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Changes in microspatial organization of bacteria during development of surface microbial mats of marine lithifying stromatolites determined using combined FISH/GIS-image analyses.

A.W. Decho (1), P.T. Visscher (2), R.P. Reid (3)

(1) Dept. Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, SC. 29208. USA (awdecho@gwm.sc.edu);

(2) Dept. Marine Sciences, University of Connecticut, 1084 Shennecossett Rd., Groton, CT. 06340-6097 USA (visscher@uconnvm.uconn.edu);

(3) Div. Marine Geology Geophysics, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, 4600 Rickenbacker Causeway, Miami, FL. 33149-1098 USA (preid@rsmas.miami.edu).

Microbial mats often exhibit dense horizontal arrays of different functional groups of bacteria. In open-water marine stromatolites at Highborne Cay (Bahamas), cyanobacteria, sulfate-reducers, aerobic heterotrophs, and sulfur oxidizers interact during the tripartite cycling of surface mats that result in precipitation of micritic laminae composed of $CaCO_3$. Activities of sulfate reducing bacteria (SRB) have been closely related to precipitation events occurring within surface mats of stromatolites (Visscher *et al.* 2000). The present study examined surface mats representing points along a continuum between a non-calcifying (Type 1) to a calcifying (Type 2) state. Fluorescence *in-situ* hybridization (*FISH*), using 16S rRNA oligoprobes (*SRB385* and *dsr*AB), was used to conservatively target SRB and coupled with confocal scanning laser microscopy (CSLM) to examine changes in the *in-situ* microspatial organization of mats that occur across this continuum. Data from image analyses and geographical information systems (GIS) approaches were used to examine statistical patterns of changes in microspatial distributions. Specific microspatial distributions of bacteria, derived from image analyses/GIS, were used to create possible instruments of discrimination between Type 1 and 2 mat communities.

The results of FISH/CSLM showed that SRB were present in the upper-most (50 μm) surface layers of all mat stages (Type1, transitional-stage, and Type 2), and reside in an environment that is intermittently ($i.e.$ approx half the day) highly oxic. The early-stage (Type 1 non-calcifying) mat communities typically exhibited comparatively lower abundances and no detectable distributional patterns of SRB, and other bacteria. As mats develop into Type 2 (calcifying) communities, there was progressive organization in the microspatial distribution ($i.e.$ clustering) of SRB and other bacteria. Both the numbers and sizes of SRB clusters, increased as mats develop from a Type 1 to a Type 2 state. This resulted in statistically (P $<$ 0.001) higher abundances of SRB in the surface of Type 2 mats (when compared with Type 1), and culminates in a relatively dense horizontal layer of SRB near the mat surface. The precipitation of micritic laminae ($i.e.$ horizontal layers of $CaCO_3$) are a characteristic feature of fossil and present-day marine stromatolites. SRB may play a defining role in C and S cycling, processes that lead to micritic laminae formation in extant marine stromatolites. Our data suggest that the development of an abundant SRB community within the uppermost (oxic region) surface of stromatolite mats is closely aligned with the transition from a noncalcifying (Type 1) to a calcifying (Type 2) state.