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ScienceDirect
Organic Geochemistry 38 (2007) 1537–1546
Organic Geochemistry
www.elsevier.com/locate/orggeochem

Towards calibration of the TEX_{86} palaeothermometer for tropical sea surface temperatures in ancient greenhouse worlds

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Received 29 November 2006; received in revised form 15 May 2007; accepted 17 May 2007
Available online 5 June 2007

Abstract

Marine Crenarchaeota from tropical Indian Ocean water were incubated at temperatures ranging from 25 to 40 °C to study the changes in glycerol dibiphytanyl glycerol tetraether (GDGT) membrane lipid composition. The results show that Crenarchaeota were able to thrive at temperatures up to 40 °C. Archaeal 16S ribosomal DNA analysis revealed that different species proliferated with moderate (25 °C) and high (36 °C) optimum growth temperatures. Analysis of the GDGT distribution shows a similar linear correlation of TEX_{86} with incubation temperature as demonstrated previously for incubation experiments at lower temperatures [Wuchter, C., Schouten, S., Coolen, M.J.L., Sinninghe Damsté, J.S., 2004. Paleoceanography 19, PA4028, doi:10.1029/2004PA001041]. Our results show that Crenarchaeota can thrive at temperatures warmer than present day tropical sea surface temperatures, such as the high temperature (up to 40 °C) inferred for tropical oceans in ancient greenhouse worlds. Our results also imply that the TEX_{86} is still applicable in this regime. However, the crenarchaeol regioisomer in the GDGT distribution obtained from the incubation experiments is substantially less than in sediments deposited in exceptionally warm oceans of the geological past, so our laboratory results cannot be directly used to convert TEX_{86} values from these sediments into temperature.

1. Introduction

Proxies for reconstructing ancient sea water temperatures are important for reconstructing the impact of climate change in Earth history. An organic seawater temperature proxy that has become increasingly used is the TetraEther indeX of 86 carbon atoms, the TEX_{86}. This palaeothermometer is based on the relative distribution of archaeal glycerol dibiphytanyl glycerol tetraether (GDGT) lipids (Schouten et al., 2002). They are biosynthesized by marine Crenarchaeota, which occur ubiquitously in the marine water column and are one of the dominant prokaryotes in today's oceans (Karner et al., 2001). Marine Crenarchaeota biosynthesize different types of GDGTs: those containing 0–3 cyclopentane moieties (GDGT 0-3; Fig. 1) and crenarchaeol (GDGT 4) whose molecular structure includes one

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doi:10.1016/j.orggeochem.2007.05.014

