PROCEEDINGS
OF THE
FIFTH SYMPOSIUM
ON THE
NATURAL HISTORY OF THE BAHAMAS

Edited by
Lee B. Kass

Conference Organizer
Daniel R. Suchy

Bahamian Field Station, Ltd.
San Salvador, Bahamas
1994

Cover photo by
Sandra Buckner

Printed in USA by Don Heuer

ISBN 0-935909-52-4

SULPHATE-REDUCING BACTERIA ASSOCIATED WITH THE RHIZOSPHERE OF TROPICAL SEAGRASSES

Wendy P. Wong and Garriet W. Smith
Department of Biology
University of South Carolina at Aiken
Aiken, SC 29801

ABSTRACT

Anaerobic sulphate-reduction by bacteria occurring around root-rhizomes of the tropical seagrasses *Halodule wrightii*, *Syringodium filiforme*, and *Thalassia testudinum* obtained from the oligotrophic, marine waters and carbonate sediments of San Salvador, Bahamas were shown to exist. Bacteria were extracted from root-rhizome segments suspended in an artificial seawater solution and inoculated into a medium specific for sulphate-reducing bacteria. Under the conditions given, the presence of anaerobic sulphate-reducing bacteria was evident by the appearance of FeS in the growth medium. The purpose of the work described in this paper is to determine the existence of anaerobic sulphate-reducing bacteria associated with three species of tropical seagrasses and to analyze their relative abundance at different sampling sites on the island. Results show seasonal differences in the occurrence of sulphate-reducing bacteria associated with the seagrasses.

INTRODUCTION

Seagrass meadows form the basis of complex coastal marine ecosystems by establishing the base of the food web and acting as nursery grounds for many species of marine organisms. Seagrasses often are abundant in shallow-water sediments throughout the world's tropical and subtropical oceans (Hartog, 1970). These submergent, vascular plants (found in both eutrophic and oligotrophic waters) appear to have obligate and complex nutritional interactions with bacteria (Smith, 1987). An overall increase in nutrient status, diversity, and stability of the ecosystem results from seagrass-bacterial interactions (Smith, 1987). Tropical seagrasses play an important role in processing and cycling nutrients through high rates of productivity and maintaining a substantial associated microflora (Short, 1987). This is especially vital to the seagrasses occurring in the nutrient-poor waters and carbonate sediments of San Salvador, Bahamas. Despite the nutritional importance of the seagrass rhizosphere microflora, this population has not been well characterized (Ducate and Smith, 1992).

With the exception of beaches exposed to a strong surf, marine sediments tend to be anaerobic and chemically reducing beneath a relatively thin oxidized layer (Fenchel and Riedl, 1971). The high productivity of seagrass beds results in a high input of organics to the sediments and thus increases the demand for hydrogen acceptors (Fenchel and Riedl, 1971).

Sulphate, the most oxidized form of sulphur, is one of the major anions in seawater and is used by the sulphate-reducing bacteria, a group which is widely distributed in nature (Brock and Madigan, 1991). The ability to utilize sulphate as an electron acceptor for energy-generating processes is restricted to a very specific group of obligately anaerobic bacteria, the sulphate-reducing bacteria (Brock and Madigan, 1991). Seagrasses also decrease the availability of oxygen in the sediments by protecting the sediments from strong water turbulence thus increasing silt deposition (Fenchel and Riedl, 1971). Much of the seagrass detritus is therefore mineralized under anaerobic conditions (Fenchel and Riedl, 1971). Many of the bacteria associated with the anaerobic environment, e.g., the bacteria of the sulphur cycle, such as *Desulfovibrio* are also known to be nitrogen-fixers (Fenchel and

Riedl, 1971). Nitrogen-fixation by sulphate-reducing bacteria was suggested by Sisler and Zobell (1951). Postgate (1970) demonstrated nitrogen-fixation activity by three out of five *Desulfovibrio* strains. Therefore, the existence of the sulphur bacteria in the rhizosphere of tropical seagrasses may play a role in supplying nitrogen to plants living in carbonate sediments.

Decomposition of seagrasses is a series of anaerobic processes which take place in the region underneath the oxidized layer of all marine sediments. Sulphate-reduction is one of these vital anaerobic processes. This is due to the abundance of nonreduced SO_4 in seawater. The environment is affected chemically and biologically by the reduction of SO_4 to sulphide. In the presence of sulphides, phosphates become chemically available to the ecosystem. Phosphate is required for DNA synthesis and high energy compounds in all cells of the plant (Fenchel and Riedl, 1971).

The purpose of this study was to show the existence of anaerobic sulphate-reducing bacteria around the rhizosphere of tropical seagrasses and to investigate seasonal distribution at different sites.

