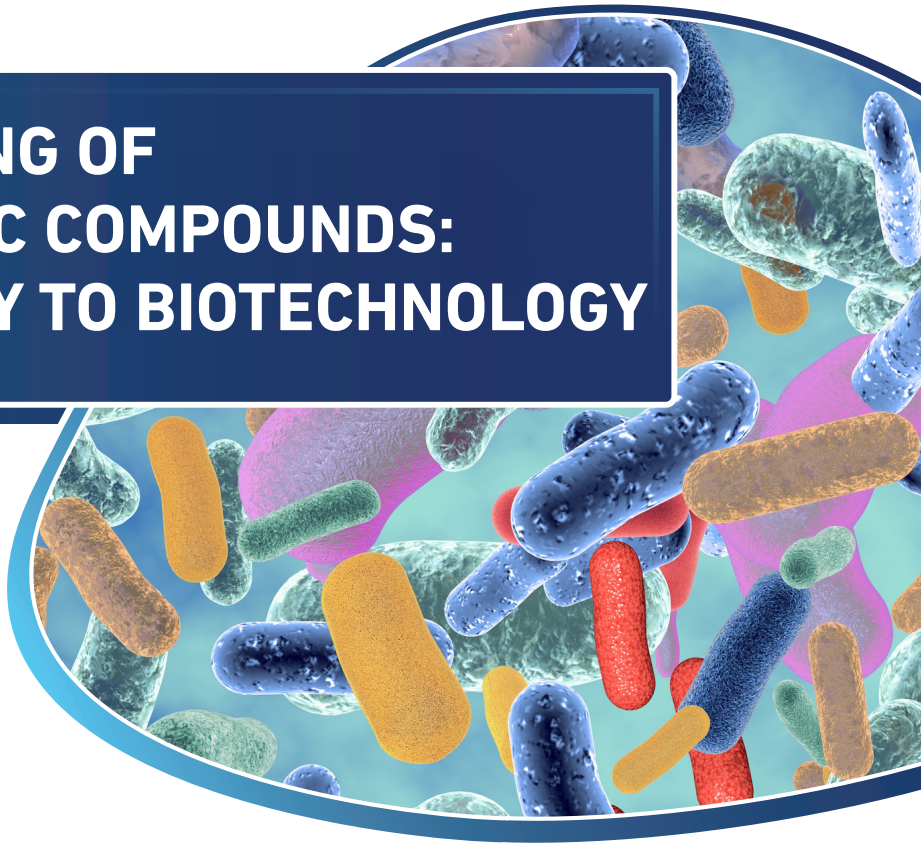
MICROBIOLOGY SOCIETY

MICROBIAL CYCLING OF VOLATILE ORGANIC COMPOUNDS: BIOGEOCHEMISTRY TO BIOTECHNOLOGY



POSTER ABSTRACT BOOK

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Novel Alphaproteobacteria Responsible for Chloromethane Degradation in a Forest Soil – How far can we go with SIP and MAG reconstruction?

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Abstract

Chloromethane is the most abundant halogenated organic compound in the atmosphere. Its global budget is imbalanced due to unrecognized sinks that can be top soils[A]. Anthropogenic CH_3Cl sources became negligible after the Montreal Protocol in 1987 whereas natural sources became substantial for its global budget.

Aiming for a link between organismal identity, metabolism and ecosystem relevance, we combined DNA SIP, metagenomics, and gas flux measurements in soil samples and mapped the occurrence of new MAGs in global metagenomes.

Chloromethane consumption occurred in organic, mineral soil, leaf litter and senescent leaves as determined in microcosms. Highest rates occurred in organic soil and (0.59-1.96 mmol $CH_3Cl\ g_{DW}^{-1}\ h^{-1}$) and senescent leaves (0.32-2.05 mmol $CH_3Cl\ g_{DW}^{-1}\ h^{-1}$). Thus, top soil layers were evidently key sink compartments of a forest ecosystem. ^{13}C-labelled MAGs suggested strains closely related to the family *Beijerinckiaceae* to be involved in chloromethane degradation and thus, confirmed the taxonomic identity of a previous study[B]. The new partial genome may represent a new methylotroph family of *Alphaproteobacteria*. Nonetheless, based MAG data the only to date known pathway for aerobic chloromethane degradation (encoded on the *cmu* operon) was not detectable[C]. Hence, raising the question on the effectiveness of MAG reconstruction based on current bioinformatics tools.

[A] Jaeger N et al. 2018. ***J Environ Qual*** 47, 2, 254-262. [B] Chaignaud P et al. 2018. ***ISME J*** 12, 11, 2681-2693. [C] Bringel F et al. 2019. ***Curr Iss Mol Biol***, 33, 149-172

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Experimental evolution of methylotrophic strains for growth with chloromethane

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Abstract

Chloromethane (CH_3Cl) can be used as growth substrate by some facultative methylotrophic bacteria. However, providing methylotrophic strains with the *cmu* genes for CH_3Cl utilization is not sufficient for them to grow with CH_3Cl[1].