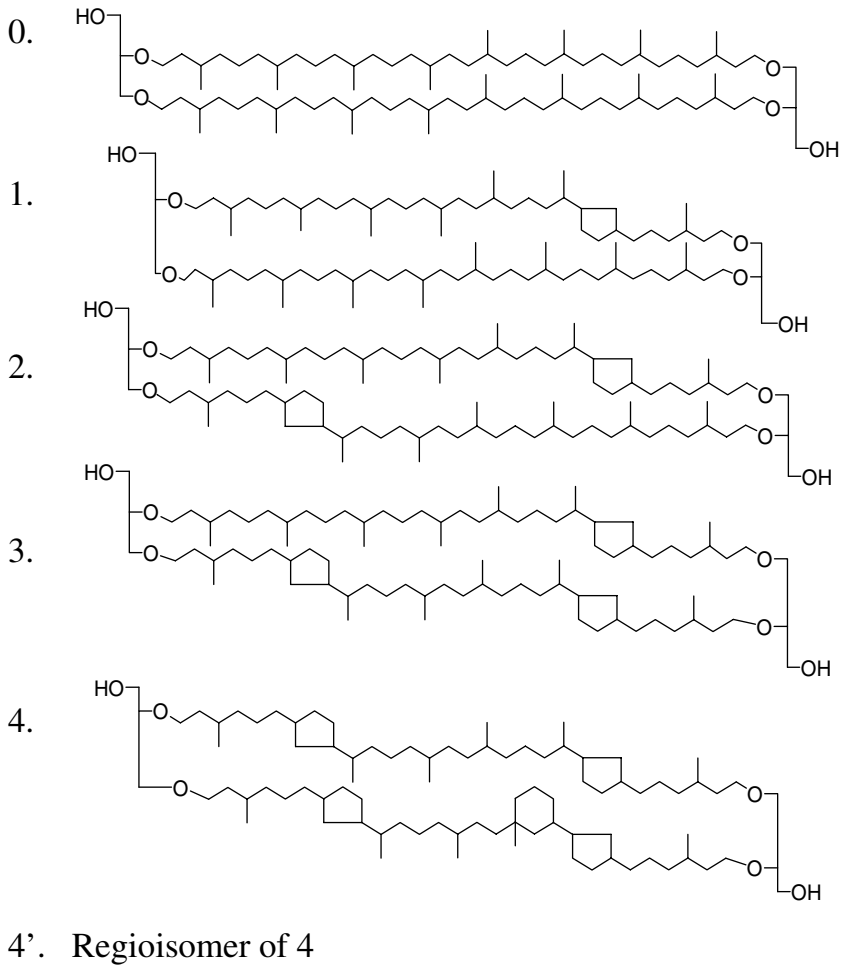

Fig. 1. Structures of GDGTs produced by marine Crenarchaeota and used for the TEX_{86} palaeothermometer (1,2,3,4′).

cyclohexane in addition to four cyclopentane moieties (Schouten et al., 2000; Sinninghe Damsté et al., 2002). Finally, they also biosynthesize small quantities of a regioisomer of crenarchaeol (GDGT 4'). From culture studies of the membrane composition of hyperthermophilic relatives of marine Crenarchaeota, it is known that the relative number of cyclopentane moieties increases with growth temperature (Gliozzi et al., 1983; Uda et al., 2001). An initial core top study based on Holocene sediments showed that marine Crenarchaeota use the same mechanism, i.e. a higher temperature of the ambient sea water results in an increase in the relative amounts of GDGTs with 2 or more cyclopentane moieties (including crenarchaeol and its regioisomer; Schouten et al., 2002). Mesocosm experiments confirmed that marine Crenarchaeota change their membrane composition with growth temperature and showed that changes in salinity and nutrients do not substantially affect the temperature signal recorded in the TEX_{86} (Wuchter et al., 2004). In contrast to the natural environment, the Crenarchaeota grown under laboratory conditions synthesized relatively much lower amounts of the crenarchaeol regioisomer (GDGT 4'). The latter compound is included in the GDGT distribution that defines the TEX_{86} (Schouten et al., 2002):

$$TEX_{86} = ([2] + [3] + [4'])/([1] + [2] + [3] + [4']) \quad (1)$$

Numbers refer to GDGT numbers in Fig. 1. The low abundance of the regioisomer of crenarchaeol in the mesocosm experiments explains why the correlation of TEX_{86} values obtained from these experiments vs. growth temperature, i.e. $TEX_{86} = 0.015^{*}T + 0.10$,

had a similar slope to the core top based equation, i.e. $TEX_{86} = 0.015^{*}T + 0.29$, but a different intercept. A survey of particulate organic matter from various oceanic sites showed that TEX_{86} values correlated well with in situ temperature at depths < 100 m (Wuchter et al., 2005) with a relationship with temperature similar to the one obtained from the core top study (Schouten et al., 2002). A recent study in the Arabian Sea demonstrated that TEX_{86} sea surface temperature (SST) estimates from descending particles collected with a sediment trap at 500 m showed a distinct seasonal pattern, in agreement with satellite SST measurements, but with an offset in temperature of ca 1–2 °C and a lag phase of ca. 1–3 weeks (Wuchter et al., 2006a). These results indicate that the TEX_{86} predominantly reflects upper water column temperatures and has a high potential for palaeoclimatic studies.

A number of studies have now applied the TEX_{86} proxy in periods of distinct climatic changes such as the last deglaciation (Huguet et al., 2006), the Paleocene–Eocene thermal maximum (Sluijs et al., 2006; Zachos et al., 2006) and Cretaceous oceanic anoxic events (Schouten et al., 2003; Dumitrescu et al., 2006; Forster et al., 2007) and indeed were able to reconstruct changes in past SST. However, a problem with some of the studies, i.e. those relating to tropical and mid latitude oceans during the Cretaceous, Paleocene and Eocene was that TEX_{86} values exceeded those observed for present day core top sediments (Schouten et al., 2002). This is due to the much higher SSTs during these periods in the geological past being characterized by much higher concentrations of greenhouse gases. To overcome this problem, Schouten et al. (2003) proposed a "high temperature" calibration line based on TEX_{86} values from core tops deposited in SSTs > 20 °C ($TEX_{86} = 0.027{*}SST - 0.016$). Extrapolation of this line could then be used to convert these high TEX_{86} values into SST. This calibration line was different from that of the regular calibration line, i.e. a steeper slope and lower intercept, possibly because Crenarchaeota have a different response at higher temperatures or are composed of a different group of Crenarchaeota. As a consequence, SSTs estimated in this way are more conservative than when the original core top calibration is used.

