-quinone oxidoreductase subunit D	0.481207	0.3176 68	0.365687	-1.45132	N	Energy production and conversion
NADP-dependent isocitrate dehydrogenase	0.571976	0.2426 22	1.697922	0.76377	N	Energy production and conversion
outer membrane protein assembly factor BamE	0.524091	0.2805 93	1.947976	0.961976	N	Cell wall/membrane/env elope biogenesis
phage major capsid protein	0.356441	0.4480 12	0.359805	-1.47471	N	Unknown function
phasin family protein	0.598105	0.2232 23	2.432342	1.282346	N	Lipid transport and metabolism
phosphopyruvate hydratase	0.69975	0.1550 57	1.475785	0.561483	N	Energy production and conversion
pyruvate dehydrogenase complex E1 component subunit beta	0.481923	0.3170 23	3.929729	1.97443	N	Energy production and conversion
pyruvate kinase	0.534315	0.2722 03	0.410874	-1.28323	N	Energy production and conversion
response regulator	0.341043	0.4671 91	1.690813	0.757717	N	Transcription/ translation
superoxide dismutase	0.998856	0.0004 97	1.000854	0.001231	N	Inorganic ion transport and metabolism
thioredoxin	0.822934	0.0846 35	1.2282	0.296546	N	Posttranslational modification, protein turnover, chaperones
translation initiation factor IF-2	0.639995	0.1938 23	0.699595	-0.51541	N	Transcription/ translation

trigger factor	0.784638	0.1053 31	0.770241	-0.37662	N	Posttranslational modification, protein turnover, chaperones
type I glutamate--ammonia ligase	0.649025	0.1877 38	1.362522	0.446279	N	Amino acid transport and metabolism
type I glyceraldehyde-3- phosphate dehydrogenase	0.439721	0.3568 23	3.106792	1.635426	N	Energy production and conversion
tyrosine-type recombinase/integrase	0.956633	0.0192 55	1.035109	0.049783	N	Transcription/ translation
xylose isomerase	0.439155	0.3573 83	3.107455	1.635733	N	Energy production and conversion

Appendix C Fold-change analysis summary using MarkerView when comparing protein abundance ratio for conditions BP3 and DMSO (BP3:DMSO) within Chapter 3.

Protein	p-value	- log10 p- value	Fold Change (BP3:DM SO)	Log2 Fold Change (BP3:DM SO)	Signa IP	Function
ABC transporter substrate-binding protein	0.12138	0.9158 54	0.340417	-1.55462	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.96961 4	0.0134 01	0.977508	-0.03282	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.44064	0.3559 16	0.524265	-0.93163	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.57940 2	0.2370 2	1.959207	0.97027	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.62302 2	0.2054 96	0.671631	-0.57426	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.19426 2	0.7116 12	1.927858	0.946999	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.77761 1	0.1092 37	1.158685	0.212488	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.22273 4	0.6522 13	0.287423	-1.79875	Y	Transmembrane transport
amino acid ABC transporter substrate-binding protein	0.26866 6	0.5707 87	0.423168	-1.2407	Y	Amino acid transport and metabolism
bifunctional 2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase	0.28105 9	0.5512 03	0.632711	-0.66038	Y	Transcription/translation
BMP family ABC transporter substrate-binding protein	0.89079 8	0.0502 21	1.128742	0.174716	Y	Cell wall/membrane/envelope biogenesis
BMP family ABC transporter substrate-binding protein	0.67477 4	0.1708 42	0.627188	-0.67303	Y	Cell wall/membrane/envelope biogenesis
BMP family protein	0.63965 7	0.1940 53	0.672421	-0.57256	Y	Cell wall/membrane/envelope biogenesis
C4-dicarboxylate TRAP transporter substrate-binding protein	0.50591 6	0.2959 22	0.617275	-0.69602	Y	General function prediction only
CAP domain-containing protein	0.40507 4	0.3924 65	0.05538	-4.17449	Y	Intracellular trafficking, secretion, and vesicular transport
carbohydrate ABC transporter substrate-binding protein	0.66640 2	0.1762 64	1.215298	0.28131	Y	Transmembrane transport
carbohydrate ABC transporter substrate-binding protein	0.30834 6	0.5109 61	1.907637	0.931786	Y	Transmembrane transport
D-xylose ABC transporter substrate-binding protein	0.67162 7	0.1728 72	1.409135	0.494809	Y	Transmembrane transport
DctP family TRAP transporter solute-binding subunit	0.43162	0.3648 99	0.517582	-0.95014	Y	Transmembrane transport
DUF3035 domain-containing protein	0.90715 8	0.0423 17	1.106611	0.146148	Y	Unknown function

DUF3576 domain-containing protein	0.637026	0.195843	0.789287	-0.34138	Y	Unknown function
extensin family protein	0.311369	0.506725	0.581548	-0.78203	Y	Cell wall/membrane/envelope biogenesis
extracellular solute-binding protein	0.818243	0.087118	0.816571	-0.29235	Y	Transmembrane transport
extracellular solute-binding protein	0.717661	0.144081	0.869823	-0.20121	Y	Transmembrane transport
Fe(3+) ABC transporter substrate-binding protein	0.367136	0.435173	0.441139	-1.18069	Y	Inorganic ion transport and metabolism
flagellar basal body L-ring protein FlgH	0.936605	0.028444	1.079624	0.110529	Y	Cell motility
flagellar basal body P-ring protein FlgI	0.404016	0.393601	0.348448	-1.52099	Y	Cell motility
hypothetical protein	0.547785	0.26139	0.337709	-1.56615	Y	Unknown function
hypothetical protein	0.560218	0.251643	0.501738	-0.995	Y	Unknown function
hypothetical protein	0.53257	0.273623	0.606659	-0.72104	Y	Unknown function
hypothetical protein	0.828612	0.081649	0.827662	-0.27289	Y	Unknown function
hypothetical protein	0.384423	0.415191	0.373583	-1.4205	Y	Unknown function
L,D-transpeptidase	0.958523	0.018397	0.946967	-0.07861	Y	Cell wall/membrane/envelope biogenesis
lytic murein transglycosylase	0.920469	0.035991	1.088612	0.12249	Y	Cell wall/membrane/envelope biogenesis
M48 family metalloprotease	0.73342	0.134647	0.747128	-0.42057	Y	Posttranslational modification, protein turnover, chaperones
OmpA family protein	0.440852	0.355707	0.441282	-1.18023	Y	Cell wall/membrane/envelope biogenesis
outer membrane protein assembly factor BamA	0.813045	0.089885	0.790169	-0.33977	Y	Cell wall/membrane/envelope biogenesis
outer membrane protein assembly factor BamD	0.34959	0.45644	0.158506	-2.65739	Y	Cell wall/membrane/envelope biogenesis
oxidoreductase	0.994137	0.002554	1.005946	0.008553	Y	Energy production and conversion
penicillin-binding protein activator	0.702786	0.153177	0.71053	-0.49303	Y	Cell wall/membrane/envelope biogenesis
penicillin-insensitive murein endopeptidase	0.861785	0.064601	0.868714	-0.20305	Y	Cell wall/membrane/envelope biogenesis
peptide ABC transporter substrate-binding protein	0.894615	0.048364	0.916272	-0.12615	Y	Transmembrane transport
peptidoglycan DD-metalloendopeptidase family protein	0.54578	0.262983	0.498511	-1.0043	Y	Cell wall/membrane/envelope biogenesis

polysaccharide biosynthesis/export family protein	0.79766 3	0.0981 81	0.827004	-0.27403	Y	Cell wall/membrane/env elope biogenesis
PQQ-like beta-propeller repeat protein	0.72754 4	0.1381 41	1.049835	0.070162	Y	General function prediction only
sn-glycerol-3-phosphate ABC transporter substrate-binding protein UgpB	0.98025 5	0.0086 61	0.97022	-0.04362	Y	Transmembrane transport
substrate-binding domain-containing protein	0.83666 1	0.0774 51	0.892434	-0.16418	Y	Transmembrane transport
sugar ABC transporter substrate-binding protein	0.54427 2	0.2641 84	0.658147	-0.60352	Y	Transmembrane transport
TAXI family TRAP transporter solute-binding subunit	0.90735 7	0.0422 22	0.910942	-0.13457	Y	Transmembrane transport
TAXI family TRAP transporter solute-binding subunit	0.68253 8	0.1658 73	0.690764	-0.53373	Y	Transmembrane transport
tetratricopeptide repeat protein	0.68836 3	0.1621 83	0.722482	-0.46897	Y	Posttranslational modification, protein turnover, chaperones
thiosulfate ABC transporter substrate-binding protein CysP	0.21713	0.6632 8	0.319104	-1.6479	Y	Transmembrane transport
Tol-Pal system protein TolB	0.83286 9	0.0794 23	1.166287	0.221923	Y	Intracellular trafficking, secretion, and vesicular transport
transporter substrate-binding domain-containing protein	0.77431 9	0.1110 8	0.842884	-0.24659	Y	Transmembrane transport
transporter substrate-binding domain-containing protein	0.60847 4	0.2157 58	0.74767	-0.41953	Y	Transmembrane transport
TRAP transporter substrate-binding protein	0.41180 1	0.3853 13	0.599843	-0.73734	Y	Transmembrane transport
TRAP transporter substrate-binding protein	0.94197 2	0.0259 62	0.957965	-0.06196	Y	Transmembrane transport
TRAP transporter substrate-binding protein	0.35375 9	0.4512 92	1.838305	0.878377	Y	Transmembrane transport
TRAP transporter substrate-binding protein DctP	0.39981 4	0.3981 42	0.478362	-1.06383	Y	Transmembrane transport
tripartite tricarboxylate transporter substrate binding protein	0.42901	0.3675 33	0.331115	-1.5946	Y	Energy production and conversion
twin-arginine translocation pathway signal	0.59773 8	0.2234 89	0.685147	-0.54552	Y	Intracellular trafficking, secretion, and vesicular transport
type II and III secretion system protein family protein	0.47144	0.3265 74	0.515922	-0.95478	Y	Cell wall/membrane/env elope biogenesis
vanadium-dependent haloperoxidase	0.4387	0.3578 32	0.518335	-0.94804	Y	Inorganic ion transport and metabolism
30S ribosomal protein S1	0.08577 9	1.0666 19	0.209953	-2.25186	N	Transcription/translation

30S ribosomal protein S13	0.41272 2	0.3843 42	0.067349	-3.89221	N	Transcription/ translation
50S ribosomal protein L1	0.26897 8	0.5702 84	0.357983	-1.48204	N	Transcription/ translation
50S ribosomal protein L3	0.37811 6	0.4223 75	0.267066	-1.90473	N	Transcription/ translation
50S ribosomal protein L5	0.28527 1	0.5447 42	0.129603	-2.94783	N	Transcription/ translation
50S ribosomal protein L7/L12	0.35545 9	0.4492 1	0.291345	-1.7792	N	Transcription/ translation
acetyl/propionyl/methylcrotonyl-CoA carboxylase subunit alpha	0.98883	0.0048 78	1.010101	0.0145	N	Lipid transport and metabolism
argininosuccinate synthase	0.40599 3	0.3914 82	0.547596	-0.86882	N	Amino acid transport and metabolism
calcium sensor EFh	0.64977 9	0.1872 34	0.536678	-0.89787	N	Intracellular trafficking, secretion, and vesicular transport
carbohydrate kinase	0.38658	0.4127 61	0.066673	-3.90674	N	Energy production and conversion
catalase/peroxidase HPI	0.52591 2	0.2790 87	1.344781	0.427372	N	Inorganic ion transport and metabolism
chaperonin GroEL	0.30844 4	0.5108 24	0.410285	-1.2853	N	Posttranslational modification, protein turnover, chaperones
cysteine synthase A	0.35132 3	0.4542 94	0.096922	-3.36703	N	Amino acid transport and metabolism
dihydrolipoyl dehydrogenase	0.26053 6	0.5841 32	0.234454	-2.09262	N	Energy production and conversion
DNA primase	0.82315 1	0.0845 2	1.168924	0.225182	N	Transcription/ translation
DNA-directed RNA polymerase subunit beta'	0.40437 8	0.3932 12	0.55998	-0.83655	N	Transcription/ translation
Do family serine endopeptidase	0.21451 6	0.6685 41	0.462341	-1.11297	N	Posttranslational modification, protein turnover, chaperones
electron transfer flavoprotein subunit alpha	0.21878	0.6599 92	0.250174	-1.999	N	Inorganic ion transport and metabolism
elongation factor Ts	0.32175 3	0.4924 77	0.322683	-1.63181	N	Transcription/ translation
elongation factor Tu	0.29258 6	0.5337 47	0.317958	-1.65309	N	Transcription/ translation
F0F1 ATP synthase subunit alpha	0.25782 6	0.5886 74	0.600716	-0.73524	N	Energy production and conversion
F0F1 ATP synthase subunit beta	0.19638	0.7069 03	0.217896	-2.19829	N	Energy production and conversion
flagellar basal-body rod protein FlgG	0.71331 1	0.1467 21	0.681795	-0.55259	N	Cell motility
flagellar hook-basal body complex protein	0.57382 5	0.2412 2	0.471531	-1.08457	N	Cell motility
flagellin	0.31679 4	0.4992 24	1.650082	0.722538	N	Cell motility

flagellin	0.41534 5	0.3815 91	1.615217	0.691728	N	Cell motility
flagellin	0.39235 7	0.4063 18	0.487639	-1.03611	N	Cell motility
flagellin	0.99545 3	0.0019 79	1.005222	0.007514	N	Cell motility
fructose-6-phosphate aldolase	0.49957	0.3014 04	0.350471	-1.51263	N	Energy production and conversion
Glu/Leu/Phe/Val dehydrogenase	0.16270 2	0.7886 08	0.137436	-2.86316	N	Inorganic ion transport and metabolism
leucyl aminopeptidase	0.96425 2	0.0158 09	1.019655	0.028081	N	Amino acid transport and metabolism
lytic transglycosylase domain-containing protein	0.97749 1	0.0098 87	1.020794	0.029691	N	Cell wall/membrane/env elope biogenesis
M23 family metallopeptidase	0.18098 7	0.7423 54	0.472657	-1.08113	N	Cell wall/membrane/env elope biogenesis
malate dehydrogenase	0.38867 8	0.4104 1	0.220803	-2.17917	N	Energy production and conversion
30S ribosomal protein S3 [Rhodobacteraceae]	0.29358 7	0.5322 64	0.127959	-2.96625	N	Transcription/ translation
DNA-directed RNA polymerase subunit alpha [Epibacterium]	0.32014 8	0.4946 49	0.14256	-2.81036	N	Transcription/ translation
HU family DNA-binding protein [Rhodobacteraceae]	0.17965	0.7455 72	0.259294	-1.94734	N	Replication, recombination and repair
NADH-quinone oxidoreductase subunit D	0.24910 1	0.6036 24	0.407036	-1.29677	N	Energy production and conversion
NADP-dependent isocitrate dehydrogenase	0.31560 6	0.5008 55	0.250262	-1.99849	N	Energy production and conversion
outer membrane protein assembly factor BamE	0.64086 9	0.1932 31	0.624541	-0.67913	N	Cell wall/membrane/env elope biogenesis
phage major capsid protein	0.17264 8	0.7628 38	2.084911	1.059986	N	Unknown function
phasin family protein	0.44998 4	0.3468 03	0.146204	-2.77395	N	Lipid transport and metabolism
phosphopyruvate hydratase	0.30258 2	0.5191 57	0.136173	-2.87649	N	Energy production and conversion
pyruvate dehydrogenase complex E1 component subunit beta	0.39481 9	0.4036 02	0.080118	-3.64173	N	Energy production and conversion
pyruvate kinase	0.13907 5	0.8567 51	0.243995	-2.03507	N	Energy production and conversion
response regulator	0.79734	0.0983 56	1.178745	0.237252	N	Transcription/ translation
superoxide dismutase	0.33231 9	0.4784 44	0.453372	-1.14123	N	Inorganic ion transport and metabolism
thioredoxin	0.46228 6	0.3350 89	0.410574	-1.28428	N	Posttranslational modification, protein turnover, chaperones
translation initiation factor IF-2	0.24159 2	0.6169 18	0.079526	-3.65243	N	Transcription/ translation

trigger factor	0.12181 2	0.9143 11	0.293822	-1.76699	N	Posttranslational modification, protein turnover, chaperones
type I glutamate--ammonia ligase	0.31683 9	0.4991 61	0.477253	-1.06717	N	Amino acid transport and metabolism
type I glyceraldehyde-3- phosphate dehydrogenase	0.42759 1	0.3689 71	0.301965	-1.72755	N	Energy production and conversion
tyrosine-type recombinase/integrase	0.27105 2	0.5669 48	0.440131	-1.184	N	Transcription/ translation
xylose isomerase	0.33556 2	0.4742 28	0.139109	-2.84571	N	Energy production and conversion

Appendix D Fold-change analysis summary using MarkerView when comparing protein abundance ratio for conditions EHMC and DMSO (EHMC:DMSO) within Chapter 3.

