"Metanogenic Diversity from *mcrA* gene in hypersaline conditions"

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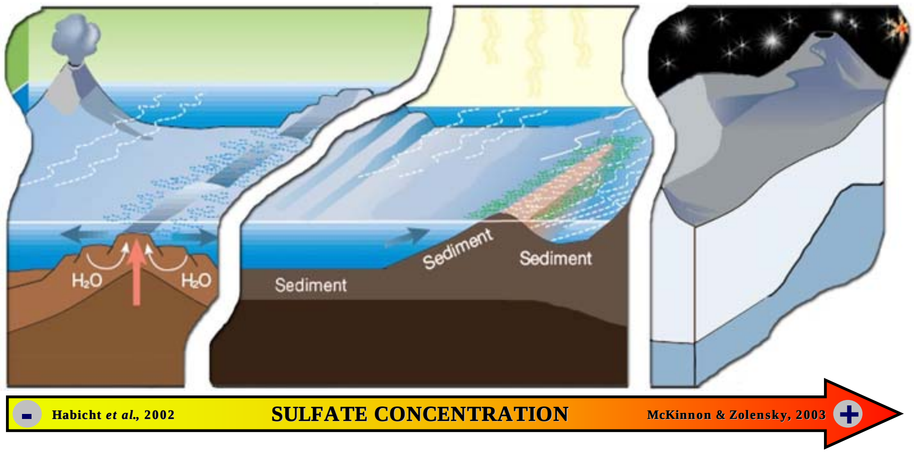


EARLY EARTH	TIREZ	EUROPA
SALTS: Between others, gypsum ($CaSO_4.2H_2O$), halite (NaCl) and dolomite as the abundant salt ($CaMg(CO_3)_2$) (González-Muñoz *et al.*, 2008)	**SALTS:** Halite (NaCl), epsomite ($MgSO_4.7H_2O$), hexahydrite ($MgSO_4.6H_2O$), mirabilite ($Na_2SO_4.10H_2O$), polihalite ($K_2MgCa_2(SO_4).2H_2O$), thenardite (Na_2SO_4), bloedite ($Na_2Mg(SO_4).4H_2O$), glauberite ($CaNa_2(SO_4)_2$), gypsum ($CaSO_4.2H_2O$) (de la Peña, 1986; Prieto-Ballesteros *et al.*, 2003)	**SALTS:** Hexahydrite ($MgSO_4.6H_2O$), Epsomite ($MgSO_4.7H_2O$), Natron ($Na_2CO_3.10H_2O$) (McCord *et al.*, 1998)
METHANOGENESIS (Proposal): *Hydrogenotrophic methanogenesis* (Hedges *et al.*, 2004)	**METHANOGENESIS (Evidence):** *Hydrogenotrophic methanogenesis* *Methylotrophic methanogenesis* (This work)	**METHANOGENESIS (Proposals):** *Hydrogenotrophic methanogenesis* (McCollom, 1999) *Methylotrophic methanogenesis* (This work)

In Europa, it has been hypothetized that biosynthesis of organic matter could be initiated chemotrophycally in ice shell (Hand *et al.*, 2007) and in ocean floor (Zolotov and Shock, 2003). The Europa' sulfate concentration predicts a completion carbon cycle (carbon mineralization) which could be supported by sulfate reducers. This scheme *prohibits* the development of hydrogenotrophic methanogenesis, although some exceptions to this *principle* have been reported (see Hoeheler *et al.* 1998). Therefore, from our findings in Tirez we suggest a expected development of the methylotrophic methanogenesis in european ocean given the high sulfate concentrations. However, the presence of a hydrogenotrophic methanogenesis would be also viable in Europa through the Hoehler's scenario.