As present day annual mean SST hardly exceeds 29 °C, it is difficult to test using core top analysis whether the crenarchaeotal membrane composition responds in a different way at temperatures > 30 °C. In addition, it is uncertain whether Crenarchaeota can actually thrive at such high temperatures as inferred for the past, i.e. up to 37 °C (Schouten et al., 2003). To address these issues we followed the approach of Wuchter et al. (2004) and performed incubation experiments with field populations of marine Crenarchaeota derived from the tropical Indian Ocean at temperatures ranging from 25 to 40 °C and monitored the concentration of archaeal lipids and the TEX_{86} vs. time.

2. Material and methods

2.1. Incubation experiments

Approximately 1250 l of surface water (ca. 29 °C at the time of sampling) were taken near the Seychelles islands in the Indian Ocean (6° 12′ S to 6° 40′ S and 52° 7′ E to 51° 37′ E) on Darwin Cruise CD153B and were stored in the dark for several months at ambient temperature. Subsequently, aliquots were incubated in two 20 l carboy tanks in a climate controlled chamber at 25 °C and in five 3 to 5 l culture flasks, which were stirred and kept in the dark at constant temperatures of 30, 32, 34, 36, 38 and 40 °C, respectively, using water mantles controlled by thermostats. Both carboy tanks and flasks were open, so air exchange remained possible. After 7 d, nutrients were added to the carboy tanks and culture flasks. Initial added concentrations were 150 mM $NaNO_3$, 149 mM NH_4Cl, 25 mM NaH_2PO_4 and 2667 mM $NaHCO_3$ (cf. Wuchter et al., 2004). In addition, a sterile mixture of 250 mg yeast and 100 mg peptone extract was added, as well as vitamins and trace elements to each tank and flask. The temperature, pH and salinity were regularly measured and kept constant. The pH was regularly adjusted to 8.2, by addition of sterile 0.1 M NaOH or HCl and, to keep salinity constant between 35 and 37‰, demineralized water was added to compensate for evaporative losses.

The tanks and flasks were sampled for lipids after 23, 36, 50, 71, 85, 101, 120 and 134 d by filtering 100 ml water over a 0.7 μm ashed glass fibre filter. Samples for DNA analysis were taken 106 d after the start of the experiments. To this end, 100 ml of water was filtered over a 0.2 μm polycarbonate filter.

2.2. Lipid analysis

Filters for lipid analysis were extracted using ultrasonic extraction and solvent of decreasing

polarity [methanol (MeOH), dicholoromethane (DCM)/MeOH 1:1 and DCM). To remove disintegrated filter material, salt crystals and water, the total extracts were cleaned over a small $NaSO_4$ column, the solvent was evaporated and the extract was re-dissolved in DCM, shaken against water for salt removal and once more filtered over a $NaSO_4$ column. The solvent was evaporated and the extract separated using Al_2O_3 column chromatography with hexane/DCM (9:1, v/v) and DCM/MeOH (1:1, v/v) sequentially as eluents. The polar fraction (DCM/MeOH) was concentrated using rotary evaporation, dissolved in hexane/isopropanol (99:1, v/v), and filtered using a PTFE 0.4 μm filter.

The liquid chromatography–mass spectrometry (HPLC/MS) method used was modified from that described by Hopmans et al. (2000), as discussed by Schouten et al. (2007). Analysis was performed using an HP (Palo-Alto, CA, USA) 1100 series HPLC/MS instrument equipped with an auto-injector and Chemstation chromatography manager software. Separation was achieved with a Prevail Cyano column (2.1 × 150 mm, 3 μm; Alltech, Deerfield, IL, USA), maintained at 30 °C. Injection volume varied from 1 to 20 μl. GDGTs were eluted isocratically with 99% A and 1% B for 5 min, followed by a linear gradient to 1.8% B over 45 min, where A: hexane and B: propanol. Flow rate was 0.2 ml/min. After each analysis the column was cleaned by back-flushing hexane/propanol (90:10, v/v) at 0.2 ml/min for 10 min. Detection was achieved using atmospheric pressure positive ion chemical ionization mass spectrometry (APCI-MS) analysis of the eluent. Conditions were: nebulizer pressure 60 psi, vaporizer temperature 400 °C, drying gas (N_2), flow 6 l/min, temperature 200 °C, capillary voltage −3 kV, corona 5 μA (~3.2 kV).