Protein	p-value	-log ₁₀ p-value	Fold Change (EHMC:DMSO)	Log ₂ Fold Change (EHMC:DMSO)	SignalP	Function
transporter substrate-binding domain-containing protein .	0.000647	3.18897	9.204297	3.202308	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.000817	3.087753	10.76621	3.428438	Y	Transmembrane transport
Fe(3+) ABC transporter substrate-binding protein	0.001092	2.961613	5.538053	2.469379	Y	Inorganic ion transport and metabolism
ABC transporter substrate-binding protein	0.002193	2.658972	13.76349	3.782775	Y	Transmembrane transport
C4-dicarboxylate TRAP transporter substrate-binding protein	0.002405	2.618894	4.295541	2.10284	Y	General function prediction only
ABC transporter substrate-binding protein	0.002702	2.568341	140.0373	7.129668	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.004341	2.362381	15.82907	3.984505	Y	Transmembrane transport
sugar ABC transporter substrate-binding protein	0.004753	2.323026	3.756365	1.909337	Y	Transmembrane transport
TAXI family TRAP transporter solute-binding subunit	0.005275	2.27778	15.18946	3.924999	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.005665	2.24677	6.047903	2.596435	Y	Transmembrane transport
carbohydrate ABC transporter substrate-binding protein	0.005864	2.231797	3.44123	1.782924	Y	Transmembrane transport
D-xylose ABC transporter substrate-binding protein	0.006124	2.21293	10.91509	3.448252	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.00617	2.209745	28.76427	4.846206	Y	Transmembrane transport
TAXI family TRAP transporter solute-binding subunit	0.006315	2.199617	12.69639	3.666346	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.006955	2.157727	57.76105	5.852025	Y	Transmembrane transport
amino acid ABC transporter substrate-binding protein	0.00834	2.078832	33.51772	5.066852	Y	Amino acid transport and metabolism
PQQ-like beta-propeller repeat protein	0.010155	1.993335	0.436147	-1.19711	Y	General function prediction only
TRAP transporter substrate-binding protein	0.012051	1.91898	25.38534	4.665924	Y	Transmembrane transport
sn-glycerol-3-phosphate ABC transporter substrate-binding protein UgpB	0.012571	1.900627	10.65107	3.412927	Y	Transmembrane transport
extracellular solute-binding protein	0.01304	1.884734	48.29108	5.593685	Y	Transmembrane transport
carbohydrate ABC transporter substrate-binding protein	0.015894	1.798757	6.04666	2.596138	Y	Transmembrane transport
TRAP transporter substrate-binding protein	0.021739	1.662764	20.18194	4.334993	Y	Transmembrane transport
BMP family ABC transporter substrate-binding protein	0.026242	1.581006	7.235807	2.855154	Y	Cell wall/membrane/envelope biogenesis

thiosulfate ABC transporter substrate-binding protein CysP	0.029 283	1.533 383	16.50036	4.044425	Y	Transmembrane transport
TRAP transporter substrate-binding protein DctP	0.029 876	1.524 672	9.623567	3.266572	Y	Transmembrane transport
TRAP transporter substrate-binding protein	0.032 811	1.483 98	2.464522	1.301308	Y	Transmembrane transport
BMP family ABC transporter substrate-binding protein	0.079 244	1.101 033	8.060826	3.010928	Y	Cell wall/membrane/envelope biogenesis
BMP family protein	0.079 732	1.098 367	5.866857	2.552588	Y	Cell wall/membrane/envelope biogenesis
lytic murein transglycosylase	0.082 548	1.083 295	3.888608	1.959254	Y	Cell wall/membrane/envelope biogenesis
polysaccharide biosynthesis/export family protein	0.090 584	1.042 947	2.3301	1.220392	Y	Cell wall/membrane/envelope biogenesis
peptide ABC transporter substrate-binding protein	0.093 626	1.028 606	40.2251	5.330024	Y	Transmembrane transport
Tol-Pal system protein TolB	0.102 027	0.991 285	2.311102	1.208581	Y	Intracellular trafficking, secretion, and vesicular transport
OmpA family protein	0.104 276	0.981 814	2.568766	1.361075	Y	Cell wall/membrane/envelope biogenesis
L,D-transpeptidase	0.119 37	0.923 104	3.178838	1.6685	Y	Cell wall/membrane/envelope biogenesis
bifunctional 2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase	0.131 563	0.880 865	8.489803	3.085731	Y	Transcription/translation
substrate-binding domain-containing protein	0.137 506	0.861 679	2.85936	1.515692	Y	Transmembrane transport
extracellular solute-binding protein	0.158 426	0.800 174	0.432005	-1.21088	Y	Transmembrane transport
oxidoreductase	0.180 645	0.743 174	19.21422	4.264102	Y	Energy production and conversion
flagellar basal body P-ring protein FlgI	0.183 132	0.737 236	2.193547	1.133266	Y	Cell motility
hypothetical protein	0.229 307	0.639 584	0.166461	-2.58675	Y	Unknown function
polyisoprenoid-binding protein	0.297 497	0.526 517	11.12899	3.47625	Y	Lipid transport and metabolism
twin-arginine translocation pathway signal	0.326 838	0.485 667	1.643106	0.716426	Y	Intracellular trafficking, secretion, and vesicular transport
DUF3576 domain-containing protein	0.373 783	0.427 381	1.478838	0.564464	Y	Unknown function
transporter substrate-binding domain-containing protein	0.390 246	0.408 662	37.55877	5.231078	Y	Transmembrane transport
CAP domain-containing protein	0.398 423	0.399 656	0.040359	-4.63098	Y	Intracellular trafficking, secretion, and vesicular transport
DctP family TRAP transporter solute-binding subunit	0.401 823	0.395 966	1.704676	0.769497	Y	Transmembrane transport

vanadium-dependent haloperoxidase	0.409 524	0.387 721	0.496072	-1.01138	Y	Inorganic ion transport and metabolism
DUF3035 domain-containing protein	0.454 944	0.342 042	1.577552	0.657687	Y	Unknown function
hypothetical protein	0.479 36	0.319 338	0.444812	-1.16873	Y	Unknown function
hypothetical protein	0.498 416	0.302 408	0.26295	-1.92714	Y	Unknown function
M48 family metalloprotease	0.498 515	0.302 322	0.524857	-0.93	Y	Posttranslational modification, protein turnover, chaperones
peptidoglycan DD-metalloendopeptidase family protein	0.523 568	0.281 027	0.477787	-1.06556	Y	Cell wall/membrane/envelope biogenesis
tetratricopeptide repeat protein	0.552 166	0.257 93	0.622825	-0.6831	Y	Posttranslational modification, protein turnover, chaperones
type II and III secretion system protein family protein	0.565 758	0.247 369	1.380162	0.464837	Y	Cell wall/membrane/envelope biogenesis
penicillin-insensitive murein endopeptidase	0.597 796	0.223 447	0.645149	-0.63229	Y	Cell wall/membrane/envelope biogenesis
outer membrane protein assembly factor BamA	0.631 588	0.199 566	0.606078	-0.72242	Y	Cell wall/membrane/envelope biogenesis
ABC transporter substrate-binding protein	0.647 153	0.188 993	1.268503	0.343126	Y	Transmembrane transport
tripartite tricarboxylate transporter substrate binding protein	0.651 897	0.185 821	0.627199	-0.673	Y	Energy production and conversion
penicillin-binding protein activator	0.663 625	0.178 077	0.682954	-0.55014	Y	Cell wall/membrane/envelope biogenesis
hypothetical protein	0.697 511	0.156 449	0.733258	-0.44761	Y	Unknown function
flagellar basal body L-ring protein FlgH	0.753 833	0.122 725	1.240558	0.310989	Y	Cell motility
outer membrane protein assembly factor BamD	0.900 118	0.045 7	1.107721	0.147595	Y	Cell wall/membrane/envelope biogenesis
extensin family protein	0.923 105	0.034 749	1.040465	0.057229	Y	Cell wall/membrane/envelope biogenesis
hypothetical protein	0.972 38	0.012 164	1.024728	0.035241	Y	Unknown function
translation initiation factor IF-2	0.001 95	2.709 945	6.63681	2.73049	N	Transcription/translation
tyrosine-type recombinase/integrase	0.009 153	2.038 43	68.99779	6.108478	N	Transcription/translation
F0F1 ATP synthase subunit alpha	0.053 579	1.271 008	0.145011	-2.78576	N	Energy production and conversion
trigger factor	0.073 919	1.131 245	0.101262	-3.30383	N	Posttranslational modification, protein turnover, chaperones

30S ribosomal protein S1	0.079 133	1.101 64	0.184513	-2.43821	N	Transcription/ translation
response regulator	0.099 28	1.003 136	17.16012	4.100988	N	Transcription/ translation
flagellin	0.117 592	0.929 624	5.804634	2.537205	N	Cell motility
flagellar basal-body rod protein FlgG	0.125 957	0.899 777	2.498383	1.320995	N	Cell motility
DNA primase	0.133 595	0.874 209	9.411771	3.234466	N	Transcription/ translation
phage major capsid protein	0.139 711	0.854 769	14.77131	3.884726	N	Unknown function
pyruvate kinase	0.145 133	0.838 234	0.251812	-1.98958	N	Energy production and conversion
Glu/Leu/Phe/Val dehydrogenase	0.155 251	0.808 966	0.115071	-3.11941	N	Inorganic ion transport and metabolism
HU family DNA-binding protein [Rhodobacteraceae]	0.159 989	0.795 911	0.20959	-2.25436	N	Replication, recombination and repair
F0F1 ATP synthase subunit beta	0.170 038	0.769 454	0.150499	-2.73218	N	Energy production and conversion
Do family serine endopeptidase	0.172 026	0.764 407	0.390521	-1.35653	N	Posttranslational modification, protein turnover, chaperones
flagellin	0.187 388	0.727 257	6.354235	2.667718	N	Cell motility
acetyl/propionyl/methylcrotony l-CoA carboxylase subunit alpha	0.197 306	0.704 86	0.024107	-5.37442	N	Lipid transport and metabolism
dihydrolipoyl dehydrogenase	0.203 561	0.691 305	0.111342	-3.16693	N	Energy production and conversion
superoxide dismutase	0.207 452	0.683 081	2.30861	1.207024	N	Inorganic ion transport and metabolism
type I glutamate--ammonia ligase	0.217 485	0.662 572	0.366006	-1.45006	N	Amino acid transport and metabolism
flagellin	0.227 16	0.643 669	7.439838	2.895271	N	Cell motility
50S ribosomal protein L5	0.252 547	0.597 657	0.052694	-4.24622	N	Transcription/ translation
electron transfer flavoprotein subunit alpha	0.264 555	0.577 485	0.337266	-1.56804	N	Inorganic ion transport and metabolism
50S ribosomal protein L7/L12	0.271 804	0.565 745	0.130602	-2.93676	N	Transcription/ translation
NADH-quinone oxidoreductase subunit D	0.277 721	0.556 392	0.452887	-1.14278	N	Energy production and conversion
30S ribosomal protein S3 [Rhodobacteraceae]	0.288 825	0.539 365	0.11853	-3.07667	N	Transcription/ translation
NADP-dependent isocitrate dehydrogenase	0.295 032	0.530 13	0.21195	-2.2382	N	Energy production and conversion
DNA-directed RNA polymerase subunit alpha [Epibacterium]	0.318 583	0.496 778	0.139196	-2.84481	N	Transcription/ translation
50S ribosomal protein L3	0.335 486	0.474 326	0.188187	-2.40976	N	Transcription/ translation

DNA-directed RNA polymerase subunit beta'	0.335	0.473	0.475089	-1.07373	N	Transcription/translation
elongation factor Ts	915	77				
	0.341	0.467	0.346704	-1.52822	N	Transcription/translation
	1	118				
calcium sensor EFh	0.378	0.422	2.171186	1.118484	N	Intracellular trafficking, secretion, and vesicular transport
	436	007				
fructose-6-phosphate aldolase	0.380	0.420	0.132171	-2.91952	N	Energy production and conversion
	136	061				
phasin family protein	0.387	0.411	0.006518	-7.26145	N	Lipid transport and metabolism
	287	967				
elongation factor Tu	0.390	0.407	0.463009	-1.11089	N	Transcription/translation
	923	909				
cysteine synthase A	0.434	0.361	0.262322	-1.93059	N	Amino acid transport and metabolism
	777	733				
30S ribosomal protein S13	0.445	0.351	0.138688	-2.85009	N	Transcription/translation
	423	228				
carbohydrate kinase	0.453	0.343	0.206396	-2.27651	N	Energy production and conversion
	932	01				
chaperonin GroEL	0.471	0.326	0.590411	-0.76021	N	Posttranslational modification, protein turnover, chaperones
	928	124				
phosphopyruvate hydratase	0.473	0.324	0.427683	-1.22539	N	Energy production and conversion
	924	292				
catalase/peroxidase HPI	0.499	0.301	1.380678	0.465377	N	Inorganic ion transport and metabolism
	712	28				
leucyl aminopeptidase	0.505	0.296	0.662988	-0.59295	N	Amino acid transport and metabolism
	6	193				
malate dehydrogenase	0.507	0.294	0.419462	-1.25339	N	Energy production and conversion
	987	148				
flagellin	0.523	0.281	1.900552	0.926418	N	Cell motility
	335	22				
M23 family metallopeptidase	0.532	0.273	2.027203	1.01949	N	Cell wall/membrane/envelope biogenesis
	969	298				
50S ribosomal protein L1	0.545	0.263	0.680012	-0.55637	N	Transcription/translation
	396	288				
pyruvate dehydrogenase complex E1 component subunit beta	0.597	0.223	1.598152	0.676404	N	Energy production and conversion
	534	637				
flagellar hook-basal body complex protein	0.609	0.215	0.538792	-0.8922	N	Cell motility
	484	038				
argininosuccinate synthase	0.644	0.190	0.735102	-0.44398	N	Amino acid transport and metabolism
	744	613				
thioredoxin	0.654	0.184	1.350626	0.433628	N	Posttranslational modification, protein turnover, chaperones
	168	311				
type I glyceraldehyde-3-phosphate dehydrogenase	0.719	0.142	1.320169	0.400722	N	Energy production and conversion
	902	727				
outer membrane protein assembly factor BamE	0.733	0.134	0.737238	-0.4398	N	Cell wall/membrane/envelope biogenesis
	918	353				

xylose isomerase	0.780 5	0.107 627	1.247305	0.318814	N	Energy production and conversion
lytic transglycosylase domain- containing protein	0.985 849	0.006 189	0.987533	-0.0181	N	Cell wall/membrane/en velope biogenesis

Appendix E Fold-change analysis summary using MarkerView when comparing protein abundance ratio for conditions EHMC and BP3 (EHMC:BP3) within Chapter 3.

Protein	p-value	-log10 p-value	Fold Change (EHMC:BP3)	Log2 Fold Change (EHMC:BP3)	Signal	Function
C4-dicarboxylate TRAP transporter substrate-binding protein	3.52E-05	4.453244	6.958881	2.798855	Y	General function prediction only
transporter substrate-binding domain-containing protein	0.000502	3.298981	10.92001	3.448902	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.000808	3.092824	11.01393	3.461257	Y	Transmembrane transport
PQQ-like beta-propeller repeat protein	0.001132	2.946256	0.415443	-1.26728	Y	General function prediction only
sugar ABC transporter substrate-binding protein	0.001434	2.843434	5.707483	2.512855	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.001955	2.708872	11.87855	3.570287	Y	Transmembrane transport
Fe(3+) ABC transporter substrate-binding protein	0.002575	2.58914	12.55398	3.650073	Y	Inorganic ion transport and metabolism
ABC transporter substrate-binding protein	0.00274	2.562326	72.63882	6.182669	Y	Transmembrane transport
D-xylose ABC transporter substrate-binding protein	0.004531	2.343784	7.745954	2.953443	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.004753	2.32304	30.19289	4.916137	Y	Transmembrane transport
TAXI family TRAP transporter solute-binding subunit	0.00518	2.285669	16.67445	4.059567	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.006102	2.214531	100.0763	6.644956	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.006219	2.206314	17.76614	4.151059	Y	Transmembrane transport
ABC transporter substrate-binding protein	0.006427	2.191995	29.48185	4.881755	Y	Transmembrane transport
TAXI family TRAP transporter solute-binding subunit	0.007763	2.109971	18.38021	4.200081	Y	Transmembrane transport
amino acid ABC transporter substrate-binding protein	0.008142	2.089292	79.20657	6.307548	Y	Amino acid transport and metabolism
TRAP transporter substrate-binding protein	0.011432	1.941875	26.49924	4.727879	Y	Transmembrane transport
flagellar basal body P-ring protein FlgI	0.011721	1.931041	6.295197	2.654252	Y	Cell motility
extracellular solute-binding protein	0.012988	1.886466	59.13888	5.886035	Y	Transmembrane transport
sn-glycerol-3-phosphate ABC transporter substrate-binding protein UgpB	0.013058	1.884131	10.97799	3.456542	Y	Transmembrane transport
carbohydrate ABC transporter substrate-binding protein	0.019038	1.720383	4.975454	2.314828	Y	Transmembrane transport
hypothetical protein	0.019237	1.715867	0.274389	-1.86571	Y	Unknown function
TRAP transporter substrate-binding protein	0.020799	1.681957	33.64538	5.072336	Y	Transmembrane transport

polysaccharide biosynthesis/export family protein	0.023364	1.631444	2.81752	1.494426	Y	Cell wall/membrane/envelope biogenesis
OmpA family protein	0.024484	1.61118	5.821137	2.541301	Y	Cell wall/membrane/envelope biogenesis
thiosulfate ABC transporter substrate-binding protein CysP	0.026801	1.571848	51.70837	5.692326	Y	Transmembrane transport
BMP family ABC transporter substrate-binding protein	0.026827	1.571434	11.53691	3.528185	Y	Cell wall/membrane/envelope biogenesis
TRAP transporter substrate-binding protein DctP	0.027024	1.568245	20.11775	4.330397	Y	Transmembrane transport
outer membrane protein assembly factor BamD	0.030399	1.517135	6.988519	2.804987	Y	Cell wall/membrane/envelope biogenesis
type II and III secretion system protein family protein	0.036337	1.439646	2.675139	1.419614	Y	Cell wall/membrane/envelope biogenesis
extracellular solute-binding protein	0.048407	1.315088	0.496658	-1.00967	Y	Transmembrane transport
BMP family protein	0.067839	1.168522	8.724971	3.12515	Y	Cell wall/membrane/envelope biogenesis
twin-arginine translocation pathway signal	0.078434	1.105495	2.398181	1.261941	Y	Intracellular trafficking, secretion, and vesicular transport
BMP family ABC transporter substrate-binding protein	0.080697	1.09314	7.141421	2.836211	Y	Cell wall/membrane/envelope biogenesis
L,D-transpeptidase	0.086329	1.063842	3.356861	1.747113	Y	Cell wall/membrane/envelope biogenesis
lytic murein transglycosylase	0.087396	1.058511	3.572078	1.836763	Y	Cell wall/membrane/envelope biogenesis
peptide ABC transporter substrate-binding protein	0.093211	1.030533	43.90084	5.456177	Y	Transmembrane transport
Tol-Pal system protein TolB	0.10039	0.99831	1.98159	0.986658	Y	Intracellular trafficking, secretion, and vesicular transport
hypothetical protein	0.112069	0.950513	1.962768	0.97289	Y	Unknown function
carbohydrate ABC transporter substrate-binding protein	0.115963	0.935679	1.803923	0.851138	Y	Transmembrane transport
bifunctional 2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase	0.119785	0.921598	13.41814	3.746113	Y	Transcription/translation
substrate-binding domain-containing protein	0.123312	0.908993	3.204001	1.679875	Y	Transmembrane transport
DctP family TRAP transporter solute-binding subunit	0.129363	0.888189	3.293539	1.719638	Y	Transmembrane transport
oxidoreductase	0.180742	0.742941	19.10064	4.255549	Y	Energy production and conversion
ABC transporter substrate-binding protein	0.183992	0.735202	1.88869	0.917386	Y	Transmembrane transport

