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Correspondence

Effect of oil pollution on euendolithic cyanobacteria of the Arabian Gulf

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Summary

Microbial euendoliths (true borer) cyanobacteria are carbonate-boring microorganisms found in modern and ancient marine environments. Modern euendoliths include a wide range of prokaryotes as well as eukaryotes, which have been reported world-wide. The importance of euendolithic cyanobacteria concerns their role in bio-erosion of calcium carbonate substrates and as ecological indicators of shallow, tropical and subtropical marine environments. Arabian Gulf ooids from four sites along the east coast of Saudi Arabia have been bored and inhabited by several species of euendolithic cyanobacteria. This assemblage of different species exists simultaneously within the same ooid grain. Comparisons of 1989 and 1992 data reveal a drastic reduction in active euendoliths, and the average numbers of colonies in these ooids. This study reveals the harmful effect of the 1991 oil spill on these unique microorganisms residing in these ooids.

Euendolithic cyanobacteria are phototrophic microorganisms found in marine and fresh water environments (Alsumard, 1970; Anagnostidis and Pantazidou, 1988; May and Perkins, 1979; Pentecost, 1992). Their microenvironment exists within calcareous substrates of rocks, ooids or shells. Euendolithic habitats have been found since the early stages of evolution, as early fossils of euendoliths have been reported in Precambrian, silicified, oolitic rocks (Perkins and Halsey, 1971; Campbell, 1983; Knoll *et al*., 1986; Green *et al*., 1987; Zhang and Golubic, 1987). In addition to their important role in calcium carbonate bio-erosion in aquatic environments, they have been used as ecological indicators of water quality and photic depth (Golubic *et al*., 1975; Knoll, 1985; Knoll *et al*., 1986).

Several modern genera and species have been described as boring in limestone and shells (Perkins and Tsentas, 1976; Lukas and Hoffman, 1984; LeCampion-Alsumard, 1991; Gudrun Radtke, 1993). Euendolithic cyanobacteria of genus *Hyella* have several morphological characters that distinguish them from other cyanobacterial genera. These characters include binary and multiple cell division; cells remain together surrounded by polysaccharide envelopes, forming pseudofilamentous, branched thalli, which penetrate calcium carbonate substrates. Cells differentiate in form and function in the course of development. Cell types can be identified based on their shape, function and location in the pseudofilament. Five types of cells, comprising apical cells, vegetative cells, branch-point cells, baeocytes mother cells and baeocytes, could be identified.

Very few species of euendolithic cyanobacteria have been reported to bore ooids in oolitic shoals (Lukas and Golubic, 1983; Al-Thukair and Golubic, 1991a). Oolitic shoals are usually found in tropical and subtropical marine environments world-wide, including the Bahamas, the Arabian Gulf and Australia (Bathurst, 1975; Simone, 1981).

Ooids are described as spherical calcium carbonate grains, formed by the sporadic accretion of aragonite needles around a nucleus in shallow tropical marine environments (Monaghan and Lytle, 1956; May and Perkins, 1979; Simone, 1981). Their sizes range between 0.2 mm and 2.0 mm, and they are most often found at depths of 2–6 m (Bathurst, 1968; 1975; Simone, 1981). High salinity, temperature and evaporation rates are prerequisites for ooid formation.

Their sizes result from a balance between different processes such as abrasion, boring rate and accretion. As a result of their continual relocation by waves and currents, epiliths and encrusting animals rarely colonize ooid surfaces. Furthermore, they are more likely to be colonized and bored by euendoliths.

It was estimated that six million barrels of crude oil were spilled in the Gulf region during the 1991 Gulf War (UNEP, 1991). Most of this oil ended up on Saudi Arabia's eastern

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coastline. This huge amount of spilled oil caused extensive destruction of marine habitats, and made fieldwork and monitoring processes hazardous.

Field assessments after the 1991 oil spill indicated that all the sampling sites had suffered from oil contamination of varying magnitudes. However, Khafji was the most affected site, followed by Khawr, Zawr and Tarut. Concentration of petroleum hydrocarbon, measured as total hydrocarbon in marine sediments on dry-weight bases, collected from these sites approximately 9 months before our study showed a concentration of 369 μgg^{-1}, and 129 μgg^{-1} in Khafji and Khawr. Whereas, its concentration could reaches up to 100 μgg^{-1} in Zawr, and less than 20 μgg^{-1} in Tarut (Fowler *et al.*, 1993; Michel *et al.*, 1993). Ooid shoals located in the lower littoral zone and in less than 1 m of water depth were found to be more affected by oil than those at greater depths.