GDGTs were detected via single ion monitoring (SIM) of their $[M + H]^+$ ions (dwell time 234 ms) and quantified by integration of the peak areas (Schouten et al., 2007). Absolute concentrations were calculated using an external stand curve obtained with a crenarchaeol standard. The concentrations of GDGTs were corrected for the regular removal of incubation water (100 ml from 5 l) due to sampling for lipid analysis.

2.3. DNA analysis

Total DNA was extracted and archaeal 16S ribosomal DNA (rDNA) amplicons were analyzed using denaturing gel gradient electrophoresis (DGGE) as described previously (Wuchter et al., 2006b). DGGE fragments were excised from the gels and sequenced for phylogenetic comparison with reference sequences from the NCBI database using the ARB software package version 2.5b (Ludwig et al., 2004). A neighbourhood joining tree was constructed with 1000 bootstrap replicates. Archaeal 16S rDNA sequences obtained have been deposited in the National Centre for Biotechnology Information (NCBI) sequence database under accession numbers EF608481-EF608498.

3. Results and discussion

3.1. Growth of crenarchaeota

Surface water from the tropical Indian Ocean was incubated at temperatures ranging from 25 to 40 °C with added nutrients. The concentration of GDGT lipids was monitored for over 4 months (Fig. 2a). The results show quite variable changes in lipid concentration over time with the different flasks. In some, e.g. those at 34 °C and 36 °C, concentration increased substantially (up to 700 ng/l) relative to that of the initial water (7 ng/l), suggesting growth of Crenarchaeota during incubation. Other flasks, e.g. those at 30 °C and 38 °C, show only relatively little increase in lipid concentration with time, suggesting minor growth of crenarchaeotal biomass despite the fact that all incubation flasks were given the same starting conditions. The reasons for these differences are not clear, but presumably small differences in the initial setting (e.g., nutrients, salinity, pH, oxygen) and stronger competition of bacteria for nutrients at these temperatures might have been of importance. Nevertheless, the large increase in GDGT concentration even in the experiment at 40 °C suggest that Crenarchaeota are able to live at temperatures well above present day annual mean tropical SST (ca. 29 °C).

If the maximum abundance of GDGTs is plotted for each experiment (Fig. 2b), it becomes apparent that there may be two, and possibly even three, optimal growth temperatures for the Crenarchaeota in the experiments; maximum GDGT abundances are found in the experiments at 25 °C (similar to that of Wuchter et al., 2004), 34–36 °C and, possibly, 40 °C. This tentatively suggests that different species of Crenarchaeota thrived at different optimal growth temperature. To investigate this further, we analysed archaeal 16S ribosomal DNA (rDNA)