DUF3576 domain-containing protein	0.2387 94	0.6219 77	1.873638	0.905842	Y	Unknown function
tripartite tricarboxylate transporter substrate binding protein	0.2599 27	0.5851 48	1.894203	0.921591	Y	Energy production and conversion
polyisoprenoid-binding protein	0.2840 96	0.5465 35	17.09748	4.095712	Y	Lipid transport and metabolism
extensin family protein	0.2979 53	0.5258 52	1.78913	0.839258	Y	Cell wall/membrane/env elope biogenesis
transporter substrate-binding domain-containing protein	0.3873 96	0.4118 45	50.23441	5.650604	Y	Transmembrane transport
TRAP transporter substrate-binding protein	0.4947 07	0.3056 52	1.340649	0.422931	Y	Transmembrane transport
DUF3035 domain-containing protein	0.5294 46	0.2761 78	1.42557	0.511539	Y	Unknown function
M48 family metalloprotease	0.5702 55	0.2439 31	0.7025	-0.50943	Y	Posttranslational modification, protein turnover, chaperones
penicillin-insensitive murein endopeptidase	0.6504 59	0.1867 8	0.742649	-0.42925	Y	Cell wall/membrane/env elope biogenesis
outer membrane protein assembly factor BamA	0.6793 16	0.1679 28	0.767024	-0.38266	Y	Cell wall/membrane/env elope biogenesis
hypothetical protein	0.7070 91	0.1505 25	1.238099	0.308127	Y	Unknown function
CAP domain-containing protein	0.7432 47	0.1288 67	0.728759	-0.45649	Y	Intracellular trafficking, secretion, and vesicular transport
tetratricopeptide repeat protein	0.7939 32	0.1002 17	0.862063	-0.21413	Y	Posttranslational modification, protein turnover, chaperones
hypothetical protein	0.8114 43	0.0907 42	0.778631	-0.36099	Y	Unknown function
flagellar basal body L-ring protein FlgH	0.8368 14	0.0773 71	1.149065	0.20046	Y	Cell motility
hypothetical protein	0.8961 48	0.0476 2	0.886543	-0.17374	Y	Unknown function
vanadium-dependent haloperoxidase	0.9308 36	0.0311 27	0.95705	-0.06333	Y	Inorganic ion transport and metabolism
penicillin-binding protein activator	0.9352 4	0.0290 77	0.96119	-0.05711	Y	Cell wall/membrane/env elope biogenesis
peptidoglycan DD-metalloendopeptidase family protein	0.9413 36	0.0262 55	0.958428	-0.06126	Y	Cell wall/membrane/env elope biogenesis
thioredoxin	0.0031 43	2.5026 73	3.289601	1.717913	N	Posttranslational modification, protein turnover, chaperones
translation initiation factor IF-2	0.0040 46	2.3929 41	83.45442	6.382917	N	Transcription/translation
tyrosine-type recombinase/integrase	0.0089 95	2.0459 94	156.7665	7.292473	N	Transcription/translation

flagellar basal-body rod protein FlgG	0.014065	1.851858	3.664422	1.873586	N	Cell motility
F0F1 ATP synthase subunit alpha	0.034509	1.462062	0.241397	-2.05052	N	Energy production and conversion
cysteine synthase A	0.035065	1.455127	2.706526	1.436442	N	Amino acid transport and metabolism
xylose isomerase	0.052589	1.279103	8.966365	3.164523	N	Energy production and conversion
type I glyceraldehyde-3-phosphate dehydrogenase	0.054819	1.261068	4.371924	2.128268	N	Energy production and conversion
pyruvate dehydrogenase complex E1 component subunit beta	0.056867	1.245142	19.94754	4.318139	N	Energy production and conversion
acetyl/propionyl/methylcrotonyl-CoA carboxylase subunit alpha	0.065554	1.183398	0.023866	-5.38892	N	Lipid transport and metabolism
malate dehydrogenase	0.07421	1.129536	1.899712	0.925781	N	Energy production and conversion
superoxide dismutase	0.093314	1.030052	5.092085	2.348256	N	Inorganic ion transport and metabolism
phosphopyruvate hydratase	0.098067	1.008477	3.140733	1.651101	N	Energy production and conversion
response regulator	0.101349	0.99418	14.55795	3.863736	N	Transcription/translation
phasin family protein	0.1031	0.986742	0.044579	-4.4875	N	Lipid transport and metabolism
30S ribosomal protein S13	0.118894	0.924841	2.059254	1.042122	N	Transcription/translation
fructose-6-phosphate aldolase	0.123761	0.907417	0.377123	-1.40689	N	Energy production and conversion
DNA primase	0.139824	0.854418	8.051651	3.009285	N	Transcription/translation
calcium sensor EFh	0.140954	0.850922	4.045607	2.016356	N	Intracellular trafficking, secretion, and vesicular transport
flagellin	0.158234	0.8007	3.517786	1.814668	N	Cell motility
phage major capsid protein	0.163066	0.787635	7.084865	2.82474	N	Unknown function
carbohydrate kinase	0.175129	0.756643	3.095628	1.630232	N	Energy production and conversion
50S ribosomal protein L1	0.185401	0.731889	1.899561	0.925666	N	Transcription/translation
flagellin	0.187689	0.726562	6.321224	2.660204	N	Cell motility
trigger factor	0.221919	0.653806	0.344638	-1.53684	N	Posttranslational modification, protein turnover, chaperones
elongation factor Tu	0.229856	0.638545	1.456194	0.542202	N	Transcription/translation
50S ribosomal protein L7/L12	0.230301	0.637705	0.448271	-1.15756	N	Transcription/translation
flagellin	0.264156	0.578139	4.606092	2.203543	N	Cell motility
50S ribosomal protein L5	0.294732	0.530573	0.406581	-1.29838	N	Transcription/translation

F0F1 ATP synthase subunit beta	0.316859	0.499134	0.690692	-0.53389	N	Energy production and conversion
leucyl aminopeptidase	0.318904	0.49634	0.650208	-0.62103	N	Amino acid transport and metabolism
flagellin	0.320819	0.49374	3.897458	1.962533	N	Cell motility
M23 family metallopeptidase	0.362314	0.440914	4.288949	2.100624	N	Cell wall/membrane/envelope biogenesis
50S ribosomal protein L3	0.370535	0.431171	0.704647	-0.50503	N	Transcription/translation
chaperonin GroEL	0.418065	0.378756	1.439027	0.525093	N	Posttranslational modification, protein turnover, chaperones
electron transfer flavoprotein subunit alpha	0.4414	0.355167	1.348126	0.430956	N	Inorganic ion transport and metabolism
dihydrolipoyl dehydrogenase	0.529143	0.276427	0.4749	-1.0743	N	Energy production and conversion
argininosuccinate synthase	0.579093	0.237251	1.342416	0.424832	N	Amino acid transport and metabolism
HU family DNA-binding protein [Rhodobacteraceae]	0.611172	0.213837	0.808308	-0.30702	N	Replication, recombination and repair
DNA-directed RNA polymerase subunit beta'	0.6212	0.206769	0.848402	-0.23718	N	Transcription/translation
30S ribosomal protein S1	0.657503	0.182102	0.87883	-0.18634	N	Transcription/translation
type I glutamate--ammonia ligase	0.667774	0.17537	0.766901	-0.38289	N	Amino acid transport and metabolism
Do family serine endopeptidase	0.668731	0.174749	0.844659	-0.24356	N	Posttranslational modification, protein turnover, chaperones
Glu/Leu/Phe/Val dehydrogenase	0.675311	0.170496	0.837265	-0.25624	N	Inorganic ion transport and metabolism
NADH-quinone oxidoreductase subunit D	0.753551	0.122887	1.112648	0.153997	N	Energy production and conversion
outer membrane protein assembly factor BamE	0.792467	0.101019	1.180448	0.239334	N	Cell wall/membrane/envelope biogenesis
NADP-dependent isocitrate dehydrogenase	0.793953	0.100205	0.846912	-0.23972	N	Energy production and conversion
flagellar hook-basal body complex protein	0.847711	0.071752	1.142644	0.192376	N	Cell motility
elongation factor Ts	0.896121	0.047633	1.074439	0.103584	N	Transcription/translation
30S ribosomal protein S3 [Rhodobacteraceae]	0.921338	0.035581	0.926314	-0.11043	N	Transcription/translation
lytic transglycosylase domain-containing protein	0.950431	0.022079	0.967417	-0.04779	N	Cell wall/membrane/envelope biogenesis
DNA-directed RNA polymerase subunit alpha [Epibacterium]	0.957726	0.018759	0.976401	-0.03445	N	Transcription/translation

catalase/oxidase HPI	0.9579 13	0.0186 74	1.026693	0.038005	N	Inorganic ion transport and metabolism
pyruvate kinase	0.9703 61	0.0130 67	1.032037	0.045495	N	Energy production and conversion

Appendix F Proteins identified within the complete characterised proteome of *Epibacterium mobile* [*Tritonibacter mobilis*] with associated signal peptides (export from SignalP- 6.0).

Definitions: SP, "standard" secretory signal peptides transported by the Sec translocon and cleaved by Signal Peptidase I (*Lep*); LIPO, lipoprotein signal peptides transported by the Sec translocon and cleaved by Signal Peptidase II (*Lsp*); TAT, Tat signal peptides transported by the Tat translocon and cleaved by Signal Peptidase I (*Lep*); TATLIPO, Tat lipoprotein signal peptides transported by the Tat translocon and cleaved by Signal Peptidase II (*Lsp*); PILIN, Pilin and pilin-like signal peptides transported by the Sec translocon and cleaved by Signal Peptidase III (*PilD/PibD*).
(Definitions retrieved from: <https://services.healthtech.dtu.dk/services/SignalP-6.0/>)

Protein	Predict ion	OTHE R	SP(Se c/SPI)	LIPO(Sec /SPII)	TAT(T at/SPI)	TATLIPO O(Tat/SP II)	PILIN(Se c/SPIII)
L,D-TPase catalytic domain- containing protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
2_3_-cyclic-nucleotide 2_- phosphodiesterase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Outer membrane protein assembly factor BamD OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Transporter OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Peptidoglycan-associated lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Flagellar L-ring protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Chitooligosaccharide deacetylase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
L,D-TPase catalytic domain- containing protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Multicopper oxidase CueO OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
L,D-TPase catalytic domain- containing protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
DUF3576 domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
DUF3035 domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lytic transglycosylase OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Copper oxidase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Twin-arginine translocation pathway signal protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00

17 kDa surface antigen OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
MBL fold metallo-hydrolase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Twin-arginine translocation pathway signal OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
D-alanyl-D-alanine carboxypeptidase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Transcriptional initiation protein Tat OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Sugar ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Excalibur calcium-binding domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Twin-arginine translocation pathway signal protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Peptide ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
DUF4157 domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Branched-chain amino acid ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
MBL fold metallo-hydrolase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Tat pathway signal protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00

Twin-arginine translocation pathway signal OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
OmpA-like domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Tetratricopeptide repeat protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
GDSL family lipase OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Glycoside hydrolase OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Transporter OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Copper resistance protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Esterase_lipase superfamily enzyme OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
SCP domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Peptide ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
C4-dicarboxylate ABC transporter OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Sporulation domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
ABC-type transport auxiliary lipoprotein component domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Peptide ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
BMP family ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00

Copper oxidase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Copper oxidase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Metal-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Polysaccharide biosynthesis protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Phosphonate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
YjbF family lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
RmmL_Fragment_OS_Tritonibacter mobilis	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Glucan biosynthesis protein G OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Peptidase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	TATLI	0.00	0.00	0.01	0.00	0.99	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	PO						
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Peptidase M48, Ste24p OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
CHAP domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Thiol_disulfide interchange protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Protein-methionine-sulfoxide reductase catalytic subunit MsrP OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	0.98	0.02	0.00
Penicillin-insensitive murein endopeptidase OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Peptidase M48 OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Entericidin OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Manganese transporter OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Quinoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Formate dehydrogenase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	0.97	0.03	0.00
L,D-TPase catalytic domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	0.77	0.00	0.23	0.00

ABC transporter OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Twin-arginine translocation pathway signal OS_Tritonibacter mobilis F1926	TATLI PO	0.00	0.00	0.02	0.01	0.98	0.00
Lytic transglycosylase OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	0.73	0.00	0.27	0.00
Peptidase M23 OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
L,D-TPase catalytic domain- containing protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	0.95	0.05	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
Copper-containing nitrite reductase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	0.65	0.00	0.35	0.00
Diguanylate cyclase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	0.96	0.04	0.00
Cell envelope protein SmpA OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	1.00	0.00	0.00	0.00
L,D-TPase catalytic domain- containing protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.04	0.96	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.03	0.97	0.00	0.00	0.00
FAD-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	1.00	0.00	0.00
VacJ lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.07	0.93	0.00	0.00	0.00
OmpA-like domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.01	0.98	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.01	0.99	0.00	0.00	0.00
Peptidoglycan-binding protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.00	0.55	0.01	0.44	0.00
Transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Imidazoleglycerol-phosphate dehydratase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Putrescine-binding periplasmic protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cytochrome C OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Hydratase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cytochrome c domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Autotransporter domain- containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Thioredoxin domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Formate dehydrogenase subunit gamma OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Metal ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Type-4 uracil-DNA glycosylase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
sn-glycerol-3-phosphate-binding periplasmic protein UgpB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Camphor resistance protein CrcB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Fe ₃ ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Tol-Pal system protein TolB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Pseudoazurin OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
histidine kinase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
PRC-barrel domain protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cytochrome c1 OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Putrescine-binding periplasmic protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

Peptide methionine sulfoxide reductase MsrA OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Fe_B12 periplasmic-binding domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Thioredoxin domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Bordetella uptake protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Serine protease OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Sugar ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Spermidine putrescine ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Metal-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	0.99	0.00	0.00
Peptide ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
D-xylose ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Periplasmic mercury ion-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Pentapeptide MXKDX repeat protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptide ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cytochrome c-type biogenesis protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Endonuclease OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidyl-prolyl cis-trans isomerase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Mechanosensitive ion channel protein MscS OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Flagellar P-ring protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

Cell envelope biogenesis protein OmpA OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Tat pathway signal sequence domain protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF2155 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Polyphosphate kinase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
EF-hand domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Metal-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
YHS domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Lysozyme inhibitor OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF3108 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
sn-glycerol-3-phosphate-binding periplasmic protein UgpB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Putrescine-binding periplasmic protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Amino acid ABC transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Phosphodiester glycosidase domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF3833 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Sulfate transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Calcium-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Flagellar motor protein MotB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF4189 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

SIMPL domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptide ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidase M23 OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Tail length tape measure protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Outer membrane lipoprotein Blc OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
YARHG domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
TRAP ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF3047 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF1036 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Flagellar motor protein MotA OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cytochrome C OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Copper chaperone PCu_A_C OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Ribonuclease T OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Invasion protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

DUF4394 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Sugar ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Arylesterase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF1223 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Flagella basal body P-ring formation protein FlgA OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Copper chaperone PCu_A_C OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter permease OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidoglycan-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Photosystem reaction center subunit H OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Flagellar motor protein MotB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Efflux transporter periplasmic adaptor subunit OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Copper-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidoglycan-binding protein LysM OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
TRAP ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidase M75 OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
LacI family transcriptional regulator OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Lipopolysaccharide transport periplasmic protein LptA OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Phosphatidylethanolamine-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Calcium-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF2282 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Urea ABC transporter permease subunit UrtB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Oxidoreductase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Thiamine pyrimidine synthase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Polyhydroxybutyrate depolymerase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Tripartite tricarboxylate transporter substrate binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Lytic murein transglycosylase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Branched-chain amino acid ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cupredoxin OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
N-acetylmuramoyl-L-alanine amidase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Sugar transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
TonB-dependent siderophore receptor OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Amino acid ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

Transcriptional initiation protein Tat OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	0.89	0.11	0.00
Cytochrome c-type biogenesis protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cytochrome-c peroxidase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
BMP family ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Urea ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Lipid polyisoprenoid-binding YceI-like domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Lytic transglycosylase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
LacI family transcriptional regulator OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF995 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Amino acid ABC transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
LacI family transcriptional regulator OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Probable sugar-binding periplasmic protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptide ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Multifunctional 2,3_-cyclic- nucleotide 2_- phosphodiesterase_5_- nucleotidase_3_-nucleotidase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Major royal jelly protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
TMAO reductase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

C4-dicarboxylate ABC transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cell division coordinator CpoB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptide ABC transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Ligand-gated channel OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cytochrome-c oxidase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Beta-lactamase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
TonB-dependent receptor OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Glutamine ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Muramidase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Integral membrane protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cadmium carbonic anhydrase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
High-affinity zinc uptake system protein ZnuA OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Thioredoxin OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Autotransporter domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
YHS domain protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
OmpA-like domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
NADH dehydrogenase subunit E OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Iron ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Alpha-glucoside ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

