Applicability of bacterial membrane lipids as environmental proxies in lacustrine settings

1) Study context

Investigating past climatic variations is essential to understand and predict future environmental changes. Paleoclimate studies are chiefly carried out for marine environments because environmental proxies were mainly developed and used for oceanic settings. However, it is essential to develop new proxies also applicable to continental (both terrestrial and aquatic) environments to assess climatic variability over the continents and improve our understanding of both past environment and global climate. Membrane lipids produced by some microorganisms can be used to this aim. Environmental stresses (e.g. modifications of temperature, pH, salinity) can alter membrane fluidity and permeability, thus affecting the proper functioning of the cell. Microorganisms (bacteria, archaea and some eukaryotes) are able to adjust their membrane composition in response to the prevailing environmental conditions in order to maintain an appropriate fluidity and ensure the optimal state of the cellular membrane. Thus, the structure of glycerol dialkyl glycerol tetraethers (GDGTs) – membrane lipids biosynthesised by archaea and some bacteria – is known to be related to environmental parameters[1].

Archaeal membranes are mainly constituted of isoprenoid alkyl chains ether-linked to glycerol. In aquatic settings, the relative abundance of the archaeal GDGTs was correlated with water surface temperature, leading to the development of the TEX_{86} temperature proxy[2]. More recently, GDGTs with branched alkyl chains were discovered in peat. Branched GDGTs (brGDGTs) were suggested to be produced by bacteria and are ubiquitous in aquatic and terrestrial environments. Their analysis in soils[3], peats[4] and lake sediments [5] distributed worldwide showed that their structure varies mainly with air temperature and pH, making them increasingly used as paleoproxies. BrGDGTs are the only microbial organic proxies which can be used for temperature reconstructions in both aquatic and terrestrial settings. Nevertheless, paleoenvironmental data derived from brGDGTs have to be interpreted with care, as these compounds may have allochthonous and autochthonous sources in aquatic settings and their source microorganisms are unknown. The development of new environmental proxies, independent and complementary to brGDGTs, is crucial to improve the reliability/accuracy of continental reconstructions.

3-Hydroxy fatty acids (3-OH FAs), membrane lipids predominantly produced by Gram-negative bacteria (GNB), could be used as such a proxy. They occur in both aquatic and terrestrial environments. Significant local correlations were initially obtained between the relative abundance of these compounds and temperature/pH in 26 soils collected along Mt. Shennongjia (China) [6]. We very recently extended this dataset and similarly observed significant relationships at the global level from ca. 170 soil samples collected all over the world[7, 8, 9]. A similar conclusion was very recently obtained by Wang et al. from a complementary soil dataset (with ca. 190 soil samples)[10]. The recent application of 3-OH FA based-proxies to a Holocene Chinese stalagmite reinforces the potential of these compounds as terrestrial paleoenvironmental proxies [11].

These promising results were mainly obtained from soils[6, 7, 8, 9], with a two very recent, one study in marine settings[12] and the another one in lakes[13]. The influence of environmental parameters on 3-OH FA distribution in lakes and the applicability of 3-OH FAs as temperature/pH proxies in such settings deserve further investigations, based on the high sensitivity of lacustrine archives as recorders of past environmental conditions[14]. It is now essential to obtain accurate information on the adaptation of 3-OH FA source microorganisms to temperature/pH changes in lakes to potentially developing robust and universal (paleo)environmental proxies applicable to both aquatic and terrestrial settings.

2) Details of the project

The main objectives of this work will be to **investigate the applicability of 3-OH FAs as new temperature and pH proxies in lakes and to concomitantly compare these new proxies to the existing ones based on GDGTs**. To this aim, the **source(s) of microbial lipids in** lakes will first be assessed. We then envision to develop **calibrations between temperature/pH and distribution of microbial lipids** in surface lacustrine sediments previously collected worldwide. Last, these calibrations

will be applied to long-term **paleoenvironmental reconstructions** from two alpine lacustrine cores.

A. Origin of microbial lipids in French Alpine lakes

The first part of this project will aim **to assess the origin of microbial membrane lipids (3-OH FAs and GDGTs) in two well-documented lakes** from the French Alps representing contrasted environmental conditions: Lake la Thuile[15] (874 m altitude; 8 m depth), with predominant terrigenous inputs and Lake Robert[16] (1998 m altitude; 12 m depth), with predominant autochthonous production. Sediment traps will be installed at 3 regular depths in each lake. The sampling strategy will involve the seasonal collection (3 times a year over 2 years, when lakes are not frozen) of (i) suspended particulate matter (SPM) from the different sediment traps and (ii) sediment at the bottom of the water column. Temperature data logger will also be installed in the lake at different depth to record temperature variations. Physicochemical water profiles will be systematically determined. After lipid extraction from sediment, 3-OH FAs and GDGTs will be analyzed using gas chromatography coupled to mass spectrometry and high-performance liquid chromatography coupled to mass spectrometry[7], respectively. These analyses will provide key information on the location (upper/bottom part of the water column and/or sediment) and period of preferential lipid production in the two lakes compared to temperature records. Soils will also be collected in the lake watersheds. The comparison of 3-OH FA and GDGT distribution and concentration in soils, suspended matter and sediment will allow constraining the main source(s) (allochthonous/autochthonous) of these specific lipids in the two investigated lakes. In parallel to lipid analyses, the spatio-temporal variability of the microbial biodiversity in the SPM, sediments and soils will be investigated by metabarcoding of 16S rRNA genes. Such work will **bring precise information on the ecology of 3-OH FA and GDGT producers in lakes.**

B. Abundance and distribution of microbial lipids in worldwide lacustrine sediments

The objective of the second part of the project will be to assess **if the conclusions drawn up from Lake Robert and La Thuile sediments can be generalized.** We will first examine the sensitivity of 3-OH FAs to the variations of environmental parameters in lake sediments of the French Alps 3-OH FAs will be analyzed in sediments previously collected from 22 French Alpine lakes located between 250 and 2500 m altitude associated to well-documented and instrumented climate-topo-sequences. The different sampling sites are well-known, as part of them belong to the network of high altitude French alpine lakes (http://www.lacs-sentinelles.org/) and the others at lower altitude (along the Bauges, Belledonne, Vanoise and Ecrins Massifs) are currently investigated for the reconstruction of past environments using environmental DNA and pollen preserved in sediments.