An experimental evolution approach was used to identify adaptations for growth with CH_3Cl. Plasmid pJM105 with the *cmu* gene cluster of *Hyphomicrobium* sp. MC1 was introduced into *Methylorubrum extorquens* strains AM1 and DM4, as a proxy to mimic horizontal gene transfer of the *cmu* pathway. Evolved cultures able to grow with CH_3Cl as the sole carbon and energy source were obtained after about 200 generations, following weekly transfers into fresh medium with CH_3Cl as the main carbon source. A total of 67 mutations (SNPs and small indels) were found in 48 loci compared to the original strains in the 13 evolved strains selected for genome sequencing. Larger deletion and IS element insertions were also identified. AM1-derived evolved strains featured different amino acid substitutions in the CmuA protein of CH_3Cl dehalogenase. Growth with CH_3Cl in both AM1 and DM4 genetic contexts was often associated with different mutations that inactivate *ftfL* coding an enzyme essential for assimilation of formaldehyde-derived carbon.

Our approach highlights some features of the *cmu* pathway for CH_3Cl utilisation, and contributes to define possible modes of bacterial adaptation towards use of a new growth substrate following acquisition of the corresponding pathway by horizontal gene transfer.

[1]Michener JK, Vuilleumier S, Bringel F, Marx CJ (2016) *Front Microbiol* **7**, art. 1116.

3

Smelly sediment dwellers —— Characterization of microorganisms driving DMSO reduction in anoxic sediments

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Abstract

Microorganisms in marine sediments have a significant influence in controlling the amount of dimethyl sulfide (DMS) that is emitted to the atmosphere since the production and degradation of DMS depend on the activities of diverse microorganisms and a number of different metabolic processes. One of these is the respiratory reduction of dimethylsulfoxide (DMSO) to DMS under anoxic conditions. Although aspects of the biochemistry of DMSO reduction have been characterised, the organisms driving this process in the environment are still poorly understood. This project aims to characterize DMSO-reducing microorganisms, both in terms of their identity and activity in the environment, as well as their physiology and genetics. In particular, the ability of certain sulfate-reducing bacteria (SRB) to couple the catabolism of organic carbon with the respiratory reduction of DMSO will be investigated. SRBs containing DMSO reductases were selected through data mining and screening, and their ability to reduce DMSO was tested during anaerobic cultivation under various conditions. Ongoing analyses are addressing the identity of the reductase genes responsible for DMSO reduction and their regulation in response to the availability of different terminal electron acceptors. This will help to understand how the ability to reduce DMSO impacts their ecology and environmental distribution.

4

In vivo genome editing in type I and II methanotrophs using a CRISPR/Cas9 system

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Abstract

Methanotrophic bacteria are Gram-negative, aerobic organisms that use methane as sole source of carbon and energy. They play a pivotal role in regulation of methane concentration in the environment and additionally have important biotechnological applications in the production of single cell protein and the bioplastic precursor polyhydroxybutyrate (PHB). Methanotrophs are broadly divided into type I and II based on the method of carbon assimilation they employ and the location of their intracytoplasmic membrane. In this study, we constructed and exemplified a CRISPR/Cas9 gene editing system and used it to successfully make gene deletions and insertions in the type I methanotroph *Methylococcus capsulatus* Bath and the type II methanotroph *Methylocystis parvus* OBBP. High frequencies of gene deletion (up to 90%) and insertion (up to 50%) were achieved in both methanotrophs. The outcome of our work demonstrated for the first time an efficient CRISPR/Cas9 system in methanotrophs.

5

Genetic potential of aerobic methanotrophs for arsenic transformation and resistance

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Abstract

Aerobic methanotrophs are increasingly recognised to be capable of environmentally significant transformation of metals and metalloids, contributing methane-driven cycling of diverse minor elements in the environment and the possibility of using methane as the feedstock for methane-fuelled bioremediation and transformations of biotechnological value. Here we have analysed available genomes from representatives across the biodiversity of known aerobic methanotrophs to assess their ability to transform, resist and respond to arsenic species. All genomes analysed suggest the capacity to reduce arsenate to arsenite and to expel the arsenite from the cell. There is substantial diversity of the abundance of arsenic-related genes and their genetic organisation. For example, *Methylococcus capsulatus* Bath may have just two isolated genes involved in reduction and efflux of arsenic species, whereas *Methylosinus trichosporium* OB3b has a cluster of six genes apparently encoding arsenate reduction and efflux of arsenite and regulatory functions. *Methylocystis* sp. Rothwell has the largest number of arsenic-related genes observed, with three gene clusters potentially involved in arsenate reduction and arsenite efflux. These results suggest a significant role for methanotrophs in global arsenic transformations and may be important in developing methanotrophs for bioremediation and other applications.

6

Signs of Extra-Terrestrial life? Applying Volatile Organic Compound (VOC) Analysis to Environmental and Astrobiological Targets

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Abstract

Background: VOCs from humans and plants are widely studied, but their use in life detection for astrobiological targets is still in its infancy. With orbiting spacecraft (e.g. ExoMars) and the James Webb Space telescope capable of detecting VOCs within the martian atmosphere and beyond, it is critical to understand the production and cycling of VOCs within habitable environments. Here, we use samples from a terrestrial analogue environment (a location that resembles geological/biological conditions similar to other planetary bodies) to investigate VOCs associated with the drying of lakes similar to those identified on Mars.

