

TEMPERATURE CONTROL ON LADDERANE LIPID CHAIN LENGTH IN ANAMMOX BACTERIA

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A recent addition to the pool of source diagnostic biomarkers are the membrane lipids of anaerobic ammonium oxidizing (anammox) bacteria. Anammox bacteria oxidize NH_4^+ with NO_2^- to N_2 under anoxic conditions and create the intermediate compound hydrazine. Anammox bacteria have a prokaryotic organelle surrounded by a membrane bilayer composed of unusual 'ladderane' lipids. Ladderanes consist of C_{18} and C_{20} alkyl chains possessing 3 or 5 linearly concatenated cyclobutane rings, bound by ether and/or ester linkages to a glycerol backbone. Modelling experiments have shown that ladderane lipids may provide a denser cell membrane than conventional lipids (Sinninghe Damsté et al., 2002). The increased density is believed to prevent proton and hydrazine loss from the organelle. Ladderane lipids have been shown to be excellent tracers for the presence of anammox bacteria in water columns, sediment traps and sediments (e.g. Kuypers et al., 2003). However, the factors controlling the relative abundance of the different ladderane lipid isomers (i.e. genetic and/or environmental) were unknown until now.

In this study we analysed the ladderane lipid composition of enrichment cultures (four genera of anammox bacteria), particulate organic matter and surface sediments to ascertain the impact of environmental conditions on the relative distribution of ladderane lipids. Analyses were conducted using our recently developed novel HPLC-APCI-MS/MS technique (Hopmans et al., 2006). On plotting a ratio between ladderane lipids containing 5 cyclobutane rings and hydrocarbon chain lengths of 18 and 20 carbon atoms, respectively, a highly significant sigmoidal relationship ($R^2 = 0.93$) is observed between ladderane lipid chain length and *in situ* temperature (Figure 1). This relationship indicates that there is an increase in the concentration of ladderane lipids containing shorter alkyl chains at lower temperatures and vice versa, particularly between 10 and 25°C. Phylogenetic analysis showed that different genera contribute to the different ladderane pools, suggesting that this mechanism of temperature adaptation is present in all anammox bacteria. Temperature stimulated production

of shorter chained lipids has been previously documented in other bacteria and is thought to lead to increased membrane fluidity under cold conditions by reducing carbon to carbon interaction between lipid chains (Russell et al., 1990). Our results may be used to discriminate between the origin of ladderane lipids in marine sediments, i.e. if ladderanes are produced *in situ* in cold surface sediments or if they originate from the warmer upper water column.

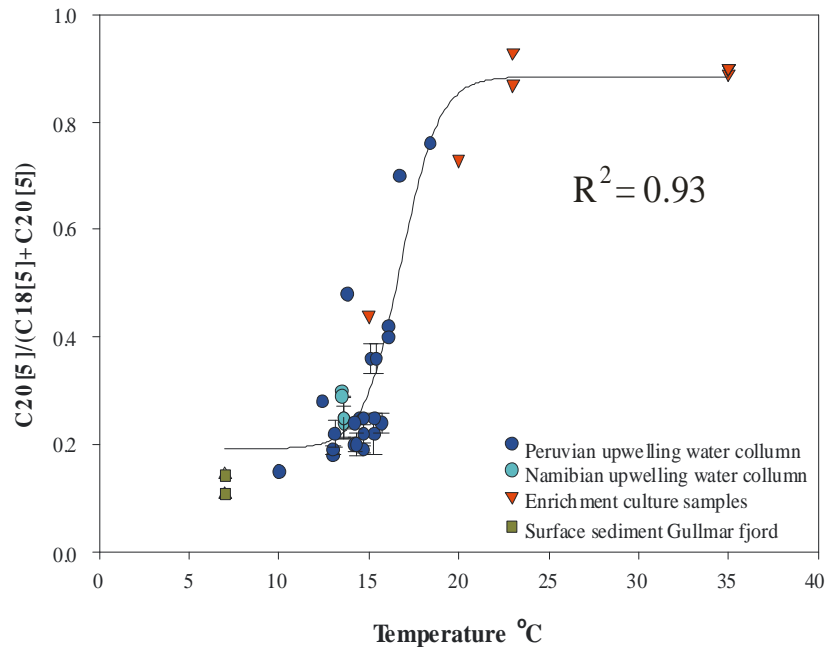


Figure 1. Sigmoidal relationship between ladderane lipid ratio and *in situ* water column, reactor medium and sediment temperature

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