Altitude is the main factor influencing air temperature. We will assess if the cooling of the air with altitude has an effect on the structure of the 3-OH FAs in lake sediments at the regional level and if a local temperature calibration can be developed. 3-OH FAs will also be analyzed in ca. 30 sediment cores from lakes distributed worldwide (including Guadeloupe, Réunion, Turkey, Slovenia, Italy, Peru, Bolivia, Chile, China, Bhutan) and available from the EDYTEM core repository, https://cybercarotheque.fr/). **The final goal will be to develop a global correlation between mean annual air temperature and 3-OH FA distribution in lake sediments available for subsequent paleoclimate reconstructions.** The influence of pH on 3-OH FA distribution will also be investigated statistically.

In parallel to 3-OH FAs, GDGTs will be analyzed in lake samples. The relationships between temperature/pH and brGDGT distribution in lakes will be compared with those obtained from 3-OH FAs. In addition, the temperature and pH estimates derived from the two families of lipids will be compared to values measured in situ. This part of the work will allow assessing the reliability and complementarity of brGDGTs with 3-OH FAs as temperature / pH proxies in lake sediments.

C. Applicability of microbial membrane lipids as paleoproxies in lacustrine archives

The aim of this last part of the project will be to examine the **applicability of microbial membrane lipids as paleoproxies.** Sediment cores will be collected in the deepest parts of lakes La Thuile and Robert, also investigated for the origin of the microbial lipids. These sites were chosen as they were the object of detailed geochemical and paleoenvironmental investigations [15, 16]. The lacustrine cores from Lakes la Thuile and Robert will provide a continuous sediment record of the last 14,000 and 12,000 cal BP, respectively, appropriate for paleoreconstruction. Samples collected along the cores will be

analyzed for their bacterial biodiversity and viability to potentially detect in situ production of 3-OH FAs in sediments. This will help to know if potentially active GNB can also be identified along the cores, and if 3-OH FAs can be produced in deep sediments, as this would bias paleoreconstructions. 3-OH FAs will be analyzed in sediments from the 2 cores. The calibrations previously established between 3-OH FA distribution and temperature/pH will then be applied to these cores to finely reconstruct environmental changes in this Northern alpine area during the Last Glacial / Holocene periods. GDGTs will also be analyzed in these cores. The lipid data from the 2 archives will be interpreted using information already available on the cores and will be compared. This will allow evaluating the applicability of 3-OH FAs as paleoproxies (temperature and pH) in lakes, independently of and concomitantly with GDGT proxies. As these two lake are in the same regional context (45km from each other) we could directly compare short and long term temperature variability reconstitutions.

References

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3) Innovative aspects and link with biology

This PhD project will focus on microbial compounds recognized recently as potential environmental proxies in terrestrial settings but not investigated in detail in lakes yet. It is based on a multidisciplinary approach, combining state-of-the-art molecular biology and organic geochemistry techniques and (paleo)environmental application (earth science), this multidisciplinary approach represents a first step in view of developing a new paleoproxy. The very good knowledge of the sampling area will allow a detailed and accurate investigation of the natural adaptation strategy of lake microorganisms, especially GNB. In the perspective of major environmental changes, the impact of environmental stresses on microorganisms has to be properly documented. It is essential to acquire a better knowledge of the vulnerability and response of organisms to different pressures of global change, including during the past 2 millennium under high human pressure, as it was the case in the French Alps [14, 15]. We will respond to this demand by determining the (Gram-negative) bacterial biodiversity/activity in lakes at different time periods and by assessing if potentially active GNB can also be identified in the lacustrine cores. On the whole, the information obtained from the development of new environmental proxies based on 3-OH FAs will benefit to the whole scientific community, especially microbiologists, organic geochemists, ecologists, paleoclimatologists and earth surface scientists.

4) Supervision

The PhD student to be hired will benefit from the distinct and complementary expertise of the two partner labs: in organic geochemistry and microbiology at METIS (Sorbonne Université) and paleoenvironment from lake sediment at EDYTEM (Université Savoie Mont Blanc). The PhD thesis perfectly fits with the activities and research topics of the two labs. The Biogeochemistry Department of METIS has a recognized expertise in the analysis of microbial lipids (in culture and environmental settings) and characterization of microbial communities. The EDYTEM lab has a perfect knowledge of the sites to be investigated and has the required expertise in the interpretation of the (paleo)environmental data of the project. The laboratories of the two PIs (METIS and EDYTEM) have all the analytical equipment and material available to make the project successful.

5) Profile of the Applicant (skills/diploma…)

The candidate will have a MSc degree in geosciences, analytical chemistry or environmental chemistry. Skills in organic geochemistry would be a plus. The candidate should be motivated by laboratory experiments and field campaigns. He/she should have good skills in English.