Methods: Lake sediment from the Makgadikgadi Pans, Botswana was homogenised with lake water media and decanted into microcosms to simulate the lake drying environment. An untreated (biotic) and a killed control (abiotic) experiment were set up. Microcosms were incubated at 30°C in controlled growth chambers with a diurnal cycle. Headspace VOCs were regularly sampled over 3 weeks with thermal desorption (TD) tubes. These tubes were analysed using TD Gas Chromatography-Mass Spectrometry (TD-GC-MS). VOCs were identified using dedicated spectral libraries and retention times.

Results: Preliminary results indicate the presence of a variety of VOC profiles from biotic and abiotic samples. Total VOC profiles (rather than individual signature compounds) could offer a fingerprint unique to specific environmental conditions or microbial community structures that could be used by returned missions searching for indication of life's presence. Machine learning classification software will be used to further explore the data, and to aid identification of these signatures.

7

Real-time monitoring of sulphur BVOCs emitted from Ulva Intestinalis (Green Gutweed) using Soft Chemical Ionisation Mass Spectrometry (SCIMS)

Fraser Reich, Daniel Blenkhorn

Kore Technology Ltd., Ely, United Kingdom

Abstract

Real-time monitoring of sulphur BVOCs emitted from Ulva Intestinalis (Green Gutweed) using Soft Chemical Ionisation Mass Spectrometry (SCIMS)

Fraser Reich and Daniel Blenkhorn
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Soft chemical ionisation mass spectrometry (SCI-MS) is a technique offering sensitive analysis of VOCs down to ppt levels in real-time, making it invaluable when seeking to follow concentration changes over periods of minutes or hours.

Whereas a GC-MS uses a pre-separation stage to separate VOCs in a mixture, in a SCIMS instrument the VOCs appear in the mass spectrum simultaneously (thus enabling real-time analysis). Using a high mass resolution TOF spectrometer the exact masses of the observed peaks are used to identify the detected ions, with confirmation from isotope patterns and a peak fitting algorithm.

In this poster we show mass spectral data acquired from the seaweed Ulva Intestinalis (green gutweed) to demonstrate real-time detection of H_2S, methanethiol and dimethyl sulphide from small amounts of the seaweed (<1g) with no preparation other than rinsing off the seawater beforehand. The BVOCs are sampled by a pipe close to the sample that draws in air at 2 atmosphere cc/second. In an example to demonstrate this capability we monitored the emissions from 0.1g of the ulva intestinalis over a period of 16 hours, observing the initially high release rate of various sulphur molecules followed by a steady decay in their signals as the sample dried out.

The poster is offered to stimulate discussion on other potential applications in microbiology.

8

Volatile metabolite profiling of morphological changes in *Candida albicans*

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Abstract

Candida albicans is a versatile fungus, known for its ability to switch between yeast and hyphal forms based on external stimuli. The sesquiterpenoid farnesol has shown to regulate this dimorphic switch however other volatile organic compounds may also be involved in signalling or other metabolic pathways. In this study we will apply an untargeted metabolomics approach to profile the volatilome of *C. albicans* cultures under conditions which promote filamentous growth. A method will be developed to capture volatile metabolites from the headspace and supernatant of *C. albicans* cultures using sampling probes thermal desorption-gas chromatography-mass spectrometry. The results from this study may be useful for multiple industrial and clinical biotechnology applications.

9

The role of organosulphur compounds in marine chemosynthetic symbioses

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Abstract

Organosulphur transformations are crucial for various marine symbiotic systems with diverse roles including communication, attraction, and nutrition. Clams of the family lucinidae are the most species-rich family of animals hosting chemosynthetic sulphur-oxidizing bacteria, which they harbour inside gill epithelial cells and rely on for nutrition. Lucinid clams, together with their symbionts, are important players in both sulphur and carbon cycling in marine sediments worldwide. They may be also involved in the organosulphur transformations, as a previous study reported remarkably high expression of a DMSO reductase gene, leading to potential production of the climate-active gas dimethylsulphide. However, these observations have never been tested experimentally. In particular, little is known about the role of this and other organosulphur transformations by sulphur-oxidizing symbionts, or the potential involvement of the host in providing its symbionts with organosulphur compounds. To address this, we measured the concentration of dimethylsulfoniopropionate (DMSP) in two lucinid host species Loripes orbiculatus and Loripinus fragilis at three sites in the Mediterranean Sea (one in Elba, Italy; and two in Piran, Slovenia) using alkaline lysis and gas chromatography. We measured values up to 356 µmol/g in the symbiont-harbouring gills and 247 µmol/g in the symbiont-free visceral mass and foot. These values are significantly higher than any previously reported DMSP-accumulating bivalve which may suggest active synthesis of the DMSP by the host. We hypothesize that DMSP may be an important compound in marine sulphur-oxidizing symbioses and we are currently investigating the possibility that DMSP may be produced by the animal host.

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