METHODOLOGY

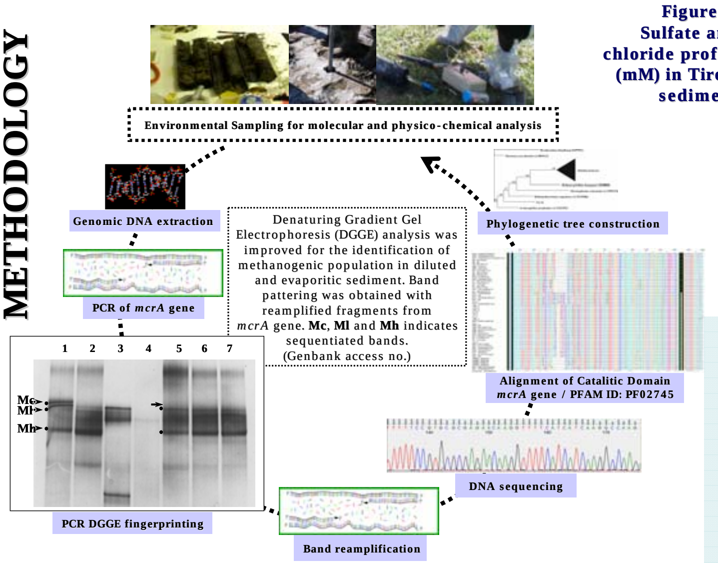
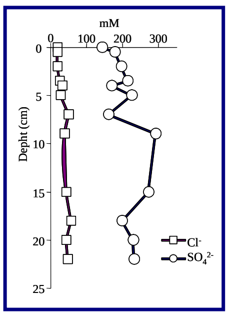


Figure 1. Sulfate and chloride profile (mM) in Tirez' sediment



The concentration of sulfate and chloride ions was determined in sediment by ionic chromatography (mM). In all sampled depths, sulfate exceeded chloride and its concentration increased from one to ten orders of magnitude with respect to the marine sediment concentration; in fact, it is considered a highly sulfated sediment.

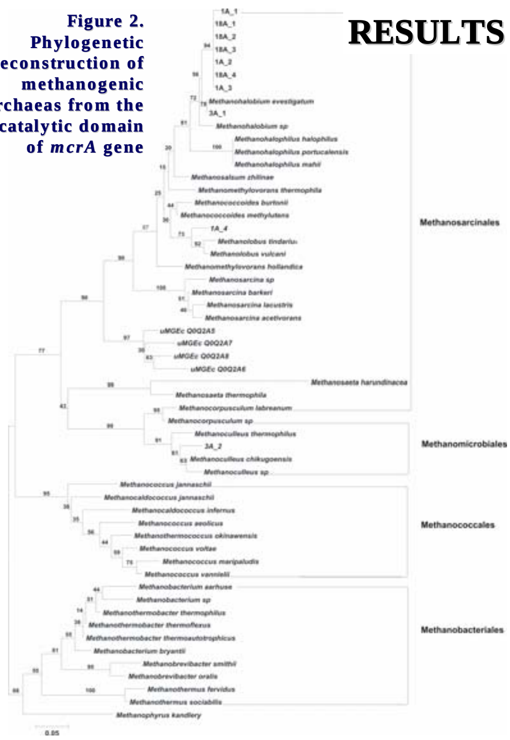
INTRODUCTION

Methanogenesis is one of the main metabolisms that were present in the early anoxigenic Earth. To date, this metabolism has been physiological and phylogenetically developed only by archaean methanogens. The ecological role of methanogenesis is specially important in sedimentary environments because this metabolism supports the carbon mineralization cycle generating methane from H_2, methylated compounds or acetate.

The ecological distribution of methanogenesis is mainly constrainted by redox potential and sulfate concentration, both can be evaluated in Tirez sediment, an athalassohaline lagoon. Besides, Tirez has been referred as an analog of Europa due to hypersalinity and geochemical properties (Prieto-Ballesteros *et al.*, 2003). Therefore, Tirez' sediment would show us how european seafloor ecosystem function; here we focused on methanogenesis as a metabolic *signature* of possible living systems in the anoxigenic and sulfated european seafloor.

RESULTS

Figure 2. Phylogenetic reconstruction of methanogenic archaeas from the catalytic domain of *mcrA* gene



The phylogenetic analysis revealed the presence of three phylotypes belonging to different taxonomic groups of methanogens: *Methanoculleus* genus (*Methanomicrobiales* Order, most of them are hydrogenotrophs), which was identified in the diluted season, and the methylotrophic *Methanohalobium* and *Methanolobus* genera (*Methanosarcinales* Order) which were founded in both evaporitic and diluted season.

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