Iron oxidizing Bacteria from the Great Sippewisset Salt Marsh

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Introduction

In salt marsh sediments ferrous iron reacts with polysulfides or hydrogen sulfide to produce iron sulfide (FeS) and the insoluble mineral pyrite (FeS_2). In a dynamic and apparently seasonal cycle, pyrite is alternately deposited and dissipated as the result of oxidation (1). This so called "iron cycle" can have a strong influence on sediment chemistry. The primary reason for this is that oxidation of the ferrous ion-containing minerals causes acidification and production of high levels of sulfate according to Equation 1.

Equation 1 $$FeS_2 + 7.5O_2 + H_2O \rightleftharpoons Fe_2(SO_4)_3 + H_2SO_4$$

As a result of this oxidation reaction the pH in marsh sediments can drop below 4 (1). At neutral pH under oxic conditions the rate of oxidation can result in half-lives for ferrous ion on the order of minutes (2). This high rate of autooxidation, and the relatively small amount of free energy released upon oxidation (on the order of 10 kcal/mole ferrous ion), makes this transformation a particularly challenging one for microbes to use as a life sustaining energy source. Never the less, there are some acidophillic bacteria (primarily from the genus *Thiobacillus*), a few archea (*Sulfolobus* and *Acidianus*) and a neutrophilic bacterium (*Gallionella ferruginea*) that can use ferrous ion as an energy source (3, 4, 5). None of these organisms have been isolated from marine environments, and it is not know if ferrous oxidation in salt marsh sediments is microbially catalyzed or strictly abiotic. This project was an attempt to use enrichment culture techniques to determine if Great Sippewisset Marsh sediments contain microbes that oxidize ferrous ion. The presence of such organisms would suggest that iron oxidation, which profoundly affects sediment chemistry, and presumably ecology, could be biologically catalyzed in salt marsh sediments.

Methods

Enrichment cultures were established based on modifications to a medium commonly used to culture *Thiobacillus* species. Constituents (per liter) were 0.4g $(NH_4)_2SO_4$, 0.4g $MgSO_4$, 0.4g K_2HPO_4, 112g $FeSO_4.7H_2O$, 20g NaCl, 0.1mg $MnCl_2$, 0.19mg $CoCl_2$, 0.144mg $ZnSO_4$, 0.006mg H_3BO_3, 0.024mg $NiCl_2.6H_2O$, 0.002mg $CuCl_2.H_2O$, 0.036mg $NaMoO_4.2H_2O$, pH adjusted to 5.0 with HCl. The $FeSO_4.7H_2O$ (freshly prepared filter sterilized aqueous solution pH 5.0) was added after all other components were autoclaved, And some cultures also contained 20 mM Na_2S. The medium was cooled under an atmosphere containing 20% CO_2/80% N_2. 5 ml cultures (in tubes sealed with butyl rubber stoppers) were inoculated with approximately 50mg of sediment collected from areas of Great Sippewisset marsh characterized by dense *Spartina* grass growth, or sediments collected from tidal pools surrounded by *Spartina*. After inoculation liquid cultures were unstoppered and loosely capped to allow establishment of oxic conditions.

For solid agar "shakes" molten agar (55C, plant tissue culture grade) was added and mixed to a final concentration of 0.5%, after which the tubes were loosely capped to establish an oxygen gradient. 220 liquid and 220 solid agar cultures using four different inocula were assembled. Both liquid and solid agar cultures were incubated in the dark at room temperature and at 30C. Secondary enrichments of iron-precipitating colonies were made on identical media in serum bottles containing 20% CO_2/80%N_2 (13 psi) or 20% CO_2/80% N_2 plus 10 ml air (final pressure approximately 18 psi).

Results

After 10 days, yellow/light orange precipitates formed approximately 4 mm below the surface of solid agar cultures (incubated at room temperature) using one of the four inocula. After 15 days incubation at room temperature identical precipitates formed in cultures using the other three inocula. The precipitates formed in shapes (ellipsoids, spheres) and sizes (from less than 0.5 mm to 3 mm) consistent with colonial growth of bacteria. Most enrichments (approximately 85%) did not form these precipitates, and those that did contained only one or a few apparent iron precipitating colonies. No precipitates formed in uninoculated media, in any of the liquid cultures, or in cultures incubated at 30C after 17 days. The color of the precipitated material in enrichment cultures was identical to that formed by autooxidation of ferrous sulfate stock solutions and had the same phase-dark appearance using phase contrast microscopy. The upper 5-6 mm of solid agar cultures containing 20 mM Na_2S, which were opaque/black in color due to FeS precipitation, turned opaque/white after 15 days, indicating the establishment of oxic conditions in the upper layer and thus an oxygen gradients in the tubes. A few of the Na_2S containing cultures formed a layer (less than approximately 0.5 mm thick) of the yellow/light orange precipitates very near the agar surface. The pH within the agar regions containing the precipitates was (pH paper) in all cases less than 4, and in some cases closer to 3.

Several morphotypes of bacteria were detected microscopically in every sample where iron precipitates formed. Although cocci were apparent, the predominant morphology was rods, with the majority being non-motile while a few were highly motile. In several samples high densities of morphologically indistinguishable rods were imbedded in what appeared to be the iron precipitates. These cells stained green/blue with acridine orange but had no detectable autofluorescence.

Secondary enrichment cultures using 5 microliter inocula from iron oxidizing colonies and incubated either in the primary culture medium (solid or liquid) or primary medium under a CO_2 rich atmosphere (see above) failed to show growth after 4 or 5 days. After 2 days cells carried over from the inoculum in these secondary enrichments appeared as phase light under phase contrast microscopy but retained the rod morphology. No cells were visible in these enrichments after 4 days.

Conclusions

Substantial evidence was collected in support of Sippewisset Marsh microaerophilic, acid tolerant bacteria that catalyze oxidation of ferrous ion. This evidence includes bacterial growth under very acidic conditions with concomitant formation of what are presumably ferric precipitates. Although the putative iron-oxidizing bacteria were not cultured past the primary stage, this is probably due to the presence of an essential component in the primary inoculum, most likely a source of organic carbon.

The existence of iron-oxidizing bacteria in marsh sediments suggests they might play important roles in sediment chemistry and microbial ecology. Although it has been speculated that microbes in salt marsh sediments might be responsible for ferrous mineral oxidation (1), this work apparently represents the first attempt to experimentally address this issue. The demonstration that iron-oxidizing bacteria can be cultured from marsh sediments opens opportunities for future work in this area.

References

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