In Khafji, a thick layer of tar and weathered oil covered the upper portion of the ooid shoals, immobilizing and trapping oil-soaked sediment beneath it. A black, thick, sticky layer of oil covered the top layer of ooids. This layer had killed euendoliths within the ooids and turned the frequently white ooids to black. On the other hand, ooids located at the bottom of the ooid shoals were less covered by oil, and consisted of very few live euendolithic colonies.

Khawr and Zawr were less exposed to less oil contamination than Khafji, yet oil was also found trapped within the oolitic sediment. Both sites represent high-energy setting beaches, on which oolitic sediments are frequently deposited. Oolites located on the surface, and in less than 1 m of water depth have been subjected to continuous oil fouling when the sediment is stirred during sampling or by strong waves. Oil droplets and a shiny film were formed on the water surface. Agitation of the upper layer of the sediment caused a release of entrapped oil.

The interstitial oil of partially exposed ooilitic shoals has been minimized as a result of direct solar exposure and leaching during tide intervals. It is common to see a thin layer of oil (30–50 µm thick) partially surrounding or affixing ooid samples taken from these two sites. The oolitic shoals of Tarut were minimally exposed to oil pollution.

Sediments consisting of ooid samples from four locations (Tarut, Zawr, Khawr and Khafji) were collected on August 20, 1989 and August 23, 1992 at depths of < 1, 1–2 and >2 m. These sites differ in current, and wave exposure. Khawr and Zawr are protected lagoon settings, whereas Khafji and Tarut are exposed to the open waters of the Gulf (Fig. 1). Ooid samples were collected from the top 1 cm layer of the sediment using 100 ml wide-mouth jars. These jars were used to scoop ooid grains from the sea bed, and were half filled with sediments. Upon arrival at the laboratory, each sample was divided into two subsamples. One subsample was fixed with 2% formaldehyde in environmental water in preparation for light

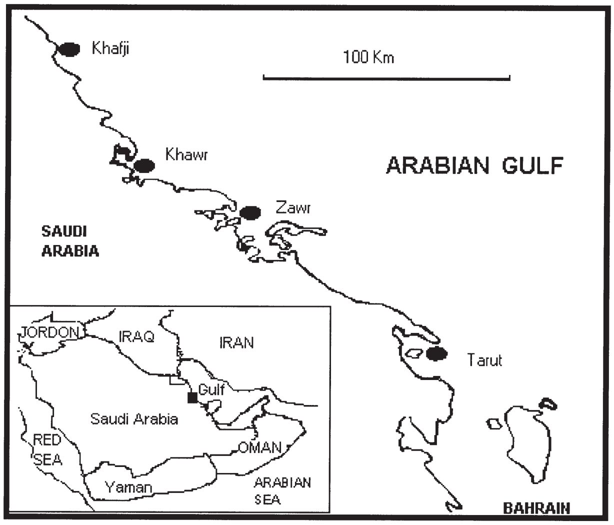

Fig. 1. Location of the sampling sites along the east coast of Saudi Arabia.

microscopy study, whereas the other was fixed with 1.2–2.5% formaldehyde in a cacodylate (0.2 M) buffer for resin embedding and scanning electron microscopy (SEM).

A total of 50 ooids were picked at random and examined with a dissecting microscope (Wild Heerbrugg), and placed on slides individually as drops of 3% HCl were added.

After all the calcium carbonate was dissolved, a coverslip was positioned over the specimen. To make a semi-permanent slide, a drop of glycerine was placed at the edge of the coverslip and allowed to enter by capillary action, and permount was used for sealing. Slides were viewed with a compound microscope (Litz, Ortholux II), and six major species of euendolithic cyanobacteria (*Hyella reptans, H. conferta, H. salutans, H. stella, H. inconstans, H. immanis*) as well as other species *(H. arbuscula, H. racemus, Solantia sangunia* and *Ostrobium* sp.*)* were identified.

The number of colonies and the percentages for each species were calculated at each depth (Al-Thukair and Golubic, 1991a; b). The percentages of *H. arbuscula, H. racemus, S. sangunia, and Ostrobium* sp. were summed together as their individual percentages were small.

Resin-replication techniques render borings visible in three-dimensional display. Randomly selected fixed ooids were dehydrated using 25%, 50%, 75% and 95% alcohol, and acetone was used in the final step of the dehydration process (Golubic *et al.*, 1970). The dehydrated ooids were embedded in Spurr's low-viscosity medium and cured.

Resin blocks with embedded ooids were cut open, and calcium carbonate was dissolved in 3% HCl. This procedure exposes the borings as resin casts, which often include embedded resident euendoliths. The percentages of bored ooids are calculated by the presence or absence of bore holes in these etched ooids under a light microscope without any attempt to quantify them. Selected etched ooids consisting of embedded euendoliths are mounted on SEM stubs, coated and analysed using a Jeol JSM 840 model scanning electron microscope. A three-dimensional display of the resin-cast boring patterns was photomicrographically documented.