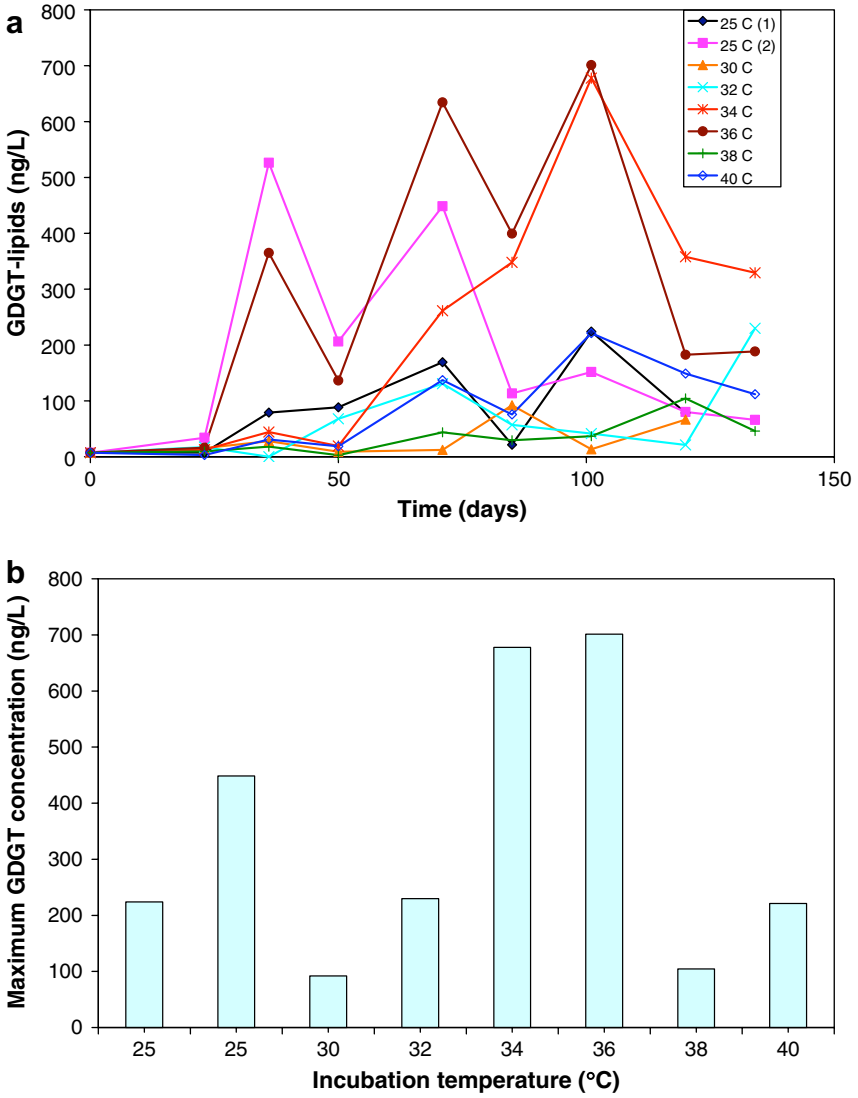

Fig. 2. Concentration of GDGTs in incubation experiments. (a) Summed concentration at days 23, 36, 50, 71, 85, 101, 120 and 134 for mesocosms at different temperature and (b) maximum summed concentrations for different incubation experiments corrected for initial concentration of Indian Ocean water.

from water samples obtained at day 106 from each experiment by amplification by PCR of a 410 base pairs fragment using general archaeal primers (Wuchter et al., 2004). This was followed by DGGE analysis of the amplified fragments and subsequent phylogenetic analysis of excised bands (cf Wuchter et al., 2004). Archaeal 16S rDNA could be amplified from most incubation flasks. Phylogenetic analysis showed that the different fragments all belong to the group 1.IA Crenarchaeota (Schleper et al., 2005) (Fig. 3). However, there is a notable difference between the sequences detected for the different incubation experiments. The sequences in the initial Indian Ocean water and 25 °C incubation experiments are closely related (99% sequence homology) to the crenarchaeote enriched from North Sea waters (Wuchter et al., 2006b) and the only crenarchaeote available in culture up to now, *Candidatus* "Nitrosopumilus maritimus" (Könneke et al., 2005). Sequences from the 32 and 34 °C incubation experiments are less closely related to those in the Indian Ocean water and 25 °C incubation experiments, but still fall in the same phylogenetic cluster. In contrast, the sequences from the 36, 38 and 40 °C