MATERIALS AND METHODS

Core samples of the three seagrass species (*Halodule wrightii*, *Thalassia testudinum*, and *Syringodium filiforme*) were obtained from Graham's Harbor (Fig. 1) and French Bay in the summer and winter of 1992. One gram of root-rhizome tissue from each species/site combination was aseptically removed and placed in 9 ml of sterile artificial seawater. This suspension was vortexed for 3 minutes to remove bacteria adhering to the root rhizomes. A series of 5 tenfold dilutions were made of the inoculant. Glass screw cap tubes containing 4.5 ml of liquified Medium E (Postgate, 1979), (a medium specific for culturing sulphate-reducing bacteria) was inoculated with 0.5 ml of the root rhizome/seawater solution. After the medium solidified, one ml of 18% liquid agar solution was added to serve as a plug to restrict oxygen diffusion. A series of 5 tenfold dilutions were made of the inoculant. The tubes were incubated at 37°C. The appearance of FeS, a black substance in the medium, indicates sulphate-reduction, was assayed for daily. Subcultures were taken from tubes showing the most sulphate-reduction (the black accumulation of FeS).

A method was devised to approximate the rate of anaerobic sulphate-reduction. Serum vials containing 30 ml of modified Medium E (agar was eliminated) were inoculated under N_2 (to ensure an anaerobic environment) with bacteria from a subculture tube. These vials were placed on a shaker and rates of reduction were read spectrophotometrically at 600 nm over time.

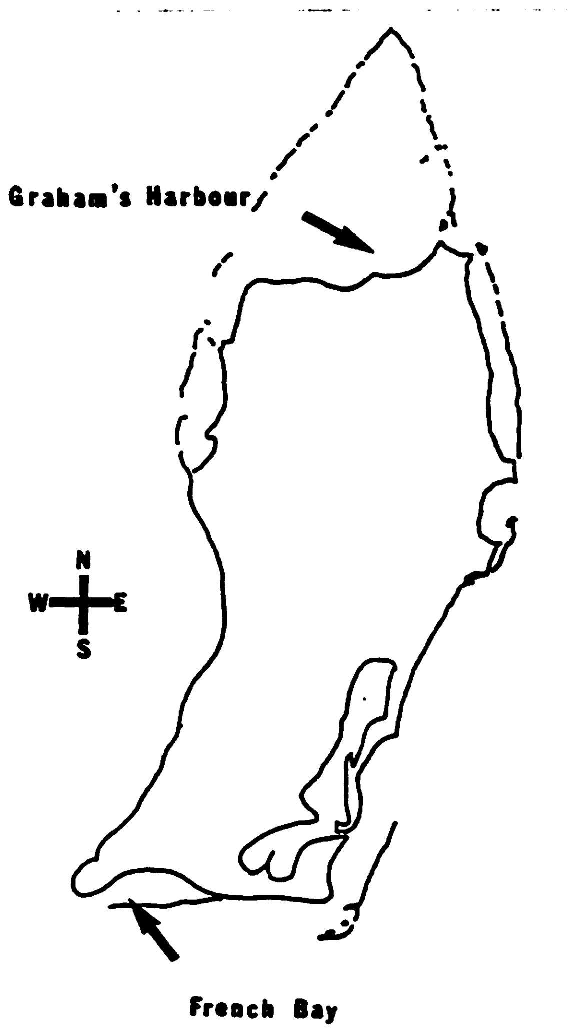

Figure 1. San Salvador Island, Bahamas

RESULTS AND DISCUSSIONS

Anaerobic sulphate-reducing bacteria were shown to exist on the root-rhizomes of the three tropical species; *Halodule wrightii*, *Syringodium filiforme*, and *Thalassia testudinum* obtained from San Salvador, Bahamas. The occurrence of anaerobic sulphate-reduction by seagrass bacteria were observed for two seasons. Results from the 1992 seasonal sulphate-reduction experiment by seagrass bacteria from French Bay and Graham's Harbour are shown in Table 1. Of the summer assay, a greater occurrence of

Table 1.1992 Seasonal Sulphate-Reduction by Seagrass Bacteria at French Bay and Graham's Harbour.

French Bay

		Summer			Winter		
		Day 0	Day 5	Day 10	Day 0	Day 7	Day 14
Thalassia	10^{-1}	0	4	4	0	4	4
	10^{-2}	0	0	0	0	0	0
	10^{-3}	0	0	0	0	0	0
	10^{-4}	0	0	0	0	0	0
	10^{-5}	0	0	0	0	0	0
		Day 0	Day 5	Day 10	Day 0	Day 7	Day 14
Syringodium	10^{-1}	0	3	3	0	3	3
	10^{-2}	0	1	1	0	0	0
	10^{-3}	0	0	0	0	0	0
	10^{-4}	0	1	1	0	0	0
	10^{-5}	0	0	0	0	0	0