EXCEL and SIGMA statistical programs were used in table preparation, statistical analysis and data evaluation. ANOVA (Kruskal–Wallis One Way Analysis of Variance) and the Student–Newman–Keuls statistical analysis methods of comparison were used.

Euendoliths occupying ooids have been studied extensively as nine new species of euendolithic cyanobacteria were reported between 1991 and 1996 (Al-Thukair and Golubic, 1991a,b; Al-Thukair *et al*., 1994; Al-Thukair and Golubic, 1996; Golubic *et al*., 1996). The combined casting and embedding techniques of euendoliths in ooids revealed characteristic species-specific boring patterns (Fig. 2). Cell morphologies and boring patterns have been used for species description and identification (Golubic *et al*., 1970; Al-Thukair and Golubic, 1991a,b; Al-Thukair *et al*., 1994). The percentages of bored ooids for all sites were calculated for 1989 and 1992. These percentages give us an indication of the status of euendolithic cyanobacteria for each site. Any decrease in the number of bored ooids provides a good indicator that populations of euendolithic cyanobacteria have declined as boring decreases, and there has been a significant decline in bored ooids for Khafji and Khawr between 1989 and 1992 (Table 1).

A persistent decrease in percentages of 15.48%, 11.58%, 5.74% and 5.55% has occurred for Khafji, Khawr, Zawr and Tarut consecutively, although this decline seems small in some sites when the data is compared. The long-term effects should be considered as the existing microbores in these ooids represent a cumulative record of microbial boring activities over time. A similar decline was detected in the number of live euendoliths and the number of colonies per ooid. In a rapid survey of randomly selected ooids from each site in 1989, approximately 50% of these ooids were bored and 25% were

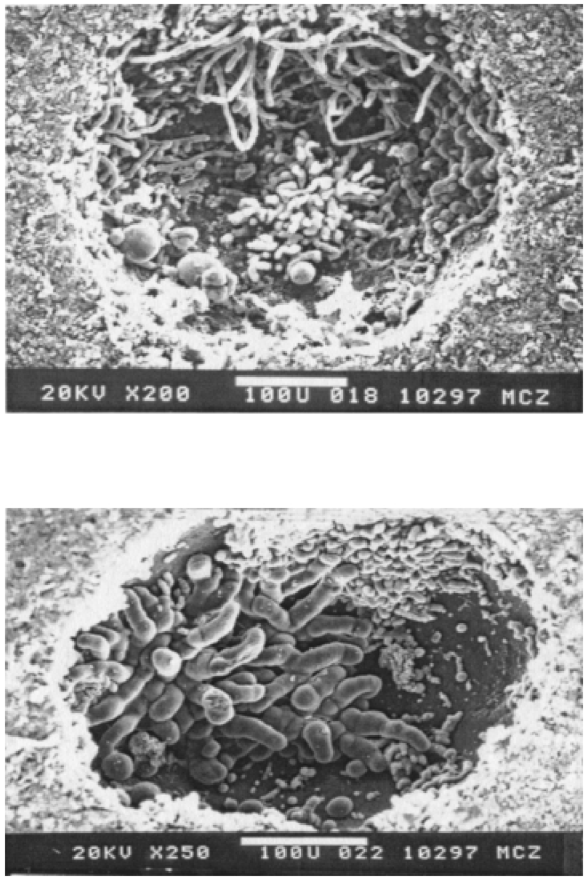

Fig. 2. A and B. Assemblage of species-specific euendolithic cyanobacteria microboring in two ooid grains from the shoals of Tarut and Khawr, respectively, visualized by resin-casting, followed by decalicification and SEM.
A. A mixed assemblage of microborings belonging to the following euendolithic cyanobacteria: *Hyella conferta*, *H. reptans*, *H. salutans*, *H. stella*, *Cyanosacus* sp.
B. A heavily bored spherical ooid grain, with microboring casts of *H. immanis*, *H. conferta* and *H. reptans*. Scale bars = 100 μm.

Table 1. Percentages of bored ooids in August 20, 1989, and August 23, 1992 sampling.

	Tarut % Bored (n)		Zawr % Bored (n)		Khawr % Bored (n)		Khafji % Bored (n)		Total % Bored (n)	
Depth	1989	1992	1989	1992	1989	1992	1989	1992	1989	1992
<1 m	71.32 (286)	66.11 (180)	37.75 (98)	28.75 (80)	79.36 (63)	67.69 (65)	50.77 (514)	35.29 (85)	57.44 (961)	52.68 (410)
1–2 m	62.90 (62)	56.67 (120)	42.20 (417)	37.87 (75)	49.69 (163)	37.76 (143)	*	*	46.10 (642)	44.37 (338)
>2 m	45.94 (111)	47.00 (100)	48.09 (131)	41.60 (137)	38.46 (39)	29.62 (54)	*	*	45.90 (281)	41.23 (291)
Total	64.05 (459)	58.50 (400)	42.72 (646)	36.98 (292)	55.09 (265)	43.51 (262)	50.77 (514)	35.29 (85)	51.85 (1911)	46.77 (1039)
% Decrease	5.55		5.74		11.58		15.48		5.08	

*No sample was taken.