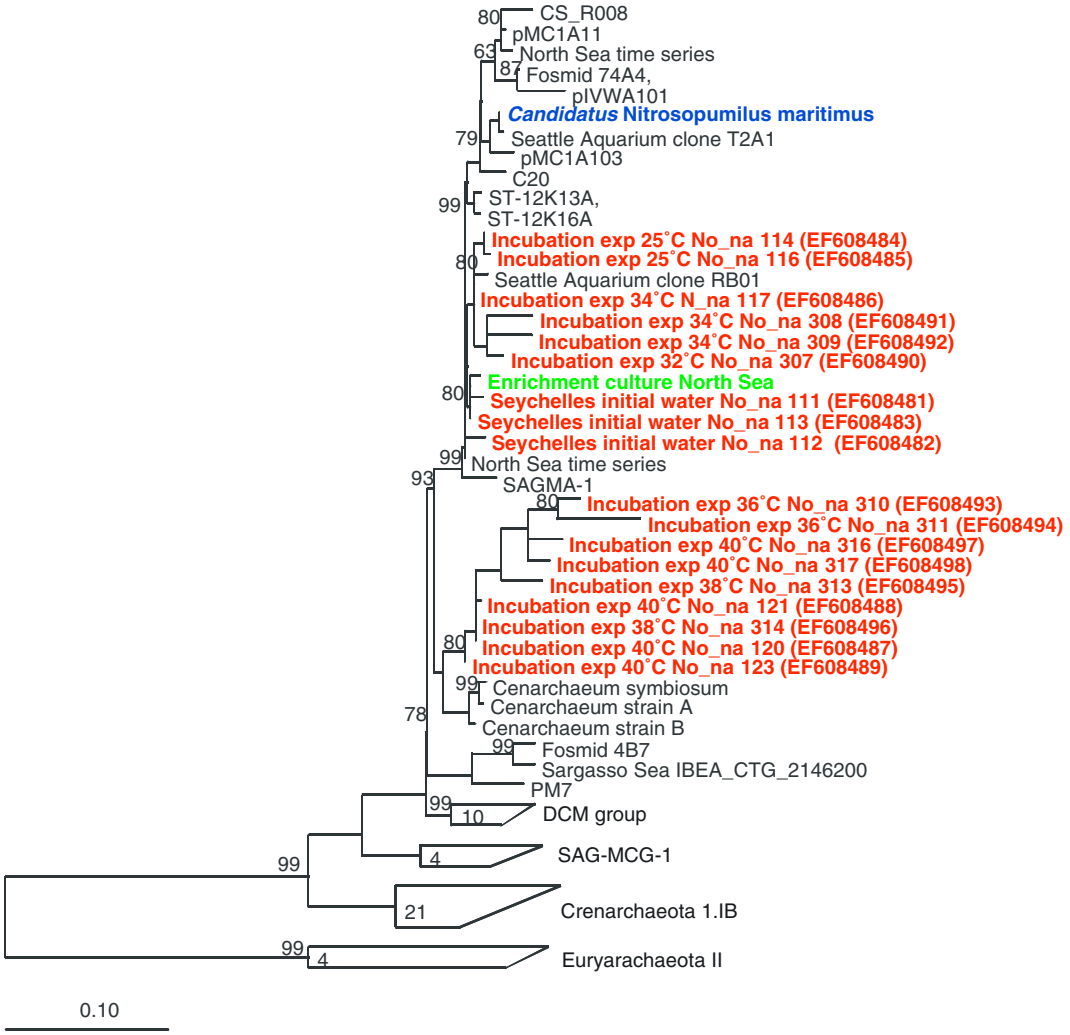

Fig. 3. Neighbour joining tree of a 400 base pair fragment of the 16S rDNA of group 1.IA Crenarchaeota excised from the DGGE gels, showing the affiliation of crenarchaeotal 16S rDNA recovered from the incubation experiments (red coloured) with *Nitrosopumilus maritimus* (blue; Könneke et al., 2005) and the North Sea enrichment culture (green) of Wuchter et al. (2006b). Numbers indicate boot strap values (%) of 1000 trees with values < 50 not shown. Classification of clades according to Schleper et al. (2005). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

experiments fall into a different phylogenetic cluster (Fig. 3; bootstrap values > 99%) and differ by 3% in sequence identity from those in the Indian Ocean water and at 25 °C. Thus, our results suggest that genetically slightly different Crenarchaeota proliferated at the different incubation temperatures, suggesting the presence of Crenarchaeota with different optimal growth temperature.

3.2. TEX_{86} analysis

Following Wuchter et al. (2004) we determined TEX_{86} values at the time of maximum abundance of Crenarchaeota in order to ensure that the membrane lipid composition had completely adapted to the new temperature regime. When the values are plotted against incubation temperature there is a general tendency of increasing TEX_{86} with temperature (dashed line in Fig. 4a), but with a large degree of scatter ($r^2 = 0.34$). However, when the values obtained for experiments where GDGT concentration remained low (< 100 ng/l, incubation temperature of 30 °C and 38 °C) are excluded, then a substantially improved correlation ($r^2 = 0.81$); solid line in Fig. 4a) is obtained between TEX_{86} and growth temperature. This suggests that there was