ABC transporter ATP-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Antifreeze glycopeptide polypeptide OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Efflux transporter periplasmic adaptor subunit OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
BNR repeat protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Ig-like domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cytochrome C OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Phosphonate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidase M23 OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Sugar ABC transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Thiosulfate transporter subunit OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Oligopeptide ABC transporter substrate-binding protein OppA OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptide ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Hemin ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Nex18 symbiotically induced protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
General secretion pathway protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidase S9 prolyl oligopeptidase catalytic domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Phosphonate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

Autoinducer 2-binding protein LsrB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF302 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Thiamine-binding periplasmic protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Acetolactate synthase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Spermidine_putrescine ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Autotransporter secretion inner membrane protein TamB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Phosphonate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Derrienterobactin-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
TonB-dependent receptor OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Integral membrane protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ASPIC_UnbV domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
BMP family ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Parvulin-like PPIase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Trypsin OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Sugar ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidoglycan peptidase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Disulfide bond formation protein DsbA OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Aromatic hydrocarbon degradation protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

Mucoidy inhibitor MuiA family protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Putative auto-transporter adhesin head GIN domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Metal-binding protein ZinT OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Transporter OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
2-isopropylmalate synthase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.04	0.96	0.00	0.00	0.00
Ligand-gated channel OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Transmembrane protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Flagellar motor protein MotB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidoglycan-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cell envelope biogenesis protein LolA OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Lytic transglycosylase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Methylamine utilization protein MauG OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidase C1 OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF4174 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Pseudoazurin OS_Tritonibacter mobilis F1926	SP	0.00	0.99	0.01	0.00	0.00	0.00
Right handed beta helix domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Sugar ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

Invasion associated locus B family protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Acetyltransferase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Molybdate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	0.98	0.02	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	0.58	0.42	0.00	0.00	0.00
TonB-dependent receptor OS_Tritonibacter mobilis F1926	SP	0.00	0.60	0.40	0.00	0.00	0.00
Energy transducer TonB OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Amidohydrolase OS_Tritonibacter mobilis F1926	SP	0.00	0.81	0.19	0.00	0.00	0.00
Major royal jelly protein OS_Tritonibacter mobilis F1926	SP	0.00	0.93	0.07	0.00	0.00	0.00
DUF2946 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Outer membrane protein beta-barrel domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Cytochrome C OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Copper-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Spermidine_putrescine ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	0.80	0.20	0.00	0.00	0.00
DUF2125 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Electron transport protein SCO1_SenC OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Murein transglycosylase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF4399 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
DUF2059 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Calcium sensor EFh OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Auto-transporter adhesin head GIN domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
C4-dicarboxylate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00

peptidoglycan lytic exotransglycosylase OS_Tritonibacter mobilis F1926	SP	0.00	0.97	0.03	0.00	0.00	0.00
Thiol_disulfide interchange protein DsbD N-terminal domain- containing protein OS_Tritonibacter mobilis F1926	SP	0.00	0.81	0.19	0.00	0.00	0.00
Glucose_Sorbose dehydrogenase domain- containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Ferrichrome-iron receptor OS_Tritonibacter mobilis F1926	SP	0.00	0.87	0.13	0.00	0.00	0.00
Cytochrome c-like protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Phosphonate ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
D-galactarate dehydratase OS_Tritonibacter mobilis F1926	LIPO	0.00	0.19	0.80	0.00	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	0.95	0.04	0.00	0.00	0.00
Peptidoglycan binding-like domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	0.56	0.43	0.00	0.00	0.00
DUF2219 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Hydrolase OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptide ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	TAT	0.00	0.38	0.00	0.61	0.00	0.00
ABC transporter substrate- binding protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Zinc protease OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Isoquinoline 1-oxidoreductase OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	0.99	0.00	0.00
Outer membrane protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
PA14 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Porin domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
AAA_ family ATPase OS_Tritonibacter mobilis F1926	SP	0.00	0.99	0.00	0.00	0.00	0.00
Penicillin-binding protein 2 OS_Tritonibacter mobilis F1926	TAT	0.00	0.00	0.00	0.71	0.28	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Peptidase S41 OS_Tritonibacter mobilis F1926	SP	0.00	1.00	0.00	0.00	0.00	0.00
Mechanosensitive ion channel protein MscS OS_Tritonibacter mobilis F1926	SP	0.00	0.98	0.01	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.00	0.99	0.00	0.00	0.00	0.00

Sulfurtransferase OS_Tritonibacter mobilis F1926	SP	0.00	0.99	0.00	0.00	0.00	0.00
DUF192 domain-containing protein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.28	0.71	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.00	0.33	0.67	0.00	0.00	0.00
Protein HflC OS_Tritonibacter mobilis F1926	SP	0.00	0.99	0.00	0.00	0.00	0.00
Phytase-like domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.01	0.97	0.02	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.01	0.22	0.78	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.01	0.22	0.77	0.00	0.00	0.00
Iron ABC transporter permease OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
Ubiquinol-cytochrome c reductase iron-sulfur subunit OS_Tritonibacter mobilis F1926	TAT	0.01	0.00	0.00	0.97	0.02	0.00
Lipid A 3-O-deacylase OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
Peptidase S8_S53 domain- containing protein OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
LPS-assembly protein LptD OS_Tritonibacter mobilis F1926	SP	0.01	0.93	0.00	0.06	0.00	0.00
Spermidine putrescine ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
Outer membrane protein assembly factor BamA OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
Extensin-like protein OS_Tritonibacter mobilis F1926	LIPO	0.01	0.21	0.78	0.00	0.00	0.00
Haem-binding uptake Tiki superfamily ChaN domain- containing protein OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	SP	0.01	0.89	0.10	0.00	0.00	0.00
Efflux transporter periplasmic adaptor subunit OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
Thiol-disulfide oxidoreductase OS_Tritonibacter mobilis F1926	SP	0.01	0.99	0.00	0.00	0.00	0.00
Twin-arginine translocation pathway signal protein OS_Tritonibacter mobilis	SP	0.01	0.99	0.00	0.00	0.00	0.00
Glutathione hydrolase proenzyme OS_Tritonibacter mobilis F1926	SP	0.01	0.98	0.00	0.00	0.00	0.00

Porin OS_Tritonibacter mobilis F1926	SP	0.02	0.97	0.00	0.00	0.00	0.00
Amino acid ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.02	0.98	0.00	0.00	0.00	0.00
Calcium sensor EFh OS_Tritonibacter mobilis F1926	SP	0.02	0.98	0.00	0.00	0.00	0.00
FAD ₂ protein FMN transferase OS_Tritonibacter mobilis F1926	TATLI PO	0.02	0.02	0.30	0.21	0.46	0.00
Entericidin EcnAB OS_Tritonibacter mobilis F1926	SP	0.02	0.52	0.45	0.00	0.00	0.00
Murein transglycosylase OS_Tritonibacter mobilis F1926	SP	0.03	0.54	0.43	0.00	0.00	0.00
Peptidoglycan-binding protein OS_Tritonibacter mobilis F1926	SP	0.03	0.97	0.00	0.00	0.00	0.00
Beta-mannosidase OS_Tritonibacter mobilis F1926	SP	0.04	0.96	0.00	0.00	0.00	0.00
Alkaline phosphatase OS_Tritonibacter mobilis F1926	SP	0.04	0.52	0.43	0.01	0.00	0.00
BaiN-like insert domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.04	0.87	0.09	0.00	0.00	0.00
Rhodanese OS_Tritonibacter mobilis F1926	TATLI PO	0.04	0.00	0.00	0.46	0.49	0.00
Alpha ₂ beta hydrolase OS_Tritonibacter mobilis F1926	SP	0.05	0.95	0.00	0.00	0.00	0.00
Parvulin-like PPIase OS_Tritonibacter mobilis F1926	SP	0.05	0.94	0.01	0.00	0.00	0.00
ABC transporter permease OS_Tritonibacter mobilis F1926	LIPO	0.05	0.01	0.94	0.00	0.00	0.00
serine-type D-Ala-D-Ala carboxypeptidase OS_Tritonibacter mobilis F1926	SP	0.05	0.94	0.00	0.00	0.00	0.00
Thiol oxidoreductase OS_Tritonibacter mobilis F1926	SP	0.05	0.94	0.00	0.00	0.00	0.00
Protein HflC OS_Tritonibacter mobilis F1926	SP	0.05	0.94	0.00	0.00	0.00	0.00
Lipoprotein OS_Tritonibacter mobilis F1926	LIPO	0.05	0.06	0.88	0.00	0.01	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.06	0.92	0.02	0.00	0.00	0.00
Tip attachment protein J domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.06	0.93	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.06	0.94	0.00	0.00	0.00	0.00
Ligand-binding protein SH3 OS_Tritonibacter mobilis F1926	SP	0.06	0.92	0.01	0.00	0.00	0.00
MAPEG family protein OS_Tritonibacter mobilis F1926	SP	0.07	0.93	0.00	0.00	0.00	0.00
Spermidine ₃ putrescine ABC transporter substrate-binding protein OS_Tritonibacter mobilis F1926	SP	0.07	0.93	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.07	0.70	0.23	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.09	0.91	0.00	0.00	0.00	0.00

Ubiquinol-cytochrome c reductase iron-sulfur subunit OS_Tritonibacter mobilis F1926	TAT	0.09	0.00	0.00	0.88	0.03	0.00
Tip attachment protein J domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.09	0.90	0.00	0.00	0.00	0.00
ATP-dependent transcriptional regulator OS_Tritonibacter mobilis F1926	LIPO	0.10	0.15	0.75	0.00	0.00	0.00
Flagellar biosynthetic protein FlhP OS_Tritonibacter mobilis F1926	SP	0.12	0.87	0.00	0.00	0.00	0.00
Protein-glutamine gamma-glutamyltransferase-like C-terminal domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.15	0.85	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.15	0.84	0.00	0.00	0.00	0.00
Ferroxidase OS_Tritonibacter mobilis F1926	SP	0.18	0.81	0.00	0.00	0.00	0.00
Protein hupE OS_Tritonibacter mobilis F1926	SP	0.19	0.80	0.00	0.00	0.00	0.00
Pilus assembly protein OS_Tritonibacter mobilis F1926	PILIN	0.19	0.00	0.00	0.00	0.00	0.81
Cytochrome c oxidase subunit 2 OS_Tritonibacter mobilis F1926	SP	0.20	0.79	0.00	0.00	0.00	0.00
Pilus assembly protein OS_Tritonibacter mobilis F1926	PILIN	0.20	0.01	0.00	0.00	0.00	0.79
Tat pathway signal sequence domain protein OS_Tritonibacter mobilis F1926	SP	0.20	0.79	0.00	0.00	0.00	0.00
Lipoprotein signal peptidase OS_Tritonibacter mobilis F1926	SP	0.22	0.77	0.00	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	LIPO	0.22	0.03	0.73	0.00	0.01	0.00
MFS transporter OS_Tritonibacter mobilis F1926	SP	0.22	0.77	0.01	0.00	0.00	0.00
PEP-utilizing protein OS_Tritonibacter mobilis F1926	SP	0.23	0.77	0.00	0.00	0.00	0.00
Biopolymer transporter ExbD OS_Tritonibacter mobilis F1926	SP	0.23	0.77	0.00	0.00	0.00	0.00
YjbH domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.24	0.71	0.04	0.00	0.00	0.00
Conjugal transfer protein TraR OS_Tritonibacter mobilis F1926	SP	0.26	0.71	0.01	0.02	0.00	0.00
Ammonium transporter OS_Tritonibacter mobilis F1926	SP	0.26	0.73	0.01	0.00	0.00	0.00
Uncharacterized protein OS_Tritonibacter mobilis F1926	SP	0.29	0.55	0.16	0.00	0.00	0.00
Chemotaxis protein OS_Tritonibacter mobilis F1926	LIPO	0.30	0.05	0.63	0.00	0.00	0.01
Electron transport protein SCO1_SenC OS_Tritonibacter mobilis F1926	TAT	0.32	0.00	0.02	0.51	0.14	0.00
Diguanylate phosphodiesterase OS_Tritonibacter mobilis F1926	SP	0.32	0.66	0.01	0.00	0.00	0.00
Pilus assembly protein OS_Tritonibacter mobilis F1926	PILIN	0.34	0.02	0.00	0.00	0.00	0.64
Aspartyl-trna synthetase OS_Tritonibacter mobilis F1926	SP	0.35	0.63	0.02	0.00	0.00	0.00

DUF2303 family protein	SP	0.35	0.64	0.01	0.00	0.00	0.00
OS_Tritonibacter mobilis F1926							
Uncharacterized protein	TAT	0.37	0.06	0.03	0.42	0.12	0.00
OS_Tritonibacter mobilis F1926							
DUF2946 domain-containing protein OS_Tritonibacter mobilis F1926	SP	0.37	0.63	0.00	0.00	0.00	0.00
Pilus assembly protein TadE	PILIN	0.37	0.01	0.00	0.00	0.00	0.61
OS_Tritonibacter mobilis F1926							
ATP synthase subunit b	SP	0.39	0.60	0.00	0.00	0.00	0.00
OS_Tritonibacter mobilis F1926							
Copper oxidase OS_Tritonibacter mobilis F1926	SP	0.41	0.59	0.00	0.00	0.00	0.00
sn-glycerol-3-phosphate transport system permease protein UgpA	SP	0.42	0.57	0.00	0.00	0.00	0.00
OS_Tritonibacter mobilis F1926							
Uncharacterized protein	SP	0.44	0.53	0.03	0.00	0.00	0.00
OS_Tritonibacter mobilis F1926							
Chalcone isomerase domain-containing protein	SP	0.44	0.56	0.00	0.00	0.00	0.00
OS_Tritonibacter mobilis F1926							
Rap1a immunity protein domain-containing protein	SP	0.44	0.56	0.00	0.00	0.00	0.00
OS_Tritonibacter mobilis F1926							
peptidoglycan glycosyltransferase	SP	0.46	0.50	0.04	0.00	0.00	0.00
OS_Tritonibacter mobilis F1926							
NirV OS_Tritonibacter mobilis F1926	SP	0.46	0.54	0.00	0.00	0.00	0.00
Lipoprotein signal peptidase	SP	0.46	0.53	0.00	0.00	0.00	0.00
OS_Tritonibacter mobilis F1926							
Uncharacterized protein	SP	0.46	0.53	0.00	0.00	0.00	0.00
OS_Tritonibacter mobilis F1926							
Threonine transporter RhtB	SP	0.47	0.53	0.00	0.00	0.00	0.00
OS_Tritonibacter mobilis F1926							
Peptide ABC transporter permease OS_Tritonibacter mobilis F1926	SP	0.48	0.52	0.00	0.00	0.00	0.00

Appendix G Proteins identified within the complete characterised proteome of *Epibacterium mobile* [*Tritonibacter mobilis*] which may be secreted through the TIISS (export from LiSC).

Id	Description	Score	is secreted
tr A0A1B0ZY46 A0A1B0ZY46_9RHOB	Nodulation protein NodH OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZYF6 A0A1B0ZYF6_9RHOB	N-formylglutamate amidohydrolase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZYH1 A0A1B0ZYH1_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926 OX=1265309	1	TRUE
tr A0A1B0ZYT6 A0A1B0ZYT6_9RHOB	Cyclopropane-fatty-acyl-phospholipid synthase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZE5 A0A1B0ZZE5_9RHOB	Rhodanese OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZF6 A0A1B0ZZF6_9RHOB	L-carnitine dehydrogenase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZZ7 A0A1B0ZZZ7_9RHOB	Imidazoleglycerol-phosphate dehydratase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A004 A0A1B1A004_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A056 A0A1B1A056_9RHOB	Protein HflK OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A061 A0A1B1A061_9RHOB	Serine aminopeptidase S33 domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A072 A0A1B1A072_9RHOB	Peptidoglycan-binding protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A095 A0A1B1A095_9RHOB	Sulfate transporter OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0B0 A0A1B1A0B0_9RHOB	Pseudouridine synthase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0M7 A0A1B1A0M7_9RHOB	Betaine-aldehyde dehydrogenase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0N3 A0A1B1A0N3_9RHOB	Aminotransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0V4 A0A1B1A0V4_9RHOB	AAA+ ATPase domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0V7 A0A1B1A0V7_9RHOB	Cobalamin biosynthesis protein CbiX OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A146 A0A1B1A146_9RHOB	Site-specific DNA-methyltransferase (adenine-specific) OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A195 A0A1B1A195_9RHOB	Calcium-binding protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1L1 A0A1B1A1L1_9RHOB	DUF502 domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1N2 A0A1B1A1N2_9RHOB	Heme A synthase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1V3 A0A1B1A1V3_9RHOB	MarR family transcriptional regulator OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A2Q0 A0A1B1A2Q0_9RHOB	Molecular chaperone DnaK OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A2W8 A0A1B1A2W8_9RHOB	Acyl-CoA thioesterase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A388 A0A1B1A388_9RHOB	PE-PGRS family protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3A6 A0A1B1A3A6_9RHOB	RNA-binding transcriptional accessory protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3F4 A0A1B1A3F4_9RHOB	Large ribosomal subunit protein bL28 OS=Tritonibacter mobilis F1926	1	TRUE