Graham's Harbour

		Summer			Winter		
		Day 0	Day 5	Day 10	Day 0	Day 9	Day 16
Thalassia	10^{-1}	0	4	4	0	3	2
	10^{-2}	0	0	2	0	0	0
	10^{-3}	0	0	1	0	0	0
	10^{-4}	0	0	4	0	0	0
	10^{-5}	0	0	2	0	0	0
		Day 0	Day 5	Day 10	Day 0	Day 9	Day 16
Syringodium	10^{-1}	0	4	4	0	4	4
	10^{-2}	0	1	4	0	0	0
	10^{-3}	0	0	3	0	0	0
	10^{-4}	0	0	3	0	0	0
	10^{-5}	0	0	1	0	0	0
		Day 0	Day 5	Day 10	Day 0	Day 5	Day 16
Halodule	10^{-1}	0	0	1	0	0	4
	10^{-2}	0	2	4	0	0	0
	10^{-3}	0	0	1	0	0	0
	10^{-4}	0	4	4	0	0	0
	10^{-5}	0	4	3	0	0	0

NOTE: Readings are based on the number of tubes showing sulphate-reduction out of four tubes per dilution over time.

Table 2. Rates of Sulphate-Reduction by Seagrass Bacteria Isolated from French Bay Samples

		Day 4	Day 5	Day 8
Thalassia	1a	.199	.065	.494
	1b	.077	.051	.168
	1c	.141	.074	.262
	1d	.163	.157	.248
Syringodium	1a	.414	.308	.460
	1b	.442	.396	.558

sulphate-reducers were shown to exist as compared to French Bay. By day 10, sulphate-reducers were shown to exist in at least one tube out of each dilution. Of the winter assay, these previous observations did not occur. The existence of sulphate-reducers were only shown to occur in the first dilution (10^{-5}).

A comparison of relative abundance was made within each site. In French Bay, a slightly higher occurrence of sulphate-reducers was shown in the summer than in the winter. In Graham's Harbour, there is a significant difference in the occurrence of sulphate-reducers in the summer than in the winter. Winter and Summer French Bay tubes (10^{-1}) showed sulphate-reduction within one day of inoculation.

Occurrence of anaerobic sulphate-reducing bacteria from French Bay were shown to be higher than those of Graham's Harbor. These results may be explained by the environmental differences between the two sites. Located on the north end of San Salvador is Graham's Harbor, a shallow-water (1 to 2 m), low-energy, semi-enclosed lagoon. French Bay is located on the southern end of the island where current velocities are higher. The increased water volume may provide more nutrients to the plants, increasing root exudation rates of organic compounds and thus decreasing dissolved oxygen concentrations in the sediment. This could lead to higher sulphate-reducing populations at the southern site. Sulphate-reduction by seagrass bacteria isolated from French Bay samples of *Thalassia* and *Syringodium* seemed to show an overall increase in abundance over an eight day period (Table 2). Research is in progress to understand the role of the sulphurbacteria in the seagrass ecosystem.

ACKNOWLEDGEMENTS

This work was supported by a grant from The Center for Field Research, The Bahamian Field Station and a NSF grant to the Ruth Patrick Science Education Center. We would like to thank Dr. Jeffrey Priest and Dr. James R. Yates for scientific insight and criticism. This is contribution No. 997 of the Belle W. Baruch Institute of Marine Biology and Coastal Research.

LITERATURE CITED

Brock, T.D., and M.T. Madigan. 1991. Biology of Micro-organisms. 6th ed. Prentice Hall. Englewood Cliffs, New Jersey 07632. p.588.

Ducate, L., and G.W. Smith. 1992. Pure Culture studies of seagrass rhizoplane isolates. *In* Proc. 4th Sym. Nat. His. Bahamas. pp.23-29.

Fenchel, T., and R. Riedl. 1971. The sulfide system. A new biotic community underneath the oxidized layer of marine sand-bottoms. Mar. Biol. 7: 255-268.

Hartog, C. Den. 1970. The Seagrasses of the World. North Holland Publ. Co., Amsterdam.

Postgate, J.R. 1970. Biological Nitrogen Fixation. Nature, Lond., 226:22-27.

Postgate, J.R. 1979. The Sulphate-Reducing Bacteria. Cambridge University Press: Cambridge, London, New York, Melbourne.

Short, F.T. 1987. Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. Aqat. Bot. 27: 41-57.

Short, F.T., W.C. Dennison, and D.G. Capone. 1990. Phosphorus-limited growth of the tropical seagrass *Syringodium filiforme* in carbonate sediments. Mar. Ecol. Prog. Ser. 62:169-174.

Sisler, F.D. and C.E. Zobell. 1951. Nitrogen Fixation by Sulfate-reducing Bacteria Indicated by Nitrogen/Argon Ratios. Science 113: 511-12.

Smith, G.W. 1987. Microbial contributions to the growth and degradation of tropical seagrasses. *In* Proc. 2nd Symp. Bot. Bahamas. R.R. Smith (ed.). pp. 45-53.