Project Title: Cave exploration: shedding light on organic sulfur cycling in a terrestrial underground chemosynthetic system

Host University: University of Warwick

Theme: Organisms & Ecosystems

Supervisory team:
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Project Highlights
- Investigate the organic sulfur cycle of a closed cave ecosystem based on chemosynthesis
- Apply stable isotope and metagenomics approaches to characterise uncultivated bacteria

Overview
Movile Cave (Romania) was discovered in 1986 during the construction of an artificial shaft dug for geological investigations. Since its discovery, studies have proved that the subterranean ecosystem is completely isolated with no surface inputs. Despite the lack of photosynthetically-fixed carbon, the ecosystem hosts a diverse range of invertebrates, including worms, insects, spiders and crustaceans (Sarbu et al 1996, Sarbu 2000). The upper passages of the cave (~200 m long; Fig 1) are dry (as a consequence of the lack of water infiltrating from the surface), while the lower level (~40 m long) is partly flooded by thermal waters (pH 7, 21°C), containing hydrogen sulfide, methane and ammonium (Kumaresan et al 2014). The cave atmosphere contains 1-2% CH₄, 2.5% CO₂ and 7-10% oxygen. Air bells in the cave (Fig 1) create an ecological niche on the surface of the water where bacteria in floating microbial mats oxidise reduced sulfur (S) compounds and CH₄ using oxygen from the cave atmosphere.

Methylotrophy and autotrophy are the major microbial processes in Movile Cave that fix carbon and form the basis of the food web in this ecosystem. Previous work used molecular ecology techniques to characterise the diversity of aerobic methylotrophic (utilizing CH₄ and methylated amines (MA) as sole C source) and autotrophic (fixing CO₂) bacteria living in the microbial mats (Chen et al 2009, Hutchens et al 2004, Kumaresan et al 2014, Kumaresan et al 2018, Wischer et al 2015).

Figure 1: Cross section of Movile Cave and a schematic representation of some major food-web interactions.

Despite these first studies on specific microbial functional groups, little is known about the cycling of organic sulfur compounds in the food web within the microbial mats. Previous work on methylated
amines showed that these compounds, which are released from degrading biomass of microbial mats, can serve as both C and N source (Wischer et al 2015). Similarly, the degradation of biomass is likely to produce organic sulfur compounds which would be further metabolised by specialised microorganisms to complete the sulfur cycle of the Movile Cave. In this project, you will investigate the diversity of microorganisms that cycle organic sulfur compound in this ecosystem.

**Methodology**
You will use a range of experimental and analytical approaches, including microbiological, molecular biological and bioinformatic methods to isolate and characterise microorganisms and microbial communities from Movile Cave samples. There will be scope for physiological characterisation, genome sequencing and potentially genetic analysis of isolated microorganisms. You will analyse microbial community composition and function based on application of stable isotope probing with $^{13}\text{C}$-labelled compounds and high throughput sequencing approaches (amplicon sequencing as well as metagenomic sequencing) of samples raised in the project. SIP metagenomics will be used to identify $^{13}\text{C}$-DMS assimilating microorganisms (Eyice et al 2015). In addition, amplicon based, targeted analysis of functional genetic markers involved in organic sulfur cycling can be applied, for instance using primers targeting methanethiol oxidase ($\text{mtoX}$) genes can be applied (Eyice et al 2018). Similarly, organisms co-oxidising DMS to DMSO (Lidbury et al 2016) or those reducing DMSO to DMS could be characterised.

**Training and skills**
CENTA students are required to complete 50 days training throughout their PhD including a 10-day placement. In the first year, students will be trained as a single cohort on environmental science, research methods and core skills. Throughout the PhD, training will progress from core skills sets to master classes specific to CENTA research themes. Your project specific training will include a wide range of approaches as required and could include microbial cultivation and characterisation, stable isotope probing, genomics, and studying the diversity and metabolic potential of environmental microbial communities using high throughput sequencing methods and bioinformatics. You will also be trained in relevant analytic chemistry techniques such as gas and ion chromatography.

**Partners and collaboration**
Co-supervisor Dr Deepak Kumaresan (QUB) has ample experience in working with material from Movile Cave and has access to samples from this unique ecosystem.

**Possible timeline**
**Year 1:** Stable isotope probing of microorganisms assimilating methylated sulfur compounds. Bioinformatic analysis of existing metagenomics data for markers of organic sulfur metabolism. Isolation and identification of organic sulfur cycling model organisms from Movile Cave samples and characterisation of their metabolic diversity, genome sequencing of isolates.

**Years 2 & 3:** Further exploration of specific activities in organic sulfur cycle of Movile Cave, e.g. reduction of DMSO and degradation of methanethiol (MT) with use of cultivation-independent techniques.

**Further reading**


**Further details**

If you would like to discuss any aspect of this project, please feel free to contact Hendrik Schäfer (H.Schaefer@warwick.ac.uk), Deepak Kumaresan (D.Kumaresan@qub.ac.uk) or Yin Chen (Yin.Chen.25@warwick.ac.uk). Further information and a link to the online application portal can be found at [https://warwick.ac.uk/fac/sci/lifesci/study/pgr/apply](https://warwick.ac.uk/fac/sci/lifesci/study/pgr/apply)