tr A0A1B1A3H4 A0A1B1A3H4_9RHOB	Glutathione-dependent disulfide-bond oxidoreductase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3T5 A0A1B1A3T5_9RHOB	OmpA-like domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3Z9 A0A1B1A3Z9_9RHOB	ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A403 A0A1B1A403_9RHOB	Type II secretion system protein E OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A4J4 A0A1B1A4J4_9RHOB	Porphobilinogen deaminase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A4R9 A0A1B1A4R9_9RHOB	Cytochrome C oxidase subunit I OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A518 A0A1B1A518_9RHOB	ATPase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A556 A0A1B1A556_9RHOB	Calcium-binding protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A567 A0A1B1A567_9RHOB	AraC family transcriptional regulator OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A592 A0A1B1A592_9RHOB	histidine kinase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5K4 A0A1B1A5K4_9RHOB	Hedgehog/Intein (Hint) domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6B6 A0A1B1A6B6_9RHOB	Flagellar biosynthesis protein FlgJ OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6Z2 A0A1B1A6Z2_9RHOB	HNH nuclease domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A710 A0A1B1A710_9RHOB	Diacylglycerol kinase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A795 A0A1B1A795_9RHOB	Amidase domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7V0 A0A1B1A7V0_9RHOB	DUF2794 domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7Y1 A0A1B1A7Y1_9RHOB	3-phosphoshikimate 1-carboxyvinyltransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8A4 A0A1B1A8A4_9RHOB	Integrase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8F9 A0A1B1A8F9_9RHOB	Sulfur carrier protein FdhD OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8G6 A0A1B1A8G6_9RHOB	Hemolysin OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A950 A0A1B1A950_9RHOB	ADP/GDP-polyphosphate phosphotransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A991 A0A1B1A991_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9P0 A0A1B1A9P0_9RHOB	GAD-like domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9V8 A0A1B1A9V8_9RHOB	Peptide ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9W5 A0A1B1A9W5_9RHOB	HTH gntR-type domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AA15 A0A1B1AA15_9RHOB	ATPase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AA35 A0A1B1AA35_9RHOB	Solute-binding protein family 5 domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AAA7 A0A1B1AAA7_9RHOB	Membrane transport protein MerF OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A027 A0A1B1A027_9RHOB	(2Fe-2S)-binding protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1B5 A0A1B1A1B5_9RHOB	histidine kinase OS=Tritonibacter mobilis F1926	1	TRUE

tr A0A1B1A3V5 A0A1B1A3V5_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8C5 A0A1B1A8C5_9RHOB	ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8E1 A0A1B1A8E1_9RHOB	Dioxygenase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AA99 A0A1B1AA99_9RHOB	Zinc ABC transporter OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZK1 A0A1B0ZZK1_9RHOB	Thiol oxidoreductase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZL8 A0A1B0ZZL8_9RHOB	FAD-dependent oxidoreductase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZS3 A0A1B0ZZS3_9RHOB	Allophanate hydrolase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A125 A0A1B1A125_9RHOB	Replicative DNA helicase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6T4 A0A1B1A6T4_9RHOB	Oligopeptide transporter ATP-binding component OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5Q5 A0A1B1A5Q5_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7D4 A0A1B1A7D4_9RHOB	Glycosyl transferase family 1 OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7Q2 A0A1B1A7Q2_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1S4 A0A1B1A1S4_9RHOB	Sulfotransferase domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZYW7 A0A1B0ZYW7_9RHOB	Tryptophan--tRNA ligase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7A8 A0A1B1A7A8_9RHOB	Protein HflK OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZX3 A0A1B0ZZX3_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZH7 A0A1B0ZZH7_9RHOB	Molybdopterin-binding oxidoreductase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1E9 A0A1B1A1E9_9RHOB	Type I secretion protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AA08 A0A1B1AA08_9RHOB	tRNA-Thr(GGU) m(6)t(6)A37 methyltransferase TsaA OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5N4 A0A1B1A5N4_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A200 A0A1B1A200_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0S6 A0A1B1A0S6_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6L4 A0A1B1A6L4_9RHOB	phosphoserine transaminase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9A0 A0A1B1A9A0_9RHOB	Molybdopterin molybdenumtransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A676 A0A1B1A676_9RHOB	Flagellar hook-associated protein 1 OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A519 A0A1B1A519_9RHOB	fumarylacetoacetase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9M0 A0A1B1A9M0_9RHOB	CBS domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6C4 A0A1B1A6C4_9RHOB	GntR family transcriptional regulator OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A616 A0A1B1A616_9RHOB	L-rhamnonate dehydratase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7B9 A0A1B1A7B9_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE

tr A0A1B1A286 A0A1B1A286_9RHOB	sn-glycerol-3-phosphate transport system permease protein UgpE OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9S4 A0A1B1A9S4_9RHOB	Rhs element Vgr protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6P8 A0A1B1A6P8_9RHOB	Peptide ABC transporter permease OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0A0 A0A1B1A0A0_9RHOB	N-acetylmuramoyl-L-alanine amidase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3X4 A0A1B1A3X4_9RHOB	3-keto-5-aminohexanoate cleavage protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6G3 A0A1B1A6G3_9RHOB	Threonine dehydratase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AAG1 A0A1B1AAG1_9RHOB	Pyridine nucleotide-disulfide oxidoreductase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0Y1 A0A1B1A0Y1_9RHOB	Cytidine deaminase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1Z7 A0A1B1A1Z7_9RHOB	Phospho-2-dehydro-3-deoxyheptonate aldolase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0L3 A0A1B1A0L3_9RHOB	HotDog ACOT-type domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A421 A0A1B1A421_9RHOB	Pilus assembly protein CpaE OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9P6 A0A1B1A9P6_9RHOB	Hemolysin-type calcium-binding region OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1X9HW32 A0A1X9HW32_9CAUD	Uncharacterized protein OS=Ruegeria phage 45A6	1	TRUE
tr A0A1B1A7A9 A0A1B1A7A9_9RHOB	GIY-YIG nuclease family protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9V5 A0A1B1A9V5_9RHOB	Glycosyltransferase 2-like domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A468 A0A1B1A468_9RHOB	DNA processing protein DprA OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5T2 A0A1B1A5T2_9RHOB	Transposase IS4-like domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A079 A0A1B1A079_9RHOB	Probable periplasmic serine endoprotease DegP-like OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AAJ5 A0A1B1AAJ5_9RHOB	DUF2793 domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZH4 A0A1B0ZZH4_9RHOB	Corrinoid adenosyltransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A539 A0A1B1A539_9RHOB	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7Q6 A0A1B1A7Q6_9RHOB	Bacterial EndoU nuclease domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9J4 A0A1B1A9J4_9RHOB	Biotin carboxyl carrier protein of acetyl-CoA carboxylase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZYI7 A0A1B0ZYI7_9RHOB	DNA-directed RNA polymerase subunit beta' OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7Y7 A0A1B1A7Y7_9RHOB	Soluble pyridine nucleotide transhydrogenase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5M7 A0A1B1A5M7_9RHOB	Helix-turn-helix domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A4T2 A0A1B1A4T2_9RHOB	FMN dependent NADH:quinone oxidoreductase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0E4 A0A1B1A0E4_9RHOB	5-bromo-4-chloroindolyl phosphate hydrolysis protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9D2 A0A1B1A9D2_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE

tr A0A1B1A4L7 A0A1B1A4L7_9RHOB	UvrABC system protein C OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZ39 A0A1B0ZZ39_9RHOB	HAD family hydrolase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A912 A0A1B1A912_9RHOB	Probable chemoreceptor glutamine deamidase CheD OS=Tritonibacter mobilis F1926 OX=1265309 GN=cheD PE=3 SV=1	1	TRUE
tr A0A1B1A489 A0A1B1A489_9RHOB	Medium/long-chain acyl-CoA thioesterase YigI OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AAD2 A0A1B1AAD2_9RHOB	DNA invertase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6G8 A0A1B1A6G8_9RHOB	Flagellar hook-length control protein-like C-terminal domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1X5 A0A1B1A1X5_9RHOB	Thiamine phosphate synthase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A848 A0A1B1A848_9RHOB	Permease OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1Q9 A0A1B1A1Q9_9RHOB	DNA gyrase subunit A OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A4G3 A0A1B1A4G3_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A4M0 A0A1B1A4M0_9RHOB	Cbb3-type cytochrome c oxidase subunit OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A127 A0A1B1A127_9RHOB	Glucans biosynthesis glucosyltransferase H OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1S7 A0A1B1A1S7_9RHOB	Cystathionine gamma-synthase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3P7 A0A1B1A3P7_9RHOB	Protocatechuate 3,4-dioxygenase subunit beta OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A292 A0A1B1A292_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9I3 A0A1B1A9I3_9RHOB	ATPase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0C1 A0A1B1A0C1_9RHOB	NADPH:quinone oxidoreductase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A4A1 A0A1B1A4A1_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A370 A0A1B1A370_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5U8 A0A1B1A5U8_9RHOB	Phage capsid-like C-terminal domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A026 A0A1B1A026_9RHOB	Transposase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8G3 A0A1B1A8G3_9RHOB	Xylose transport system permease protein XylH OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZU9 A0A1B0ZZU9_9RHOB	DUF2867 domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7Y5 A0A1B1A7Y5_9RHOB	S-formylglutathione hydrolase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZY38 A0A1B0ZY38_9RHOB	Manganese ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5K9 A0A1B1A5K9_9RHOB	Calcium-binding protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9K2 A0A1B1A9K2_9RHOB	Hemolysin-type calcium-binding region OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8H4 A0A1B1A8H4_9RHOB	Malyl-CoA lyase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0F0 A0A1B1A0F0_9RHOB	ABC transporter OS=Tritonibacter mobilis F1926	1	TRUE

tr A0A1B0ZZH0 A0A1B0ZZH0_9RHOB	Outer membrane lipoprotein Blc OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0X6 A0A1B1A0X6_9RHOB	Methyltransferase domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8Q0 A0A1B1A8Q0_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A122 A0A1B1A122_9RHOB	ATP-dependent Clp protease ATP-binding subunit ClpX OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3Z0 A0A1B1A3Z0_9RHOB	Serine/threonine protein kinase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A248 A0A1B1A248_9RHOB	Indolepyruvate ferredoxin oxidoreductase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A197 A0A1B1A197_9RHOB	MBL fold metallo-hydrolase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3V6 A0A1B1A3V6_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6G0 A0A1B1A6G0_9RHOB	L-asparaginase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A775 A0A1B1A775_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A914 A0A1B1A914_9RHOB	Chemotaxis protein CheW OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZK8 A0A1B0ZZK8_9RHOB	BCCT transporter OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A976 A0A1B1A976_9RHOB	Glutamate--cysteine ligase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A344 A0A1B1A344_9RHOB	SUF system Fe-S cluster assembly protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AAJ7 A0A1B1AAJ7_9RHOB	YjbH domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A4Z7 A0A1B1A4Z7_9RHOB	ATP synthase subunit b OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6X1 A0A1B1A6X1_9RHOB	ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926 OX=1265309 GN=K529_016220 PE=4 SV=1	1	TRUE
tr A0A1B1A811 A0A1B1A811_9RHOB	Protein phosphatase OS=Tritonibacter mobilis F1926 OX=1265309 GN=K529_018270 PE=4 SV=1	1	TRUE
tr A0A1B0ZZY2 A0A1B0ZZY2_9RHOB	Hydroxyacylglutathione hydrolase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AA60 A0A1B1AA60_9RHOB	Sugar ABC transporter substrate-binding protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9I6 A0A1B1A9I6_9RHOB	2-oxoglutarate dehydrogenase E1 component OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A934 A0A1B1A934_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5C5 A0A1B1A5C5_9RHOB	Sulfatase modifying factor 1 (C-alpha-formylglycine-generating enzyme 1) OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1X3 A0A1B1A1X3_9RHOB	Phosphate transport system permease protein PstA OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5A7 A0A1B1A5A7_9RHOB	D-ala-D-ala transporter subunit OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6E7 A0A1B1A6E7_9RHOB	Succinyl-diaminopimelate desuccinylase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0Y9 A0A1B1A0Y9_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AAF7 A0A1B1AAF7_9RHOB	Cation transporter OS=Tritonibacter mobilis F1926	1	TRUE

tr A0A1B1A870 A0A1B1A870_9RHOB	ATPase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZR6 A0A1B0ZZR6_9RHOB	2-hydroxychromene-2-carboxylate isomerase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1M3 A0A1B1A1M3_9RHOB	histidine kinase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A544 A0A1B1A544_9RHOB	RpiR family transcriptional regulator OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZYS0 A0A1B0ZYS0_9RHOB	Ribonuclease P protein component OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A2Q8 A0A1B1A2Q8_9RHOB	Agmatinase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A2T3 A0A1B1A2T3_9RHOB	Glutamine-synthetase adenylyltransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZD6 A0A1B0ZZD6_9RHOB	Twin-arginine translocation pathway signal OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZI4 A0A1B0ZZI4_9RHOB	Multidrug export protein MepA OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0Z7 A0A1B1A0Z7_9RHOB	DNA repair protein RadA OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A025 A0A1B1A025_9RHOB	N-acetylmuramoyl-L-alanine amidase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A844 A0A1B1A844_9RHOB	DNA polymerase III subunit gamma/tau OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZYU4 A0A1B0ZYU4_9RHOB	5-carboxymethyl-2-hydroxy muconate isomerase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AAE1 A0A1B1AAE1_9RHOB	DUF3768 domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9K5 A0A1B1A9K5_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7S1 A0A1B1A7S1_9RHOB	3,4-dihydroxyphenylacetate 2,3-dioxygenase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6Z6 A0A1B1A6Z6_9RHOB	Iron ABC transporter permease OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A325 A0A1B1A325_9RHOB	Aspartate/glutamate leucyltransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A4F1 A0A1B1A4F1_9RHOB	Glycolate oxidase iron-sulfur subunit OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3G4 A0A1B1A3G4_9RHOB	Transport permease protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5E0 A0A1B1A5E0_9RHOB	DUF1203 domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6E2 A0A1B1A6E2_9RHOB	GntR family transcriptional regulator OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5N2 A0A1B1A5N2_9RHOB	Copper oxidase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6H3 A0A1B1A6H3_9RHOB	Basal-body rod modification protein FlgD OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AAB0 A0A1B1AAB0_9RHOB	Toxin OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A319 A0A1B1A319_9RHOB	Mandelate racemase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A820 A0A1B1A820_9RHOB	Taurine--pyruvate aminotransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A994 A0A1B1A994_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5I0 A0A1B1A5I0_9RHOB	SelT/selW/selH selenoprotein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZD3 A0A1B0ZZD3_9RHOB	Metal-binding protein OS=Tritonibacter mobilis F1926	1	TRUE

tr A0A1B0ZYL0 A0A1B0ZYL0_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1W2 A0A1B1A1W2_9RHOB	LuxR family transcriptional regulator OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZYD6 A0A1B0ZYD6_9RHOB	Large ribosomal subunit protein uL6 OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A985 A0A1B1A985_9RHOB	Thiol-disulfide oxidoreductase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZA8 A0A1B0ZZA8_9RHOB	Hydrogenobyrinate a,c-diamide synthase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AAH1 A0A1B1AAH1_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A196 A0A1B1A196_9RHOB	Fumarate hydratase class II OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A074 A0A1B1A074_9RHOB	Protein MgtC OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A838 A0A1B1A838_9RHOB	Threonine aldolase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZ36 A0A1B0ZZ36_9RHOB	Pyruvate, phosphate dikinase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3J6 A0A1B1A3J6_9RHOB	AraC family transcriptional regulator OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZI7 A0A1B0ZZI7_9RHOB	Aspartate aminotransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6A9 A0A1B1A6A9_9RHOB	Flagellin OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A9J9 A0A1B1A9J9_9RHOB	XRE family transcriptional regulator OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5F0 A0A1B1A5F0_9RHOB	Serine--glyoxylate aminotransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A3V8 A0A1B1A3V8_9RHOB	inorganic diphosphatase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0L5 A0A1B1A0L5_9RHOB	3-methyladenine DNA glycosylase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZYY9 A0A1B0ZYY9_9RHOB	Muramidase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A824 A0A1B1A824_9RHOB	PhoH-like protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1X9HWP7 A0A1X9HWP7_9CAUD	Peptidase S74 domain-containing protein OS=Ruegeria phage 45A6	1	TRUE
tr A0A1B1A0T2 A0A1B1A0T2_9RHOB	Alanine racemase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5V0 A0A1B1A5V0_9RHOB	Succinate-semialdehyde dehydrogenase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A2S4 A0A1B1A2S4_9RHOB	SMC-Scp complex subunit ScpB OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZZ69 A0A1B0ZZ69_9RHOB	Antibiotic biosynthesis monooxygenase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A0N8 A0A1B1A0N8_9RHOB	Exopolyphosphatase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A7I1 A0A1B1A7I1_9RHOB	Phenylacetate-CoA oxygenase subunit PaaJ OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8Z2 A0A1B1A8Z2_9RHOB	Phosphodiesterase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A2Z7 A0A1B1A2Z7_9RHOB	ABC transporter permease OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A2A6 A0A1B1A2A6_9RHOB	Glutamate--tRNA ligase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZYZ4 A0A1B0ZYZ4_9RHOB	Sigma-54-dependent Fis family transcriptional regulator OS=Tritonibacter mobilis F1926	1	TRUE

tr A0A1B1A9Q2 A0A1B1A9Q2_9RHOB	DNA topoisomerase 4 subunit B OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A766 A0A1B1A766_9RHOB	HTH araC/xylS-type domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A486 A0A1B1A486_9RHOB	RNase adaptor protein RapZ OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A2D9 A0A1B1A2D9_9RHOB	Citrate synthase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A974 A0A1B1A974_9RHOB	Response regulator receiver protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A059 A0A1B1A059_9RHOB	Histidinol-phosphate aminotransferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6D2 A0A1B1A6D2_9RHOB	Ribonuclease R OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B0ZYP7 A0A1B0ZYP7_9RHOB	5-amino-6-(D-ribitylamino)uracil--L-tyrosine 4-hydroxyphenyl transferase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1X9HVK2 A0A1X9HVK2_9CAUD	RNase H type-1 domain-containing protein OS=Ruegeria phage 45A6	1	TRUE
tr A0A1B0ZY77 A0A1B0ZY77_9RHOB	Allantoin permease OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A1T6 A0A1B1A1T6_9RHOB	Multidrug DMT transporter permease OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8A0 A0A1B1A8A0_9RHOB	Haloacid dehalogenase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1AAJ4 A0A1B1AAJ4_9RHOB	DUF2892 domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A8Z6 A0A1B1A8Z6_9RHOB	Hydroxyproline-2-epimerase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A180 A0A1B1A180_9RHOB	Flavin oxidoreductase OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A5T3 A0A1B1A5T3_9RHOB	DUF3768 domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1X9HWA2 A0A1X9HWA2_9CAUD	RecT family protein OS=Ruegeria phage 45A6	1	TRUE
tr A0A1B1A5N1 A0A1B1A5N1_9RHOB	PAN domain-containing protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A6A7 A0A1B1A6A7_9RHOB	Flagellar protein OS=Tritonibacter mobilis F1926	1	TRUE
tr A0A1B1A816 A0A1B1A816_9RHOB	Lipoprotein OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1AA66 A0A1B1AA66_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A2V2 A0A1B1A2V2_9RHOB	DUF2189 domain-containing protein OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1AA30 A0A1B1AA30_9RHOB	Multidrug resistance protein NorM OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A0G2 A0A1B1A0G2_9RHOB	DUF2267 domain-containing protein OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B0ZYQ2 A0A1B0ZYQ2_9RHOB	Glucose-6-phosphate isomerase OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A1U8 A0A1B1A1U8_9RHOB	Ubiquinone-binding protein OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B0ZZL7 A0A1B0ZZL7_9RHOB	Capsule biosynthesis protein CapA OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B0ZZY1 A0A1B0ZZY1_9RHOB	LysR family transcriptional regulator OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A3Y9 A0A1B1A3Y9_9RHOB	Glyoxalase OS=Tritonibacter mobilis F1926	0.999999	TRUE