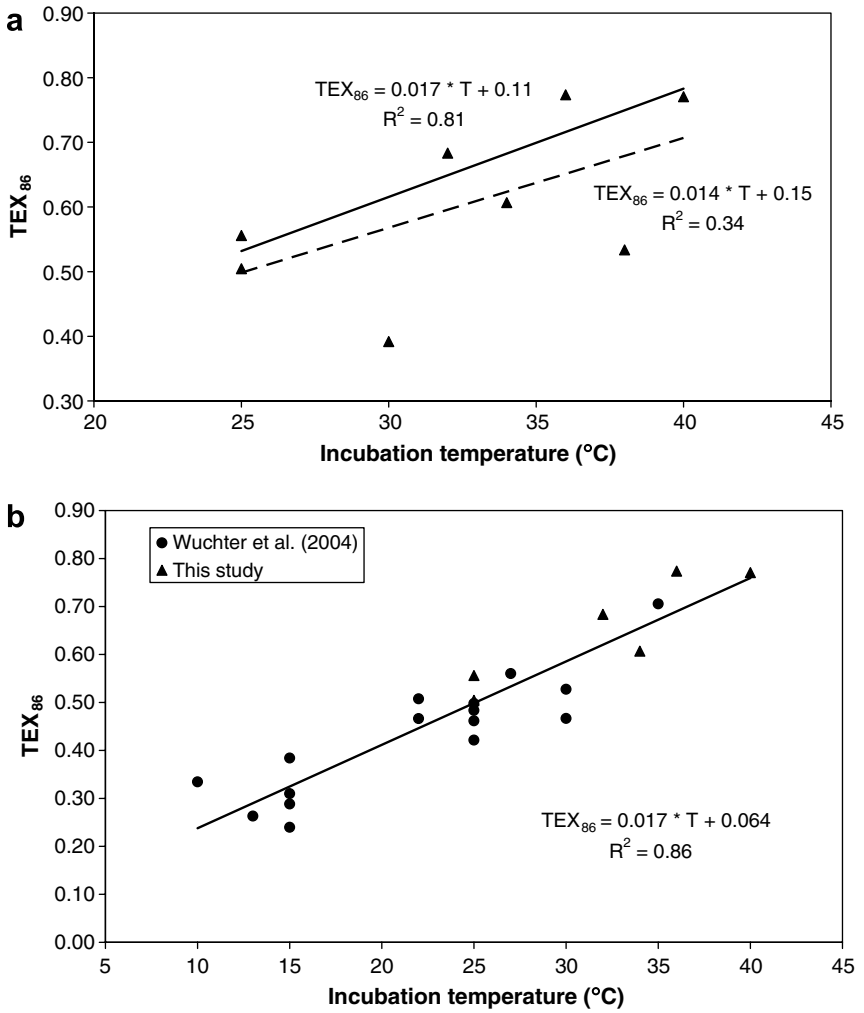

Fig. 4. Correlation of TEX_{86} with incubation temperature. (a) Correlation for all incubation experiments (dotted line) and correlation for incubation experiments excluding those at 30 and 38 °C, where there was no substantial increase in GDGT concentration. (b) Correlation of TEX_{86} from mesocom experiments of Wuchter et al. (2004, filled circles) and from this study (filled triangles).

not sufficient growth of Crenarchaeota in the experiments at 30 °C and 38 °C for the membrane composition to fully reflect incubation temperature. Interestingly, the correlation obtained ($TEX_{86} = 0.017^{*}T + 0.11$) is almost identical to that obtained by Wuchter et al. (2004), who used relatively cold (15 °C) North Sea water for their mesocosm experiments instead of Indian Ocean water as used here. When our data are plotted with those of Wuchter et al. (2004), they follow exactly the same linear trend of TEX_{86} with temperature (Fig. 4b). Taken together, our data and those of Wuchter et al. (2004) show a strong linear correlation ($r^2 = 0.85$) between TEX_{86} and temperature between 10 and 40 °C. This suggests that, despite the fact that genetically slightly different species of Crenarchaeota grew in the different incubation experiments, their membrane composition changed in a similar fashion in response to temperature.

Remarkably, as in the mesocosm experiments of Wuchter et al. (2004), the intercept of our calibration line between TEX_{86} and temperature is substantially lower than observed for core top sediments and particulate organic matter (Schouten et al., 2002; Wuchter et al., 2005). Again, this is due to the relatively lower abundance of the regioisomer of crenarchaeol (cf. Wuchter et al., 2004). Though present in relatively high amount in the water used at the start of

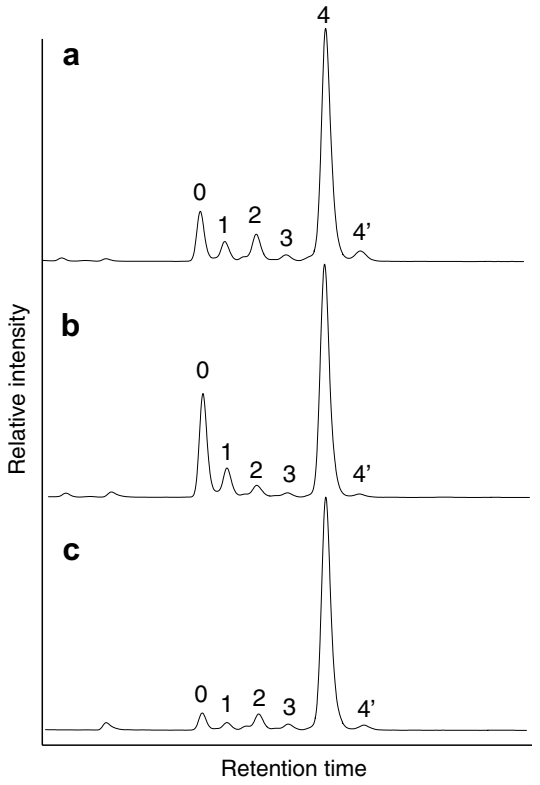

Fig. 5. Base peak chromatogram from HPLC/MS analysis of GDGTs in (a) original Indian Ocean water taken near the Seychelles, (b) incubation experiment at 25 °C at day 36 and (c) incubation experiment at 36 °C at day 71, showing the change in GDGT distribution with temperature. Note the relatively lower abundance of the crenarchaeol regioisomer in the incubation experiments compared to that in the original water.

