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EVIDENCE OF AZOTOBACTER AND RHODOSPIRILLUM AS ASSOCIATES OF THALASSIA TESTUDINUM

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ABSTRACT

Free-living nitrogen-fixing bacteria, Azotobacter agilis and Rhodospirillum rubrum were isolated from rhizomes and leaves of the seagrass Thalassia testudinum in Tampa Bay, Florida, but not from the surrounding sediments. Thiobacillus trautweinii and the anaerobes Thiobacillus denitrificans, Methanobacterium omelianskii, and Desulfovibrio desulfuricans were isolated from rhizomes and also from sediment samples taken from a site completely lacking in seagrass growth.

The presence of Azotobacter and Rhodospirillum on the seagrass but not in sediments suggests that they may contribute to the fixed nitrogen budget of the habitat.

INTRODUCTION

Although bacteria capable of fixing nitrogen have been reported in marine waters (Maruyama, et al., 1970), the species involved and quantitative data are not available.

Patriquin (1972) and Patriquin and Knowles (1972) have speculated that bacterial nitrogen fixation is a major source of nitrogen compounds for the seagreass, Thalassia testudinum (König and Sims) that covers several thousand square miles of shallow bottom along the entire Florida Gulf coast. This suggested to the writer that efforts to isolate nitrogen-fixing bacteria from seagrasses and bottom sediments of these communities might provide useful information, and such a study was carried on while the writer was in residence in the Department of Marine Science, University of South Florida, St. Petersburg Campus, during the summer of 1973.

MATERIALS AND METHODS

Leaves, rhizomes, surface sediments, and sediment cores were obtained from Tampa Bay, July 10, 1973. Surface core sediments were taken at the same time from a site which was devoid of seagrasses. The material was handled to avoid contamination from any outside source.

To determine whether or not Azotobacter or other nitrogen-fixers were present in or on the inoculum, 80 mm segments of both young leaves and rhizomes and one gram samples of surface and core sediments (taken at 3 and 6 inches) were placed in screw-cap prescription bottles containing 40 ml of a modification of Burke's N-free salt solution (Wilson and Knight, 1952). Aged sea water was used and $CaCl_2$ was substituted for $CaSO_4$. Initial incubation with inoculum present was of 24 hours duration at 22-24°C. in the dark. At the end of this period, leaf and rhizome segments were removed. Decantation was used to separate the culture from the sediment samples with transfer into clean, sterile containers and selective media.

From the original inocula, 1 ml from each was transferred by sterile pipette into the following selective media: N-free Burke's medium with 0.2% ethanol substituted for glucose as carbon source (Breed, et al, 1957); media for Thiobacillus (cultured both aerobically and anaerobically), Desulfovibrio, Methanobacterium and Clostridium (anaerobically) according to Stanier et al, 1963. Culture tubes with screw cap filled to the top were used for anaerobes, and prescription bottles with 40 ml aliquots of the medium were used for aerobes. The original inoculum was maintained for Azotobacter culture. All cultures were made in triplicate and all were incubated in the dark at 22-24°C.

At the same time, pour plates for anaerobic culture were made with the selective media by aseptically introducing 1 ml aliquots of culture in the sterile plate which was allowed to dry before pouring the sterile medium. The plates, filled to the top, so that the cover rested on the agar surface, were wrapped in foil and placed in tightly closed jars in which grass seed was sprouting.

The original inocula in N-free media were cultured for eight days after which 1 ml was transferred by pipette into fresh broth medium and plates of N-free agar were streaked. At this time 1 ml of inoculum was transferred into tubes of sterile nutrient broth. Washed colonies were examined under ultra violet light.

The Rhodospirillum culture and that of the aerobic Thiobacillus were transferred by sterile pipette after eight days of incubation into fresh selective media. Streak plates were made by introducing 0.1 ml of inoculum on top of the selective media and spreading with a sterile glass rod.

Gram staining and microscopic examination for morphology was done following four days of incubation in broth and from the plate colonies after seven days for all cultures.

To confirm positive nitrogen fixation, the following procedure was followed: Sterile N-free agar medium was aseptically dispensed in sterile plastic culture tubes with screw cap to form agar slants and agar deeps. Following fourteen days of incubation in selective medium, each culture was transferred from both broth and plate colonies by sterile needle. The cultures were examined after fourteen days of incubation.

RESULTS

Typical Azotobacter colonies were obtained from leaves and rhizomes of Thalassia. There was no evidence of Azotobacter in surface or core sediments taken at either site. Positive identification of Azotobacter agilis Beirjink was made using the following criteria (Breed, et al, 1957); gram-negative rods, 4-5 microns in length, often almost spherical; the colonies fluoresced under ultraviolet light in the range of 3600 A; no pigmentation developed after four weeks of incubation on N-free agar; nutrient broth was turbid seventy-two hours following inoculation.