tr A0A1B1A2Z5 A0A1B1A2Z5_9RHOB	DUF2852 domain-containing protein OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B0ZZG8 A0A1B0ZZG8_9RHOB	Metal-dependent hydrolase OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A3P3 A0A1B1A3P3_9RHOB	Phosphoglucomutase OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1AAC9 A0A1B1AAC9_9RHOB	Cation diffusion facilitator family transporter OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A2Q3 A0A1B1A2Q3_9RHOB	50S ribosomal protein L34 OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B0ZZM7 A0A1B0ZZM7_9RHOB	Chemotaxis protein CheA OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A7W5 A0A1B1A7W5_9RHOB	histidine kinase OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A8G5 A0A1B1A8G5_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1AA75 A0A1B1AA75_9RHOB	Aminopeptidase OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A9H1 A0A1B1A9H1_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A4U6 A0A1B1A4U6_9RHOB	Homogentisate 1,2-dioxygenase OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1X9HWA0 A0A1X9HWA0_9CAUD	Transglycosylase SLT domain-containing protein OS=Ruegeria phage 45A6	0.999999	TRUE
tr A0A1B1A9J7 A0A1B1A9J7_9RHOB	Iron transporter OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A2H3 A0A1B1A2H3_9RHOB	Aconitate hydratase OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A137 A0A1B1A137_9RHOB	XdhC/CoxI family protein OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A5J0 A0A1B1A5J0_9RHOB	N-formylglutamate amidohydrolase OS=Tritonibacter mobilis F1926	0.999999	TRUE
tr A0A1B1A2R5 A0A1B1A2R5_9RHOB	Sporulation protein SsgA OS=Tritonibacter mobilis F1926	0.999998	TRUE
tr A0A1B1A8F2 A0A1B1A8F2_9RHOB	Sugar ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	0.999998	TRUE
tr A0A1B1A0U4 A0A1B1A0U4_9RHOB	Translation initiation factor IF-3 OS=Tritonibacter mobilis F1926	0.999998	TRUE
tr A0A1B1A206 A0A1B1A206_9RHOB	Phosphate ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	0.999998	TRUE
tr A0A1B1A5X6 A0A1B1A5X6_9RHOB	tRNA threonylcarbamoyladenosine biosynthesis protein TsaE OS=Tritonibacter mobilis F1926	0.999998	TRUE
tr A0A1B1A9H5 A0A1B1A9H5_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999998	TRUE
tr A0A1B1A738 A0A1B1A738_9RHOB	MbtH-like domain-containing protein OS=Tritonibacter mobilis F1926	0.999998	TRUE
tr A0A1B1A329 A0A1B1A329_9RHOB	Alkene reductase OS=Tritonibacter mobilis F1926	0.999998	TRUE
tr A0A1B1A086 A0A1B1A086_9RHOB	Septum site-determining protein OS=Tritonibacter mobilis F1926	0.999997	TRUE
tr A0A1B1A931 A0A1B1A931_9RHOB	Alpha-glucosidase OS=Tritonibacter mobilis F1926	0.999997	TRUE
tr A0A1B0ZZ77 A0A1B0ZZ77_9RHOB	Pyridoxamine 5-phosphate oxidase OS=Tritonibacter mobilis F1926	0.999997	TRUE
tr A0A1B0ZZ59 A0A1B0ZZ59_9RHOB	Glutamate--tRNA ligase OS=Tritonibacter mobilis F1926	0.999997	TRUE
tr A0A1B1A6M7 A0A1B1A6M7_9RHOB	Phosphoserine phosphatase OS=Tritonibacter mobilis F1926	0.999997	TRUE
tr A0A1B1A1B3 A0A1B1A1B3_9RHOB	MATE family efflux transporter OS=Tritonibacter mobilis F1926	0.999997	TRUE

tr A0A1B0ZY47 A0A1B0ZY47_9RHOB	DNA mismatch repair protein MutL OS=Tritonibacter mobilis F1926	0.999997	TRUE
tr A0A1B1A8H9 A0A1B1A8H9_9RHOB	Flagellin OS=Tritonibacter mobilis F1926	0.999996	TRUE
tr A0A1B1A6D8 A0A1B1A6D8_9RHOB	Hedgehog/Intein (Hint) domain-containing protein OS=Tritonibacter mobilis F1926	0.999996	TRUE
tr A0A1B1A3U0 A0A1B1A3U0_9RHOB	Adenylyl-sulfate kinase OS=Tritonibacter mobilis F1926	0.999996	TRUE
tr A0A1B1A081 A0A1B1A081_9RHOB	Exodeoxyribonuclease 7 large subunit OS=Tritonibacter mobilis F1926	0.999996	TRUE
tr A0A1B1A157 A0A1B1A157_9RHOB	TIGR01244 family protein OS=Tritonibacter mobilis F1926	0.999996	TRUE
tr A0A1B1A9L8 A0A1B1A9L8_9RHOB	3'-phosphate/5'-hydroxy nucleic acid ligase OS=Tritonibacter mobilis F1926	0.999996	TRUE
tr A0A1B1A6Y4 A0A1B1A6Y4_9RHOB	Exopolysaccharide biosynthesis protein exod OS=Tritonibacter mobilis F1926	0.999996	TRUE
tr A0A1B0ZXW7 A0A1B0ZXW7_9RHOB	Galactose mutarotase OS=Tritonibacter mobilis F1926	0.999995	TRUE
tr A0A1B1AAB1 A0A1B1AAB1_9RHOB	DUF305 domain-containing protein OS=Tritonibacter mobilis F1926	0.999995	TRUE
tr A0A1B1A9S0 A0A1B1A9S0_9RHOB	ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	0.999995	TRUE
tr A0A1B1A4J8 A0A1B1A4J8_9RHOB	Propionate 3-nitronate monooxygenase OS=Tritonibacter mobilis F1926	0.999995	TRUE
tr A0A1B1A9Y6 A0A1B1A9Y6_9RHOB	Thioesterase OS=Tritonibacter mobilis F1926	0.999994	TRUE
tr A0A1B1A5J2 A0A1B1A5J2_9RHOB	RpiR family transcriptional regulator OS=Tritonibacter mobilis F1926	0.999994	TRUE
tr A0A1B1A436 A0A1B1A436_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999994	TRUE
tr A0A1B0ZZU1 A0A1B0ZZU1_9RHOB	Transcriptional regulator OS=Tritonibacter mobilis F1926	0.999994	TRUE
tr A0A1B1A5N5 A0A1B1A5N5_9RHOB	AMP nucleosidase OS=Tritonibacter mobilis F1926	0.999993	TRUE
tr A0A1B1A304 A0A1B1A304_9RHOB	Acetolactate synthase small subunit OS=Tritonibacter mobilis F1926	0.999993	TRUE
tr A0A1B1A2X9 A0A1B1A2X9_9RHOB	DUF4167 domain-containing protein OS=Tritonibacter mobilis F1926	0.999993	TRUE
tr A0A1B1A8C7 A0A1B1A8C7_9RHOB	ABC transporter permease OS=Tritonibacter mobilis F1926	0.999992	TRUE
tr A0A1B1A1N6 A0A1B1A1N6_9RHOB	Diguanylate cyclase OS=Tritonibacter mobilis F1926	0.999991	TRUE
tr A0A1B1A8U7 A0A1B1A8U7_9RHOB	Phosphoribosyl 1,2-cyclic phosphodiesterase OS=Tritonibacter mobilis F1926	0.999991	TRUE
tr A0A1B1A4W8 A0A1B1A4W8_9RHOB	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase OS=Tritonibacter mobilis F1926	0.999991	TRUE
tr A0A1B0ZZX0 A0A1B0ZZX0_9RHOB	DUF4265 domain-containing protein OS=Tritonibacter mobilis F1926	0.999988	TRUE
tr A0A1B1A6Y3 A0A1B1A6Y3_9RHOB	Amino acid ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	0.999985	TRUE
tr A0A1B1A8Z9 A0A1B1A8Z9_9RHOB	Dihydrodipicolinate synthase family protein OS=Tritonibacter mobilis F1926	0.999985	TRUE
tr A0A1B1A5T6 A0A1B1A5T6_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999984	TRUE
tr A0A1B1A4L3 A0A1B1A4L3_9RHOB	Universal stress protein OS=Tritonibacter mobilis F1926	0.999983	TRUE
tr A0A1B1A1Q0 A0A1B1A1Q0_9RHOB	YicC family protein OS=Tritonibacter mobilis F1926	0.999983	TRUE

tr A0A1B1A0S3 A0A1B1A0S3_9RHOB	Carbon monoxide dehydrogenase OS=Tritonibacter mobilis F1926	0.999982	TRUE
tr A0A1B0ZZA3 A0A1B0ZZA3_9RHOB	Xanthine dehydrogenase small subunit OS=Tritonibacter mobilis F1926	0.999981	TRUE
tr A0A1B1A5V7 A0A1B1A5V7_9RHOB	Diacylglycerol kinase OS=Tritonibacter mobilis F1926	0.999981	TRUE
tr A0A1B1A437 A0A1B1A437_9RHOB	Phosphotransferase OS=Tritonibacter mobilis F1926	0.99998	TRUE
tr A0A1B0ZZG0 A0A1B0ZZG0_9RHOB	Zinc-finger protein OS=Tritonibacter mobilis F1926	0.99998	TRUE
tr A0A1B1A2Q6 A0A1B1A2Q6_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999979	TRUE
tr A0A1B0ZKY1 A0A1B0ZKY1_9RHOB	Phospho-2-dehydro-3-deoxyheptonate aldolase OS=Tritonibacter mobilis F1926	0.999978	TRUE
tr A0A1B1A8J4 A0A1B1A8J4_9RHOB	CbbBc protein OS=Tritonibacter mobilis F1926	0.999977	TRUE
tr A0A1B0ZZX4 A0A1B0ZZX4_9RHOB	Aminotransferase OS=Tritonibacter mobilis F1926	0.999977	TRUE
tr A0A1B0ZZL0 A0A1B0ZZL0_9RHOB	Enamine deaminase RidA OS=Tritonibacter mobilis F1926	0.999976	TRUE
tr A0A1B0ZZ22 A0A1B0ZZ22_9RHOB	Acetyltransferase OS=Tritonibacter mobilis F1926	0.999975	TRUE
tr A0A1B0ZZX6 A0A1B0ZZX6_9RHOB	Transcriptional regulator MraZ OS=Tritonibacter mobilis F1926	0.999974	TRUE
tr A0A1B1A211 A0A1B1A211_9RHOB	Drug:proton antiporter OS=Tritonibacter mobilis F1926	0.999973	TRUE
tr A0A1B1A9V0 A0A1B1A9V0_9RHOB	UPF0276 protein K529_021310 OS=Tritonibacter mobilis F1926	0.999973	TRUE
tr A0A1B1A5W4 A0A1B1A5W4_9RHOB	Glycosyltransferase 61 catalytic domain-containing protein OS=Tritonibacter mobilis F1926	0.999968	TRUE
tr A0A1B1A1L0 A0A1B1A1L0_9RHOB	Chromate transporter OS=Tritonibacter mobilis F1926	0.999967	TRUE
tr A0A1B0ZYY4 A0A1B0ZYY4_9RHOB	histidine kinase OS=Tritonibacter mobilis F1926	0.999966	TRUE
tr A0A1B1AAL0 A0A1B1AAL0_9RHOB	Chromosome partitioning protein ParB OS=Tritonibacter mobilis F1926	0.999963	TRUE
tr A0A1B1A435 A0A1B1A435_9RHOB	Type VI secretion protein OS=Tritonibacter mobilis F1926	0.999963	TRUE
tr A0A1B0ZYI8 A0A1B0ZYI8_9RHOB	Protein translocase subunit SecE OS=Tritonibacter mobilis F1926	0.999956	TRUE
tr A0A1B1A752 A0A1B1A752_9RHOB	ABC transporter permease OS=Tritonibacter mobilis F1926	0.999953	TRUE
tr A0A1B1A6D0 A0A1B1A6D0_9RHOB	Aminotransferase OS=Tritonibacter mobilis F1926	0.999953	TRUE
tr A0A1B1A718 A0A1B1A718_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999953	TRUE
tr A0A1B1A054 A0A1B1A054_9RHOB	Histidinol phosphate aminotransferase OS=Tritonibacter mobilis F1926	0.999952	TRUE
tr A0A1B0ZYR2 A0A1B0ZYR2_9RHOB	PepSY domain-containing protein OS=Tritonibacter mobilis F1926	0.999951	TRUE
tr A0A1B1AAK2 A0A1B1AAK2_9RHOB	Glycosyltransferase 61 catalytic domain-containing protein OS=Tritonibacter mobilis F1926	0.999946	TRUE
tr A0A1B1A4Q1 A0A1B1A4Q1_9RHOB	Aminotransferase OS=Tritonibacter mobilis F1926	0.999945	TRUE
tr A0A1B1A2M9 A0A1B1A2M9_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999939	TRUE
tr A0A1B1A953 A0A1B1A953_9RHOB	ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	0.999933	TRUE
tr A0A1B1A1X0 A0A1B1A1X0_9RHOB	tRNA (cytidine-uridine-2'-O-)-methyltransferase TrmJ OS=Tritonibacter mobilis F1926	0.999933	TRUE

tr A0A1B0ZY24 A0A1B0ZY24_9RHOB	Peptide ABC transporter permease OS=Tritonibacter mobilis F1926	0.999931	TRUE
tr A0A1B1A609 A0A1B1A609_9RHOB	Dihydrodipicolinate synthase family protein OS=Tritonibacter mobilis F1926	0.99993	TRUE
tr A0A1B1A495 A0A1B1A495_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999929	TRUE
tr A0A1B1A555 A0A1B1A555_9RHOB	GntR family transcriptional regulator OS=Tritonibacter mobilis F1926	0.999929	TRUE
tr A0A1B1A1A9 A0A1B1A1A9_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999926	TRUE
tr A0A1B0ZZK9 A0A1B0ZZK9_9RHOB	Cobaltochelatase subunit CobN OS=Tritonibacter mobilis F1926	0.999924	TRUE
tr A0A1B1A921 A0A1B1A921_9RHOB	TRAP transporter small permease protein OS=Tritonibacter mobilis F1926	0.999923	TRUE
tr A0A1B0ZZ21 A0A1B0ZZ21_9RHOB	(4Fe-4S)-binding protein OS=Tritonibacter mobilis F1926	0.999921	TRUE
tr A0A1B1A1C5 A0A1B1A1C5_9RHOB	Hint domain-containing protein OS=Tritonibacter mobilis F1926	0.99992	TRUE
tr A0A1B1A7I0 A0A1B1A7I0_9RHOB	UPF0262 protein K529_017320 OS=Tritonibacter mobilis F1926	0.999914	TRUE
tr A0A1B1A2A3 A0A1B1A2A3_9RHOB	Phage tail protein OS=Tritonibacter mobilis F1926	0.999913	TRUE
tr A0A1B1A301 A0A1B1A301_9RHOB	ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	0.999911	TRUE
tr A0A1B1A8K0 A0A1B1A8K0_9RHOB	AsnC family transcriptional regulator OS=Tritonibacter mobilis F1926	0.99991	TRUE
tr A0A1B1A3R8 A0A1B1A3R8_9RHOB	L-ectoine synthase OS=Tritonibacter mobilis F1926	0.99991	TRUE
tr A0A1B1A8T3 A0A1B1A8T3_9RHOB	L-asparaginase 1 OS=Tritonibacter mobilis F1926	0.99991	TRUE
tr A0A1B1A3K4 A0A1B1A3K4_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999908	TRUE
tr A0A1B1A854 A0A1B1A854_9RHOB	Amino acid acetyltransferase OS=Tritonibacter mobilis F1926	0.999906	TRUE
tr A0A1B1A1Y0 A0A1B1A1Y0_9RHOB	Zn-dependent hydrolase OS=Tritonibacter mobilis F1926	0.999906	TRUE
tr A0A1B0ZZD7 A0A1B0ZZD7_9RHOB	Glycerol-3-phosphate dehydrogenase OS=Tritonibacter mobilis F1926	0.999905	TRUE
tr A0A1B0ZZ68 A0A1B0ZZ68_9RHOB	2-isopropylmalate synthase OS=Tritonibacter mobilis F1926	0.999901	TRUE
tr A0A1B1A3G3 A0A1B1A3G3_9RHOB	Ribosomal protein uS12 methylthiotransferase RimO OS=Tritonibacter mobilis F1926	0.9999	TRUE
tr A0A1B1A728 A0A1B1A728_9RHOB	ABC transporter permease OS=Tritonibacter mobilis F1926	0.9999	TRUE
tr A0A1B1A442 A0A1B1A442_9RHOB	TIGR01620 family protein OS=Tritonibacter mobilis F1926	0.999897	TRUE
tr A0A1X9HWB8 A0A1X9HWB8_9CAUD	Uncharacterized protein OS=Ruegeria phage 45A6	0.999896	TRUE
tr A0A1B1A6I8 A0A1B1A6I8_9RHOB	Methylenetetrahydrofolate reductase OS=Tritonibacter mobilis F1926	0.999896	TRUE
tr A0A1B1A677 A0A1B1A677_9RHOB	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase OS=Tritonibacter mobilis F1926	0.999893	TRUE
tr A0A1B1A1G2 A0A1B1A1G2_9RHOB	tRNA glutamyl-Q synthetase OS=Tritonibacter mobilis F1926	0.999892	TRUE
tr A0A1B1A3S7 A0A1B1A3S7_9RHOB	ABC transporter permease OS=Tritonibacter mobilis F1926	0.999891	TRUE
tr A0A1B0ZYL8 A0A1B0ZYL8_9RHOB	Small ribosomal subunit protein uS17 OS=Tritonibacter mobilis F1926	0.99989	TRUE
tr A0A1B0ZZF7 A0A1B0ZZF7_9RHOB	NADPH dependent quinone reductase OS=Tritonibacter mobilis F1926	0.99989	TRUE