the experiments (Fig. 5a), it was reduced upon growth of the Crenarchaeota during the incubation (Fig. 5b and c), even at very high temperatures where the regioisomer is usually present in relatively high abundance in particulate organic matter (Wuchter et al., 2005), sediment traps (Wuchter et al., 2006a) and sediments (Schouten et al., 2002). Thus, somehow our incubation conditions affected the production of the crenarchaeol regioisomer, although the reasons for this are unclear. Conditions in the incubation flasks are relatively similar to that in "regular" sea water (i.e. nutrients, pH, salinity, oxygen, etc.) except that the flasks were kept in the dark. However, it is difficult to envisage that reduced light intensity would diminish the synthesis of the regioisomer. Further investigation into the biophysical role of this crenarchaeol isomer is clearly needed to shed light on the discrepancy between laboratory experiments and the natural environment.

4. Implications

Our results show that Crenarchaeota belonging to group 1.IA are able to thrive at relatively high temperatures up to 40 °C. Hence, these Crenarchaeota were likely able to thrive at the very high SSTs thought to have occurred in mid latitude and especially tropical oceans of ancient greenhouse worlds, such as during the Cretaceous and Paleocene/Eocene. The results show that, at these high temperatures, TEX_{86} still responds in a linear fashion to increasing temperature. Unfortunately, due to the low abundance of the crenarchaeol regioisomer in our incubation experiments, it is difficult to extrapolate the results to those in the natural environment and so to past conditions where SST was substantially higher than today. Nevertheless, our incubation experiments suggest that the slope of the TEX_{86}-SST correlation line at SST $>$ 30 °C may be similar to that at $<$ 30 °C and therefore that the "high temperature" calibration line used to calculate SST in past greenhouse worlds (Schouten et al., 2003) may be too conservative and results in underestimation of past SSTs of tropical oceans. For example, TEX_{86} values for tropical oceans during the mid-Cretaceous vary from 0.84 to 0.96 for OAE 1a and 2 (Schouten et al., 2003). If we were to employ the regular temperature calibration of Schouten et al. (2002), these values would translate to SSTs between 36 and 44 °C, substantially higher than the SST reconstruction using the high temperature calibration (30–36 °C; Schouten et al., 2003) and substantially higher than most estimates using $\delta^{18}O$ of presumed planktonic foraminifera (e.g. Wilson and Norris, 2001). However, recent estimates using Mg/Ca and $\delta^{18}O$ of planktonic foraminifera, two independent proxies, also suggest peak SSTs of up to 42 °C (Bice et al., 2006), in agreement with the high TEX_{86} values of up to 0.96 measured at the same site (Forster et al., 2007). Hence, the higher SST estimates may not be inconsistent with those of planktonic foraminifera, especially considering the number of assumptions involved in calculating SST from $\delta^{18}O$ (e.g. salinity, pH, $\delta^{18}O$ of water, etc.) and the uncertainty regarding the ecology of extinct species of foraminifera. However, before we can revise our temperature estimates, there need to be established the controls on the synthesis of the crenarchaeol regioisomer and how Crenarchaeota can be grown under conditions where their lipid compositions are similar to those observed in the natural environment. Further research using, for

example, the crenarchaeote *Candidatus* "Nitrosopumilus maritimus" (now available in culture; Könneke et al., 2005) rather than mixed populations of Crenarchaeota could be of importance in this matter.

Acknowledgements

Cornelia Wuchter and Marcel van der Meer (NIOZ) are thanked for their support and input in setting up the incubation experiments, Martijn Woltering (NIOZ) for help with sampling and maintenance of the incubation experiments and GDGT analysis. Eveline van Weerlee and Jan van Ooijen (NIOZ) are thanked for analyzing the nutrient content of the incubation flasks. Lydie Herfort, Anna Noordeloos, Govert van Noort and Sjerry van der Gaast (NIOZ) are thanked for valuable advice, help and discussions concerning the experimental setup. Ben Abbas, Lydie Herfort, Harry Witte and Judith van Bleijswijk (NIOZ) are thanked for assistance with molecular analysis of archaeal rDNA. Two anonymous referees are thanked for constructive comments.

Associate Editor—**R.D. Pancost**

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