

HYPERSALINE INTERTIDAL MICROBIAL MATS AT THE COAST OF OMAN – A LIPID AND MOLECULAR MICROBIOLOGICAL STUDY

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Microbial mats are frequently found in the intertidal zones along the coast of Oman. Strong physical and chemical gradients driven by tides and extremely hot and arid climate conditions shape microbial communities, which are usually dominated by extremophilic microorganisms.

Samples and setting: In this study, lipids and microbial diversity of five microbial mats along a transect over tidal flats at the Arabian Sea shore of Oman were investigated. A gradient of increasing duration and frequency of inundation as well as a decreasing salinity gradient, indicated by decreasing thickness of salt crusts at the surface of the mats, was observed from the upper tidal line (sample O1) towards the sea (sample O5). The grain size of sediment in the mats decreased along the transect towards the sea, while the mat lamination increased.

Methods: Lipids were extracted using a modified protocol after Bligh & Dyer. Extracts were hydrolysed and separated by column chromatography into acid and neutral fractions, which were analysed as methyl esters and trimethylsilyl esters, respectively, using gas chromatography and GC-mass spectrometry. Cyanobacteria, bacteria, and archaea were analysed using MiSeq sequencing of 16S rRNA genes which yields in-depth information on community composition, richness, and diversity.

Results and discussion: Lipid composition of microbial mats showed gradual changes along the transect. Total yields of acid and neutral fraction after hydrolysis were generally low. They increased from 77 (in upper tidal samples) to 218 mg/g extract in sample O5. Concentrations of all short chain (C₁₄-C₂₂) fatty acids followed this trend (Fig 1A). Compositional changes were most evident by the increasing portion of polyunsaturated fatty acids (dominantly 18:2 ω 6) attributed to algal or cyanobacterial sources (Fig. 1B). This can be explained by the observed shift from unicellular (i.e. *Chroococcales*) cyanobacteria in the lower tidal mats towards filamentous (*Oscillatoria*) types in upper tidal mats. The ratio of monounsaturated/saturated fatty remained nearly constant. A relative increase in 10methyl-16:0 fatty acid, which is often attributed to sulfate-reducing bacteria (SRB), was observed towards the sea (Fig. 1B). This is in agreement with increasing relative sequence abundances of SRB in the lower tidal mats. In general, a shift from predominantly anoxygenic photosynthesis in the upper tidal mats towards a more diverse spectrum in metabolic activities in the lower tidal mats was observed in 16S rRNA gene analyses. Remarkably, long chain fatty acids (>C₂₂) occurred with chain lengths up to 40 carbon atoms. Their concentration and maximum chain length decreased from C₄₀ in sample O1 to C₂₈ in samples O4 and O5. Their distribution suggests rather a microbial than a terrestrial origin.

Two archaeols were the dominant neutral lipids in the three upper tidal samples. They made up ca. 50 % of all quantified neutral lipids in the upper tidal mat samples indicating the high importance of archaea in the most extreme upper tidal sites, and decreased to >10 % in sample O5. While Archaeol (diphytanylglycerol) did not show a concentration gradient, the second compound, a dialkylglycerylether with a C20 and a C25 isoprenoid side chain, strongly decreased pointing to an origin from archaea adapted to the most extreme conditions (Fig. 1C). In parallel, relative abundances of archaeal sequences belonging to unclassified archaea and Nanohaloarchaeota decreased. The occurrence of Thaumarchaeota in the lower tidal mat samples coincided with the appearance of two other ether lipid compounds, tentatively identified as 1,2-dihexadecylglycerylethers.

Sterols were almost absent in upper tidal mat samples (mainly cholesterol), but became more abundant and diverse in the lower tidal mats samples by additional occurrence of C28 and C29 sterols with $\Delta 5$, $\Delta 22$ and $\Delta 5,22$ double bonds. They indicate that algae became abundant at lower tidal sites taking advantage of decreasing salinity and increasing water availability.

In general, lipid diversity was highest in the lower tidal mat sample (O5), which contained a number of mainly bacterial lipid compounds only detected at that site (e.g. mid-chain branched C₁₅ and C₁₇ alkanes, specific isomers of 17:1 fatty acids, and cyclopropyl 19:0 fatty acids). Similarly, highest bacterial diversity and richness increased towards lower tidal sites.

In conclusion, extreme hypersaline conditions favour archaea and highly adapted specialised bacteria. General patterns analysed by means of molecular microbiological methods and changes in the lipid inventory were in agreement and provide complementary information on community composition of the microbial mats.

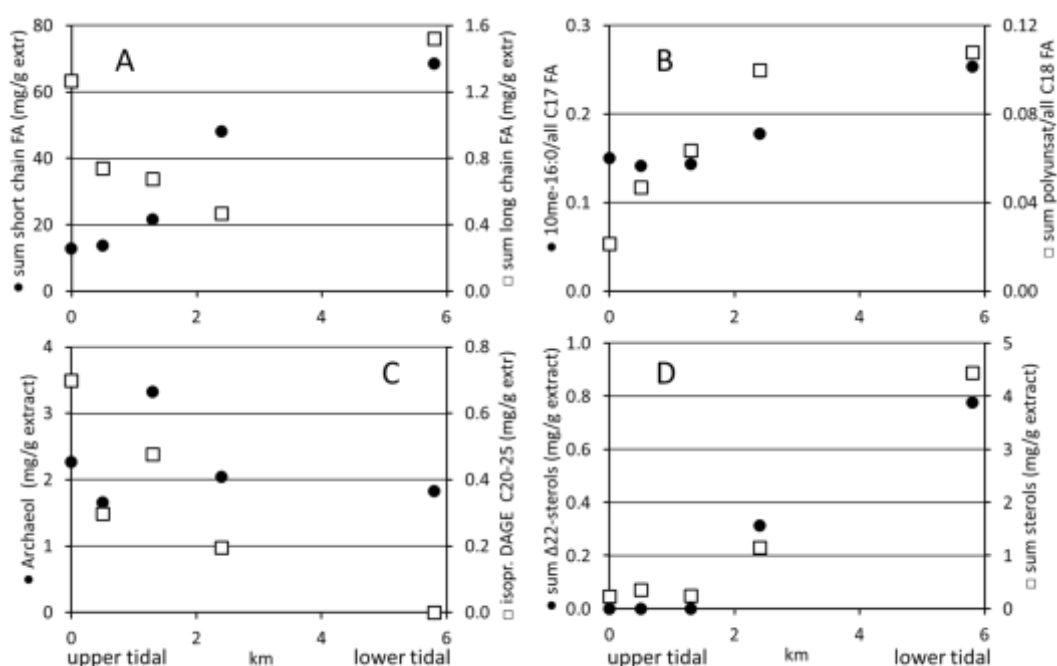


Figure 1 Lipid distributions along a transect from upper to lower tidal sites. A: short (<C₂₄, dots) and long (>C₂₄, squares) chain fatty acids; B: ratio 10-methyl-16:0/sum of all C₁₇ fatty acids (dots) and polyunsaturated C₁₈/sum of all C₁₈ fatty acids (squares); C: archaeol (dots) and isoprenoid dialkylglycerylether C₂₀-C₂₅ (squares); D: sterols with $\Delta 22$ double bond (dots) and sum of all sterols (squares). X-axis: Distance in km from sample O1 in the upper tidal area.