Table 2. Distribution of six common euendolithic species by depth and site as the percentage of the total number of euendolithic colonies within ooids in 1989 sampling.

Site	Rept	Conf	Salut	Stell	Incon	Imma	Other
Depth < 1 m							
Tarut	58.0	0.0	1.1	9.3	13.0	3.3	15.3
Zawr	45.0	0.5	1.0	4.6	8.8	10.1	30.0
Khawr	50.0	36.0	0.0	2.2	2.6	3.5	5.7
Khafji	30.0	3.4	2.6	4.3	26.5	5.1	28.1
Depth 1–2 m							
Tarut	34.0	0.0	0.0	3.4	32.2	2.7	27.7
Zawr	44.0	0.0	0.0	8.8	19.1	10.0	18.1
Khawr	48.0	0.5	24.3	0.7	9.6	1.5	15.4
Khafji	–	–	–	–	–	–	–
Depth > 2 m							
Tarut	22.0	0.0	2.7	21.2	38.9	3.5	11.7
Zawr	17.0	0.0	0.0	3.7	37.0	7.4	34.9
Khawr	40.0	0.5	2.7	6.3	27.5	8.1	14.9
Khafji	–	–	–	–	–	–	–
Average (1989)	42.2	4.6	3.0	6.1	19.7	5.9	18.5

Rept, *Hyella reptans*; Conf, *H. conferta*; Salut, *H. salutans*; Stell, *H. stella*; Incon, *H. inconstans*; Imma, *H. immanis*.

inhabited by live euendoliths, compared with 44% and 10% in 1992. Similarly, the average number of colonies per ooid declined from 1.7 to 0.8. In a species comparison of the 1989 and 1992 data as a percentage of total number of euendolithic colonies residing in these ooids using the Kruskul–Wallis one-way analysis of variance ($P = 0.0001$), it was found that there was a significant difference between species(Tables 2 and 3). However, when the Student–Newman–Keuls method for a paired comparison was used to compare the percentages of each species for 1989–92, no significant change in species composition percentages occurred, indicating that all species were affected equally by oil pollution. *Hyella reptans* remains the dominant species of most of the sites, followed by *Hyella inconstans*. Depth only slightly affected the presence of *H. reptans* and *H. inconstans*.

Table 3. Distribution of six common euendolithic species by depth and site as the percentage of the total number of euendolithic colonies within ooids in 1992 sampling.

Site	Rept	Conf	Salut	Stell	Incon	Imma	Other
Depth < 1 m							
Tarut	54.2	0.0	3.4	12.1	16.7	5.0	8.6
Zawr	50.0	2.1	1.9	3.4	10.2	12.7	19.7
Khawr	46.7	33.8	0.3	1.2	2.1	4.4	11.5
Khafji	39.1	2.7	3.3	5.0	33.2	4.8	11.9
Depth 1–2 m							
Tarut	30.6	0.9	1.9	4.8	36.0	3.4	22.4
Zawr	46.3	1.1	0.3	10.2	23.5	11.3	7.3
Khawr	52.7	0.8	20.6	0.0	12.1	2.6	12.0
Khafji	–	–	–	–	–	–	–
Depth > 2 m							
Tarut	18.4	0.0	1.3	17.8	42.9	2.4	17.2
Zawr	13.1	0.1	0.0	5.8	35.2	8.4	37.4
Khawr	46.8	0.0	1.6	2.1	22.9	10.4	16.2
Khafji	–	–	–	–	–	–	–
Average (1992)	37.7	7.0	5.3	9.8	11.4	8.0	20.8

Rept, *Hyella reptans*; Conf, *H. conferta*; Salut, *H. salutans*; Stell, *H. stella*; Incon, *H. inconstans*; Imma, *H. immanis*.

The percentage of the number of colonies of *Hyella reptans* declines with depth, whereas it increases for *H. inconstans* in some sites, such as Tarut and Zawr. Considering the above results, it is apparent that the 1991 oil pollution caused a substantial decrease in the euendolithic cyanobacteria population of Arabian Gulf ooids. It is unknown whether this reduction will continue until it reaches a critical stage, as there has been no major clean-up, restoration or long-term studies in this area. However, it is worth mentioning that whereas the current observations reflect Arabian Gulf euendoliths, they are in agreement with most of those studies of marine microorganisms threatened by major oil spill incidents.

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