tr A0A1B1A4F6 A0A1B1A4F6_9RHOB	GNAT family N-acetyltransferase OS=Tritonibacter mobilis F1926	0.999889	TRUE
tr A0A1B1A762 A0A1B1A762_9RHOB	Tautomerase OS=Tritonibacter mobilis F1926	0.999888	TRUE
tr A0A1B0ZZC9 A0A1B0ZZC9_9RHOB	Hemin uptake protein HemP OS=Tritonibacter mobilis F1926	0.999883	TRUE
tr A0A1B1A1E2 A0A1B1A1E2_9RHOB	NADH dehydrogenase OS=Tritonibacter mobilis F1926	0.999881	TRUE
tr A0A1B1A1Y7 A0A1B1A1Y7_9RHOB	Acyl-phosphate glycerol 3-phosphate acyltransferase OS=Tritonibacter mobilis F1926	0.99988	TRUE
tr A0A1B1A0F4 A0A1B1A0F4_9RHOB	Inner membrane-spanning protein YciB OS=Tritonibacter mobilis F1926	0.999861	TRUE
tr A0A1B1A4T4 A0A1B1A4T4_9RHOB	Cytochrome c oxidase accessory protein CcoG OS=Tritonibacter mobilis F1926	0.999859	TRUE
tr A0A1B1A361 A0A1B1A361_9RHOB	Hemolysin OS=Tritonibacter mobilis F1926	0.999848	TRUE
tr A0A1B1A4Z8 A0A1B1A4Z8_9RHOB	Guanylate cyclase OS=Tritonibacter mobilis F1926	0.999844	TRUE
tr A0A1X9HW79 A0A1X9HW79_9CAUD	DNA-binding protein OS=Ruegeria phage 45A6	0.999835	TRUE
tr A0A1B1A6C8 A0A1B1A6C8_9RHOB	Organic hydroperoxide resistance protein OS=Tritonibacter mobilis F1926	0.999833	TRUE
tr A0A1B1A0W0 A0A1B1A0W0_9RHOB	DUF883 domain-containing protein OS=Tritonibacter mobilis F1926	0.999831	TRUE
tr A0A1B1A2L0 A0A1B1A2L0_9RHOB	T6SS Phospholipase effector Tle1-like catalytic domain-containing protein OS=Tritonibacter mobilis F1926	0.999826	TRUE
tr A0A1B1A2Q2 A0A1B1A2Q2_9RHOB	Phosphate acyltransferase OS=Tritonibacter mobilis F1926	0.999809	TRUE
tr A0A1B1A194 A0A1B1A194_9RHOB	CsbD-like domain-containing protein OS=Tritonibacter mobilis F1926	0.999807	TRUE
tr A0A1B1A6D9 A0A1B1A6D9_9RHOB	Histidine ammonia-lyase OS=Tritonibacter mobilis F1926	0.999803	TRUE
tr A0A1B1A7G5 A0A1B1A7G5_9RHOB	Phosphonate C-P lyase system protein PhnL OS=Tritonibacter mobilis F1926	0.999792	TRUE
tr A0A1B1A2J9 A0A1B1A2J9_9RHOB	Capsid protein OS=Tritonibacter mobilis F1926	0.999792	TRUE
tr A0A1B1A695 A0A1B1A695_9RHOB	Flagellar basal-body rod protein FlgF OS=Tritonibacter mobilis F1926	0.999788	TRUE
tr A0A1B1A654 A0A1B1A654_9RHOB	Translation initiation factor IF-2 OS=Tritonibacter mobilis F1926	0.999769	TRUE
tr A0A1B1A2B5 A0A1B1A2B5_9RHOB	Cytochrome OS=Tritonibacter mobilis F1926	0.999751	TRUE
tr A0A1B1A0X1 A0A1B1A0X1_9RHOB	DUF817 domain-containing protein OS=Tritonibacter mobilis F1926	0.999734	TRUE
tr A0A1B1A7L4 A0A1B1A7L4_9RHOB	Acetyltransferase OS=Tritonibacter mobilis F1926	0.999732	TRUE
tr A0A1B1A130 A0A1B1A130_9RHOB	Acyl-CoA dehydrogenase OS=Tritonibacter mobilis F1926	0.99973	TRUE
tr A0A1B1A784 A0A1B1A784_9RHOB	Tail assembly chaperone OS=Tritonibacter mobilis F1926	0.99972	TRUE
tr A0A1B1A2C9 A0A1B1A2C9_9RHOB	protein O-GlcNAc transferase OS=Tritonibacter mobilis F1926	0.999702	TRUE
tr A0A1B1A0D0 A0A1B1A0D0_9RHOB	Peptide ABC transporter permease OS=Tritonibacter mobilis F1926	0.999693	TRUE
tr A0A1B1A6N6 A0A1B1A6N6_9RHOB	Thiazole synthase OS=Tritonibacter mobilis F1926	0.999683	TRUE
tr A0A1B1A7K5 A0A1B1A7K5_9RHOB	Ribulose-phosphate 3-epimerase OS=Tritonibacter mobilis F1926	0.999662	TRUE

tr A0A1B1A7E2 A0A1B1A7E2_9RHOB	Ribose 1,5-bisphosphate phosphokinase PhnN OS=Tritonibacter mobilis F1926	0.999629	TRUE
tr A0A1B0ZZ27 A0A1B0ZZ27_9RHOB	Glycine--tRNA ligase alpha subunit OS=Tritonibacter mobilis F1926	0.999621	TRUE
tr A0A1B1A208 A0A1B1A208_9RHOB	Citramalate synthase OS=Tritonibacter mobilis F1926	0.999621	TRUE
tr A0A1B1A473 A0A1B1A473_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999591	TRUE
tr A0A1B1A4B7 A0A1B1A4B7_9RHOB	Glycosyl transferase OS=Tritonibacter mobilis F1926	0.999588	TRUE
tr A0A1B1A564 A0A1B1A564_9RHOB	N-alpha-acetyl diaminobutyric acid deacetylase DoeB OS=Tritonibacter mobilis F1926	0.999568	TRUE
tr A0A1B1A5T1 A0A1B1A5T1_9RHOB	DUF3768 domain-containing protein OS=Tritonibacter mobilis F1926	0.999558	TRUE
tr A0A1B1A438 A0A1B1A438_9RHOB	cytochrome-c oxidase OS=Tritonibacter mobilis F1926	0.999542	TRUE
tr A0A1B1A3P2 A0A1B1A3P2_9RHOB	Oligoendopeptidase F OS=Tritonibacter mobilis F1926	0.999539	TRUE
tr A0A1B0ZZ67 A0A1B0ZZ67_9RHOB	Gamma-glutamylcyclotransferase AIG2-like domain-containing protein OS=Tritonibacter mobilis F1926	0.999527	TRUE
tr A0A1B1A787 A0A1B1A787_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999496	TRUE
tr A0A1B0ZZZ6 A0A1B0ZZZ6_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.999467	TRUE
tr A0A1B1A021 A0A1B1A021_9RHOB	Fe/B12 periplasmic-binding domain-containing protein OS=Tritonibacter mobilis F1926	0.999467	TRUE
tr A0A1B1A7G4 A0A1B1A7G4_9RHOB	Aspartate aminotransferase family protein OS=Tritonibacter mobilis F1926	0.999457	TRUE
tr A0A1B1A850 A0A1B1A850_9RHOB	TniQ protein OS=Tritonibacter mobilis F1926	0.999443	TRUE
tr A0A1B0ZXV4 A0A1B0ZXV4_9RHOB	AraC family transcriptional regulator OS=Tritonibacter mobilis F1926	0.999427	TRUE
tr A0A1B1A812 A0A1B1A812_9RHOB	AraC family transcriptional regulator OS=Tritonibacter mobilis F1926	0.999406	TRUE
tr A0A1B1A2R6 A0A1B1A2R6_9RHOB	AsnC family transcriptional regulator OS=Tritonibacter mobilis F1926	0.999389	TRUE
tr A0A1B1A9K9 A0A1B1A9K9_9RHOB	Carbohydrate kinase OS=Tritonibacter mobilis F1926	0.999372	TRUE
tr A0A1B1A174 A0A1B1A174_9RHOB	Phosphoribosylglycinamide formyltransferase OS=Tritonibacter mobilis F1926	0.999343	TRUE
tr A0A1B1A9F6 A0A1B1A9F6_9RHOB	DUF1989 domain-containing protein OS=Tritonibacter mobilis F1926	0.999311	TRUE
tr A0A1B1A4B1 A0A1B1A4B1_9RHOB	Protoheme IX farnesyltransferase OS=Tritonibacter mobilis F1926	0.999284	TRUE
tr A0A1B1A8S3 A0A1B1A8S3_9RHOB	Peptide ABC transporter substrate-binding protein OS=Tritonibacter mobilis F1926	0.999283	TRUE
tr A0A1B1A6E0 A0A1B1A6E0_9RHOB	Flagellar motor switch protein FlhN OS=Tritonibacter mobilis F1926	0.999265	TRUE
tr A0A1B1A3X7 A0A1B1A3X7_9RHOB	aspartate kinase OS=Tritonibacter mobilis F1926	0.999248	TRUE
tr A0A1B1A890 A0A1B1A890_9RHOB	LysR family transcriptional regulator OS=Tritonibacter mobilis F1926	0.99923	TRUE
tr A0A1B1A750 A0A1B1A750_9RHOB	Aminotransferase OS=Tritonibacter mobilis F1926	0.999201	TRUE
tr A0A1B1A5C9 A0A1B1A5C9_9RHOB	2-dehydro-3-deoxygluconokinase OS=Tritonibacter mobilis F1926	0.999157	TRUE
tr A0A1B1A5L6 A0A1B1A5L6_9RHOB	Chorismate synthase OS=Tritonibacter mobilis F1926	0.999127	TRUE

tr A0A1B1A5C1 A0A1B1A5C1_9RHOB	NAD-dependent succinate-semialdehyde dehydrogenase OS=Tritonibacter mobilis F1926	0.999118	TRUE
tr A0A1B0ZZ91 A0A1B0ZZ91_9RHOB	Methyltransferase OS=Tritonibacter mobilis F1926	0.999107	TRUE
tr A0A1B1A5I1 A0A1B1A5I1_9RHOB	Adenine deaminase OS=Tritonibacter mobilis F1926	0.999095	TRUE
tr A0A1B1A9V7 A0A1B1A9V7_9RHOB	Gamma-glutamyltransferase OS=Tritonibacter mobilis F1926	0.999032	TRUE
tr A0A1B1A8Y2 A0A1B1A8Y2_9RHOB	2,5-dioxovalerate dehydrogenase OS=Tritonibacter mobilis F1926	0.999016	TRUE
tr A0A1B1A7S7 A0A1B1A7S7_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.998967	TRUE
tr A0A1B1A6S1 A0A1B1A6S1_9RHOB	MBL fold metallo-hydrolase OS=Tritonibacter mobilis F1926	0.998963	TRUE
tr A0A1B1A342 A0A1B1A342_9RHOB	Amino acid amidase OS=Tritonibacter mobilis F1926	0.998901	TRUE
tr A0A1B0ZZW5 A0A1B0ZZW5_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.998872	TRUE
tr A0A1B1A537 A0A1B1A537_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.998843	TRUE
tr A0A1B1A683 A0A1B1A683_9RHOB	Acetoin utilization protein OS=Tritonibacter mobilis F1926	0.998794	TRUE
tr A0A1B1A8K5 A0A1B1A8K5_9RHOB	Sugar ABC transporter permease OS=Tritonibacter mobilis F1926	0.998759	TRUE
tr A0A1B1A0Q2 A0A1B1A0Q2_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.998712	TRUE
tr A0A1B1A8M1 A0A1B1A8M1_9RHOB	Short-chain dehydrogenase OS=Tritonibacter mobilis F1926	0.998702	TRUE
tr A0A1B1A7S0 A0A1B1A7S0_9RHOB	(S)-ureidoglycine aminohydrolase OS=Tritonibacter mobilis F1926	0.998698	TRUE
tr A0A1B1A8G2 A0A1B1A8G2_9RHOB	LysR family transcriptional regulator OS=Tritonibacter mobilis F1926	0.99866	TRUE
tr A0A1B1A5N0 A0A1B1A5N0_9RHOB	Catalase-peroxidase OS=Tritonibacter mobilis F1926	0.998611	TRUE
tr A0A1B1A6M9 A0A1B1A6M9_9RHOB	Rap1a immunity protein domain-containing protein OS=Tritonibacter mobilis F1926	0.998593	TRUE
tr A0A1B0ZZ41 A0A1B0ZZ41_9RHOB	Integral membrane protein OS=Tritonibacter mobilis F1926	0.998588	TRUE
tr A0A1B0ZZ56 A0A1B0ZZ56_9RHOB	ABC transporter OS=Tritonibacter mobilis F1926	0.998561	TRUE
tr A0A1B1A7P8 A0A1B1A7P8_9RHOB	AAA+ ATPase domain-containing protein OS=Tritonibacter mobilis F1926	0.998524	TRUE
tr A0A1B1AAD3 A0A1B1AAD3_9RHOB	Chromosome partitioning protein ParB OS=Tritonibacter mobilis F1926	0.998497	TRUE
tr A0A1B1A7T6 A0A1B1A7T6_9RHOB	Chitooligosaccharide deacetylase OS=Tritonibacter mobilis F1926	0.998459	TRUE
tr A0A1B1A7R8 A0A1B1A7R8_9RHOB	Acyl-CoA dehydrogenase OS=Tritonibacter mobilis F1926	0.998431	TRUE
tr A0A1B1A192 A0A1B1A192_9RHOB	Alkene reductase OS=Tritonibacter mobilis F1926	0.998427	TRUE
tr A0A1B1A729 A0A1B1A729_9RHOB	Carrier domain-containing protein OS=Tritonibacter mobilis F1926	0.998422	TRUE
tr A0A1B1A603 A0A1B1A603_9RHOB	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG OS=Tritonibacter mobilis F1926	0.998292	TRUE
tr A0A1B1A392 A0A1B1A392_9RHOB	Acetylornithine deacetylase OS=Tritonibacter mobilis F1926	0.998274	TRUE
tr A0A1B1A3S6 A0A1B1A3S6_9RHOB	Protocatechuate 3,4-dioxygenase subunit alpha OS=Tritonibacter mobilis F1926	0.998236	TRUE

tr A0A1B1AAH0 A0A1B1AAH0_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.998193	TRUE
tr A0A1B1A8T2 A0A1B1A8T2_9RHOB	Phosphonate ABC transporter, permease protein PhnE OS=Tritonibacter mobilis F1926	0.99811	TRUE
tr A0A1B1A767 A0A1B1A767_9RHOB	Glyoxalase OS=Tritonibacter mobilis F1926	0.997981	TRUE
tr A0A1B1AAE8 A0A1B1AAE8_9RHOB	Cytochrome c oxidase assembly protein OS=Tritonibacter mobilis F1926	0.99797	TRUE
tr A0A1B1AA39 A0A1B1AA39_9RHOB	D-alanine--poly(Phosphoribitol) ligase OS=Tritonibacter mobilis F1926	0.997836	TRUE
tr A0A1B1A8Y8 A0A1B1A8Y8_9RHOB	Iron dicitrate transport regulator FecR OS=Tritonibacter mobilis F1926	0.997756	TRUE
tr A0A1B1A1A3 A0A1B1A1A3_9RHOB	Glucan biosynthesis protein G OS=Tritonibacter mobilis F1926	0.997662	TRUE
tr A0A1B1A6X9 A0A1B1A6X9_9RHOB	Dihydrodipicolinate synthase family protein OS=Tritonibacter mobilis F1926	0.997594	TRUE
tr A0A1B0ZZJ5 A0A1B0ZZJ5_9RHOB	Sarcosine oxidase subunit alpha OS=Tritonibacter mobilis F1926	0.997544	TRUE
tr A0A1B1A6W1 A0A1B1A6W1_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.997456	TRUE
tr A0A1B1A3F5 A0A1B1A3F5_9RHOB	Mannose-6-phosphate isomerase OS=Tritonibacter mobilis F1926	0.997406	TRUE
tr A0A1B1AAB5 A0A1B1AAB5_9RHOB	DUF1440 domain-containing protein OS=Tritonibacter mobilis F1926	0.997297	TRUE
tr A0A1B1A1J2 A0A1B1A1J2_9RHOB	Uridylate kinase OS=Tritonibacter mobilis F1926	0.997113	TRUE
tr A0A1B1A3Z1 A0A1B1A3Z1_9RHOB	3-carboxy-cis,cis-muconate cycloisomerase OS=Tritonibacter mobilis F1926	0.996899	TRUE
tr A0A1B1A605 A0A1B1A605_9RHOB	Flagellin OS=Tritonibacter mobilis F1926	0.99686	TRUE
tr A0A1B1A1D6 A0A1B1A1D6_9RHOB	DUF1326 domain-containing protein OS=Tritonibacter mobilis F1926	0.996805	TRUE
tr A0A1B1A011 A0A1B1A011_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.996793	TRUE
tr A0A1B1A753 A0A1B1A753_9RHOB	Recombinase zinc beta ribbon domain-containing protein OS=Tritonibacter mobilis F1926	0.996717	TRUE
tr A0A1B1A5Y4 A0A1B1A5Y4_9RHOB	Protein-export protein SecB OS=Tritonibacter mobilis F1926	0.996537	TRUE
tr A0A1B1A691 A0A1B1A691_9RHOB	Ferrochelatase OS=Tritonibacter mobilis F1926	0.996508	TRUE
tr A0A1B1A520 A0A1B1A520_9RHOB	peptidoglycan glycosyltransferase OS=Tritonibacter mobilis F1926	0.996453	TRUE
tr A0A1X9HWB1 A0A1X9HWB1_9CAUD	Uncharacterized protein OS=Ruegeria phage 45A6	0.996406	TRUE
tr A0A1B1A2M3 A0A1B1A2M3_9RHOB	Competence protein TfoX OS=Tritonibacter mobilis F1926	0.996394	TRUE
tr A0A1B1A6I9 A0A1B1A6I9_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.996299	TRUE
tr A0A1B0ZZ45 A0A1B0ZZ45_9RHOB	2-(1,2-epoxy-1,2-dihydrophenyl)acetyl-CoA isomerase OS=Tritonibacter mobilis F1926	0.996287	TRUE
tr A0A1B1A159 A0A1B1A159_9RHOB	Amidase OS=Tritonibacter mobilis F1926	0.996179	TRUE
tr A0A1B1A8H3 A0A1B1A8H3_9RHOB	Sodium symporter small subunit domain-containing protein OS=Tritonibacter mobilis F1926	0.996118	TRUE
tr A0A1B0ZY12 A0A1B0ZY12_9RHOB	Alkyl hydroperoxide reductase C OS=Tritonibacter mobilis F1926	0.996072	TRUE
tr A0A1B1A0W6 A0A1B1A0W6_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.996023	TRUE
tr A0A1B0ZY08 A0A1B0ZY08_9RHOB	Gamma-glutamyltransferase OS=Tritonibacter mobilis F1926	0.995945	TRUE