From the N-free medium using ethanol as carbon source, Rhodospirillum rubrum (von Esmarch) Molisch was isolated but only from leaf and rhizome. There was no evidence of growth in any culture taken from surface or core sediments from either site.

On surface and core sediments from both sites and on the rhizomes of Thalassia testudinum (using the criteria of Breed, et al, 1957), the following organisms were identified: Thiobacillus trautweinni Bergey, Thiobacillus denitrificans, Beirjinck, Methanobacterium omelianskii Barker; Desulfovibrio desulfuricans Kluyver and van Neil, and Clostridium pasteurianum Winogradsky.

Following transfer into N-free media, all cultures showed growth after two weeks of incubation.

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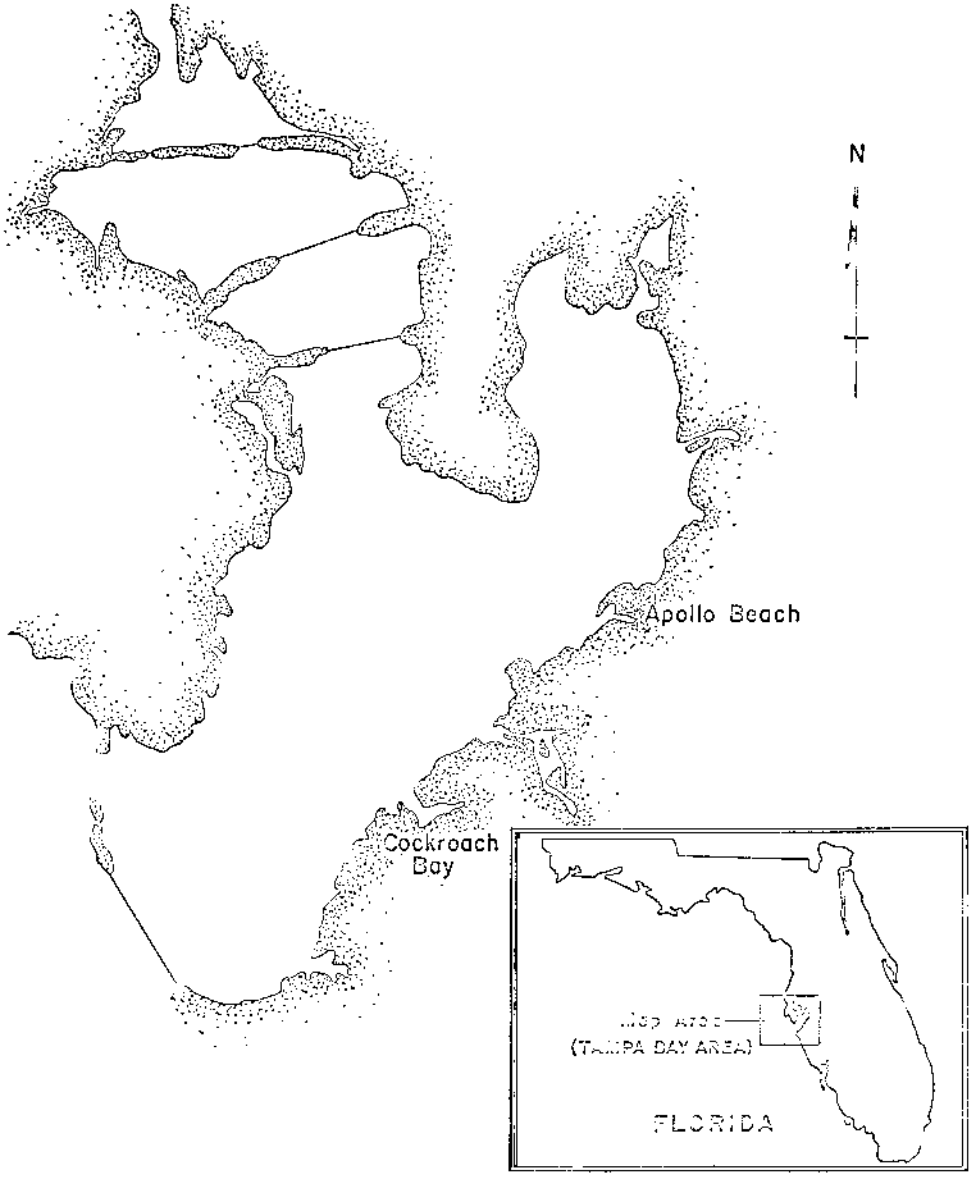
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