

**MEMBRANE LIPIDS OF *CANDIDATUS "NITROSOPUMILUS MARITIMUS"*, A CULTIVATED ISOLATE FROM THE UBIQUITOUS MARINE CRENARCHAEOTA**

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Glycerol diphytanyl glycerol tetraethers (GDGTs) are thought to be the main core membrane lipids of marine Crenarchaeota based on analysis of marine waters and sediments, lakes, soils, peats, the uni-archaeal "culture" *Cenarcheum symbiosum* (Sinninghe Damsté et al., 2002) and enrichment cultures of a marine crenarchaeote from the Wadden Sea (Wuchter et al., 2004). Marine crenarchaeota appear to produce GDGTs with 0 to 3 cyclopentyl-rings (GDGT-0 to GDGT-3, respectively), which are also synthesized by (hyper)thermophilic Crenarchaeota. In addition, crenarchaeol, a unique GDGT containing 4 cyclopentyl-rings and a cyclohexyl-ring, has been attributed to non-thermophilic Crenarchaeota and is considered a biomarker for this group (Sinninghe Damsté et al., 2002). Most research has been focused on the analysis of the core membrane lipids, while few have looked at the polar headgroups which may be attached to these lipids. Unambiguous evidence for the biosynthesis of crenarchaeol by mesophilic Crenarchaeota and their putative polar headgroups is lacking because, until recently, no cultivated and isolated representative of the marine crenarchaeota was available. In this study we analysed both the core GDGT lipid composition and the intact polar lipid composition of the recently, and only, cultivated crenarchaeote, *Candidatus "N. maritimus"* (Könneke et al., 2005).

HPLC/APCI-MS analysis of the extract released after acid hydrolysis of cell material of axenically-grown *Candidatus "N. maritimus"* revealed the presence of a range of GDGT core lipids dominated by crenarchaeol with relatively lower amounts of GDGT-0 and GDGTs 1 to 3. This establishes unambiguously that mesophilic marine group I.1A Crenarchaeota synthesize GDGTs including the characteristic GDGT crenarchaeol. The distribution of the GDGTs is in agreement with the mesophilic temperature, 28 °C, at which this archaeon was cultivated. Interestingly, only very small amounts of the crenarchaeol regio-isomer could be detected similar to what has been reported previously (Wuchter et al.

2004). Indeed, the  $TEX_{86}$  calculated for *Candidatus "N. maritimus"* was  $24.5^{\circ}\text{C}$  and is slightly lower than what would be expected based on core top calibrations.

We then determined the distribution of intact polar lipids in *Candidatus "N. maritimus"* by HPLC/ESI-MS<sup>2</sup> (Fig. 1). Peak cluster I apparently consists of monohexose-GDGTs, containing GDGTs 0-2 and crenarchaeol as core lipids. Peak cluster II was tentatively identified as dihexose-GDGTs with GDGTs 0-3 as core lipids. Peak III appears to be crenarchaeol with a phosphomonohexose headgroup, while peak cluster IV was identified as GDGTs 0-3 and crenarchaeol with both a monohexose and phosphomonohexose headgroup. These results are in agreement with a previous tentative identification of GDGTs with a dihexose headgroup in deep subsurface sediments (Biddle et al., 2006), but show that mesophilic crenarchaeota also make phospho-GDGTs.

Presently we are confirming the tentative structures of the intact polar lipids. In addition, we will examine the impact of culture conditions, specifically temperature, on the composition of the GDGT core and intact polar lipids of *Candidatus "N. maritimus"*.

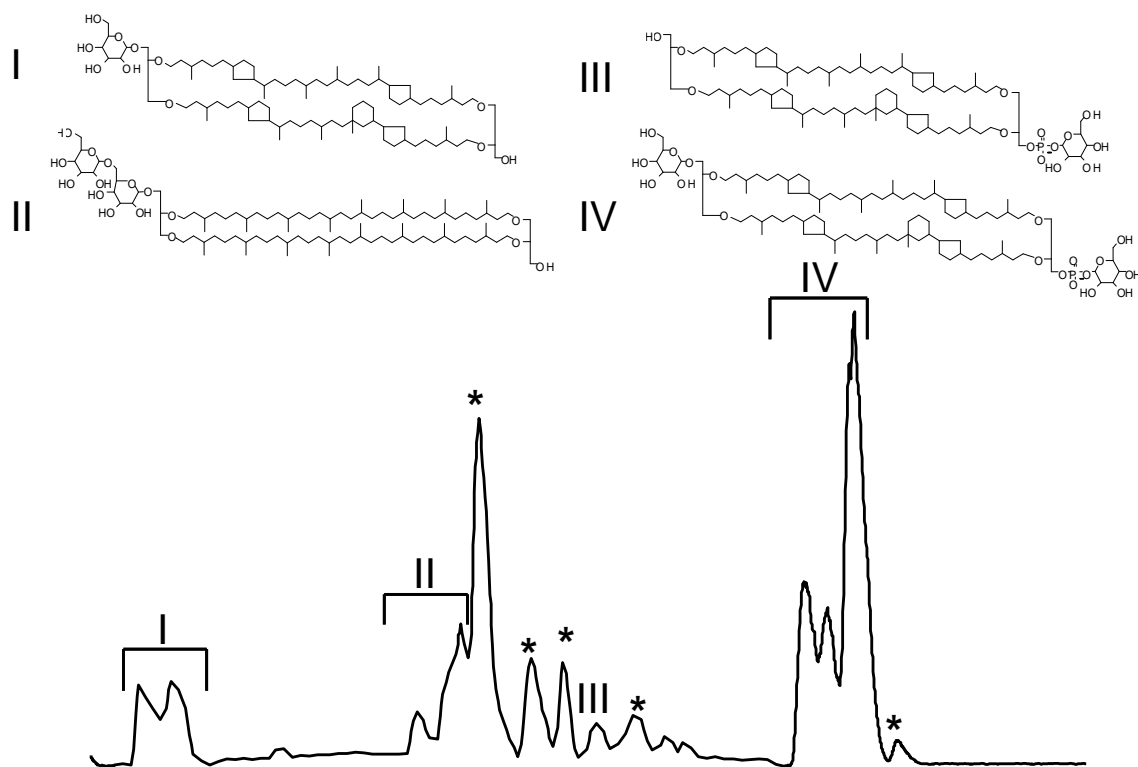


Figure 1. HPLC/ESI-MS base peak chromatogram of *Candidatus "N. maritimus"*.

## REFERENCES

Biddle et al., *PNAS* 2006; **103**: 3846.

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