tr A0A1B1A5J1 A0A1B1A5J1_9RHOB	Pyruvate dehydrogenase E1 component OS=Tritonibacter mobilis F1926	0.995937	TRUE
tr A0A1B1A7L5 A0A1B1A7L5_9RHOB	Cytochrome OS=Tritonibacter mobilis F1926	0.995926	TRUE
tr A0A1B1A798 A0A1B1A798_9RHOB	Major royal jelly protein OS=Tritonibacter mobilis F1926	0.995891	TRUE
tr A0A1B0ZZA9 A0A1B0ZZA9_9RHOB	Glutathione S-transferase OS=Tritonibacter mobilis F1926	0.99579	TRUE
tr A0A1B1A0W1 A0A1B1A0W1_9RHOB	Sulfotransferase OS=Tritonibacter mobilis F1926	0.995785	TRUE
tr A0A1B1A751 A0A1B1A751_9RHOB	Transketolase OS=Tritonibacter mobilis F1926	0.995714	TRUE
tr A0A1B1A3R4 A0A1B1A3R4_9RHOB	Diaminobutyrate--2-oxoglutarate transaminase OS=Tritonibacter mobilis F1926	0.995543	TRUE
tr A0A1B1A979 A0A1B1A979_9RHOB	Aspartate carbamoyltransferase OS=Tritonibacter mobilis F1926	0.995395	TRUE
tr A0A1B1A590 A0A1B1A590_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.995315	TRUE
tr A0A1B1A8X5 A0A1B1A8X5_9RHOB	Iron ABC transporter permease OS=Tritonibacter mobilis F1926	0.995062	TRUE
tr A0A1B0ZZ06 A0A1B0ZZ06_9RHOB	RNA polymerase sigma factor OS=Tritonibacter mobilis F1926	0.995001	TRUE
tr A0A1B1A6L7 A0A1B1A6L7_9RHOB	Winged helix domain-containing protein OS=Tritonibacter mobilis F1926	0.994888	TRUE
tr A0A1B1A7G8 A0A1B1A7G8_9RHOB	1,2-phenylacetyl-CoA epoxidase subunit B OS=Tritonibacter mobilis F1926	0.994836	TRUE
tr A0A1B1A7N0 A0A1B1A7N0_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.99461	TRUE
tr A0A1B0ZZJ2 A0A1B0ZZJ2_9RHOB	Glyoxalase-related protein domain-containing protein OS=Tritonibacter mobilis F1926	0.994531	TRUE
tr A0A1B1AA77 A0A1B1AA77_9RHOB	phosphoglycerate mutase (2,3-diphosphoglycerate-dependent) OS=Tritonibacter mobilis F1926	0.994495	TRUE
tr A0A1B1A617 A0A1B1A617_9RHOB	Ribokinase OS=Tritonibacter mobilis F1926	0.994293	TRUE
tr A0A1B1A2P8 A0A1B1A2P8_9RHOB	Protein adenylyltransferase SelO OS=Tritonibacter mobilis F1926	0.993652	TRUE
tr A0A1B1A8M5 A0A1B1A8M5_9RHOB	RecA/RadA recombinase OS=Tritonibacter mobilis F1926	0.99317	TRUE
tr A0A1B1A9R0 A0A1B1A9R0_9RHOB	Aminotransferase OS=Tritonibacter mobilis F1926	0.992668	TRUE
tr A0A1B1A1E3 A0A1B1A1E3_9RHOB	Bleomycin resistance protein OS=Tritonibacter mobilis F1926	0.992495	TRUE
tr A0A1B1A3Q5 A0A1B1A3Q5_9RHOB	L-2,4-diaminobutyric acid acetyltransferase OS=Tritonibacter mobilis F1926	0.992422	TRUE
tr A0A1B1A3T2 A0A1B1A3T2_9RHOB	Type VI secretion protein OS=Tritonibacter mobilis F1926	0.992374	TRUE
tr A0A1B1A5S0 A0A1B1A5S0_9RHOB	Terminase OS=Tritonibacter mobilis F1926	0.992319	TRUE
tr A0A1B1A7Y2 A0A1B1A7Y2_9RHOB	S-adenosylmethionine synthase OS=Tritonibacter mobilis F1926	0.992317	TRUE
tr A0A1B1A1D4 A0A1B1A1D4_9RHOB	OpgC protein OS=Tritonibacter mobilis F1926	0.992258	TRUE
tr A0A1B1A9P4 A0A1B1A9P4_9RHOB	Class I poly(R)-hydroxyalkanoic acid synthase OS=Tritonibacter mobilis F1926	0.991807	TRUE
tr A0A1B1A9P1 A0A1B1A9P1_9RHOB	Cold-shock protein OS=Tritonibacter mobilis F1926	0.991766	TRUE
tr A0A1B1A5S3 A0A1B1A5S3_9RHOB	Cytochrome b OS=Tritonibacter mobilis F1926	0.991723	TRUE
tr A0A1B1A9Q0 A0A1B1A9Q0_9RHOB	CENP-V/GFA domain-containing protein OS=Tritonibacter mobilis F1926	0.991492	TRUE

tr A0A1B1A6D5 A0A1B1A6D5_9RHOB	ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	0.991461	TRUE
tr A0A1B1A177 A0A1B1A177_9RHOB	MBL fold metallo-hydrolase OS=Tritonibacter mobilis F1926	0.991174	TRUE
tr A0A1B1A2F6 A0A1B1A2F6_9RHOB	Lauroyl acyltransferase OS=Tritonibacter mobilis F1926	0.990619	TRUE
tr A0A1B1A732 A0A1B1A732_9RHOB	Chitooligosaccharide deacetylase OS=Tritonibacter mobilis F1926	0.990498	TRUE
tr A0A1B1A7F7 A0A1B1A7F7_9RHOB	Glycosyl transferase family 28 C-terminal domain-containing protein OS=Tritonibacter mobilis F1926	0.990427	TRUE
tr A0A1B0ZYK0 A0A1B0ZYK0_9RHOB	Zn-dependent hydrolase OS=Tritonibacter mobilis F1926	0.990379	TRUE
tr A0A1B1A7R9 A0A1B1A7R9_9RHOB	ATP-dependent protease ATP-binding subunit OS=Tritonibacter mobilis F1926	0.990193	TRUE
tr A0A1B1A749 A0A1B1A749_9RHOB	C4-dicarboxylate ABC transporter permease OS=Tritonibacter mobilis F1926	0.990023	TRUE
tr A0A1B1A4U2 A0A1B1A4U2_9RHOB	Aminoglycoside phosphotransferase domain-containing protein OS=Tritonibacter mobilis F1926	0.989843	TRUE
tr A0A1B0ZYZW3 A0A1B0ZYZW3_9RHOB	LacI family transcriptional regulator OS=Tritonibacter mobilis F1926	0.989184	TRUE
tr A0A1B1A7H7 A0A1B1A7H7_9RHOB	Chloramphenicol acetyltransferase OS=Tritonibacter mobilis F1926	0.989138	TRUE
tr A0A1B1A8E3 A0A1B1A8E3_9RHOB	Transporter OS=Tritonibacter mobilis F1926	0.98861	TRUE
tr A0A1B1A9L0 A0A1B1A9L0_9RHOB	Peptidoglycan-binding protein OS=Tritonibacter mobilis F1926	0.98851	TRUE
tr A0A1B0ZZZ5 A0A1B0ZZZ5_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.988508	TRUE
tr A0A1B0ZYL4 A0A1B0ZYL4_9RHOB	Small ribosomal subunit protein uS3 OS=Tritonibacter mobilis F1926	0.988097	TRUE
tr A0A1B1A3L2 A0A1B1A3L2_9RHOB	Beta-ketoacyl-ACP synthase III OS=Tritonibacter mobilis F1926	0.987942	TRUE
tr A0A1B1A7F8 A0A1B1A7F8_9RHOB	Acyl-CoA transferase OS=Tritonibacter mobilis F1926	0.98764	TRUE
tr A0A1B0ZYG0 A0A1B0ZYG0_9RHOB	Phosphoglycerate mutase OS=Tritonibacter mobilis F1926	0.987429	TRUE
tr A0A1B1A1N5 A0A1B1A1N5_9RHOB	tRNA-dihydrouridine synthase OS=Tritonibacter mobilis F1926	0.987299	TRUE
tr A0A1B1A6F7 A0A1B1A6F7_9RHOB	Dual-specificity RNA methyltransferase RlmN OS=Tritonibacter mobilis F1926	0.987099	TRUE
tr A0A1B1A450 A0A1B1A450_9RHOB	GntR family transcriptional regulator OS=Tritonibacter mobilis F1926	0.986644	TRUE
tr A0A1B1A888 A0A1B1A888_9RHOB	Ribose ABC transporter permease OS=Tritonibacter mobilis F1926	0.986494	TRUE
tr A0A1B1A9W1 A0A1B1A9W1_9RHOB	Acyl-CoA dehydrogenase OS=Tritonibacter mobilis F1926	0.986268	TRUE
tr A0A1B1A165 A0A1B1A165_9RHOB	Integrase OS=Tritonibacter mobilis F1926	0.986233	TRUE
tr A0A1B1A2J4 A0A1B1A2J4_9RHOB	DUF1223 domain-containing protein OS=Tritonibacter mobilis F1926	0.986156	TRUE
tr A0A1B1AAL8 A0A1B1AAL8_9RHOB	Electron transporter OS=Tritonibacter mobilis F1926	0.985544	TRUE
tr A0A1B1A692 A0A1B1A692_9RHOB	Chemotaxis protein MotB OS=Tritonibacter mobilis F1926	0.985036	TRUE
tr A0A1B1A485 A0A1B1A485_9RHOB	Primosomal protein N' OS=Tritonibacter mobilis F1926	0.982768	TRUE
tr A0A1B1AAK8 A0A1B1AAK8_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.981874	TRUE
tr A0A1B1A5R4 A0A1B1A5R4_9RHOB	Thoeris protein ThsB TIR-like domain-containing protein OS=Tritonibacter mobilis F1926	0.981646	TRUE

tr A0A1B1A3F2 A0A1B1A3F2_9RHOB	GcrA cell cycle regulator OS=Tritonibacter mobilis F1926	0.981469	TRUE
tr A0A1B1A764 A0A1B1A764_9RHOB	HTH gntR-type domain-containing protein OS=Tritonibacter mobilis F1926	0.980927	TRUE
tr A0A1B1A5P2 A0A1B1A5P2_9RHOB	Type I secretion protein OS=Tritonibacter mobilis F1926	0.980822	TRUE
tr A0A1B1A0T8 A0A1B1A0T8_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.979467	TRUE
tr A0A1B1A822 A0A1B1A822_9RHOB	Error-prone DNA polymerase OS=Tritonibacter mobilis F1926	0.977387	TRUE
tr A0A1B1A348 A0A1B1A348_9RHOB	SAM-dependent methyltransferase OS=Tritonibacter mobilis F1926	0.977093	TRUE
tr A0A1B0ZYJ7 A0A1B0ZYJ7_9RHOB	Rhamnosyl transferase OS=Tritonibacter mobilis F1926	0.976974	TRUE
tr A0A1B1A9J3 A0A1B1A9J3_9RHOB	Heme ABC transporter ATP-binding protein OS=Tritonibacter mobilis F1926	0.976625	TRUE
tr A0A1B1A475 A0A1B1A475_9RHOB	Thioesterase OS=Tritonibacter mobilis F1926	0.976233	TRUE
tr A0A1B1A1I8 A0A1B1A1I8_9RHOB	Methylenetetrahydrofolate--tRNA-(uracil-5-)-methyltransferase TrmFO OS=Tritonibacter mobilis F1926	0.976044	TRUE
tr A0A1B0ZYJ6 A0A1B0ZYJ6_9RHOB	DL-methionine transporter permease subunit OS=Tritonibacter mobilis F1926	0.975795	TRUE
tr A0A1B1A686 A0A1B1A686_9RHOB	NAD(P)-dependent oxidoreductase OS=Tritonibacter mobilis F1926	0.975453	TRUE
tr A0A1B0ZZU8 A0A1B0ZZU8_9RHOB	Sarcosine oxidase subunit beta OS=Tritonibacter mobilis F1926	0.975399	TRUE
tr A0A1B1A5X2 A0A1B1A5X2_9RHOB	ABC-three component systems C-terminal domain-containing protein OS=Tritonibacter mobilis F1926	0.975183	TRUE
tr A0A1B1A3E7 A0A1B1A3E7_9RHOB	NADH-quinone oxidoreductase subunit D OS=Tritonibacter mobilis F1926	0.974847	TRUE
tr A0A1B1A3K9 A0A1B1A3K9_9RHOB	DUF6161 domain-containing protein OS=Tritonibacter mobilis F1926	0.973955	TRUE
tr A0A1B0ZYX1 A0A1B0ZYX1_9RHOB	Ribosomal RNA small subunit methyltransferase I OS=Tritonibacter mobilis F1926	0.972458	TRUE
tr A0A1B1A2A0 A0A1B1A2A0_9RHOB	Gene transfer agent protein OS=Tritonibacter mobilis F1926	0.970343	TRUE
tr A0A1B1A9M6 A0A1B1A9M6_9RHOB	4-aminobutyrate--2-oxoglutarate transaminase OS=Tritonibacter mobilis F1926	0.970256	TRUE
tr A0A1B1A771 A0A1B1A771_9RHOB	EamA domain-containing protein OS=Tritonibacter mobilis F1926	0.970138	TRUE
tr A0A1B1A6I0 A0A1B1A6I0_9RHOB	Acyl carrier protein OS=Tritonibacter mobilis F1926	0.970031	TRUE
tr A0A1B1A0G6 A0A1B1A0G6_9RHOB	Acetyltransferase OS=Tritonibacter mobilis F1926	0.969893	TRUE
tr A0A1B1A6S7 A0A1B1A6S7_9RHOB	D-tagatose 3-epimerase OS=Tritonibacter mobilis F1926	0.969491	TRUE
tr A0A1B1A0U1 A0A1B1A0U1_9RHOB	Sulfotransferase family protein OS=Tritonibacter mobilis F1926	0.965355	TRUE
tr A0A1B1A4L1 A0A1B1A4L1_9RHOB	NAD(P)H-quinone oxidoreductase OS=Tritonibacter mobilis F1926	0.963892	TRUE
tr A0A1B1A6F1 A0A1B1A6F1_9RHOB	N-formylglutamate deformylase OS=Tritonibacter mobilis F1926	0.961762	TRUE
tr A0A1B0ZYN6 A0A1B0ZYN6_9RHOB	ABC transporter permease OS=Tritonibacter mobilis F1926	0.959975	TRUE
tr A0A1B1A875 A0A1B1A875_9RHOB	Malonyl-CoA synthase OS=Tritonibacter mobilis F1926	0.95856	TRUE
tr A0A1B1A3D0 A0A1B1A3D0_9RHOB	Acetylornithine aminotransferase OS=Tritonibacter mobilis F1926	0.957044	TRUE

tr A0A1X9HX62 A0A1X9HX62_9CAUD	Cell division protein FtsK OS=Ruegeria phage 45A6	0.956889	TRUE
tr A0A1B1A559 A0A1B1A559_9RHOB	DUF1468 domain-containing protein OS=Tritonibacter mobilis F1926	0.956318	TRUE
tr A0A1B1A0S0 A0A1B1A0S0_9RHOB	ferredoxin--NADP(+) reductase OS=Tritonibacter mobilis F1926	0.956276	TRUE
tr A0A1B1A9X0 A0A1B1A9X0_9RHOB	Molybdopterin-guanine dinucleotide biosynthesis protein MobC OS=Tritonibacter mobilis F1926	0.95459	TRUE
tr A0A1B1A7J9 A0A1B1A7J9_9RHOB	AAA family ATPase OS=Tritonibacter mobilis F1926	0.954384	TRUE
tr A0A1B1A873 A0A1B1A873_9RHOB	Decarboxylase OS=Tritonibacter mobilis F1926	0.953346	TRUE
tr A0A1B1A768 A0A1B1A768_9RHOB	Uncharacterized protein OS=Tritonibacter mobilis F1926	0.952106	TRUE