Isolation of Iron-Reducing Bacteria from Christina River Basin-Critical Zone Observatory Site

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Introduction

A vast range of microorganisms have been identified with the ability to use a variety of energy sources via a number of different chemical and metabolic processes (Gold et al. 1992). Besides utilizing sunlight, water, oxygen, etc., all of which are necessities of common life forms, populations of microorganisms can also utilize unusual sources for the basis of survival. Before the dawn of photosynthesis, it has been postulated that several forms of microbial respiration, including iron reduction, were the dominant chemical processes on early Earth (Vargas et al. 1998; Weber et al. 2006).

Iron, as the fourth most abundant element in Earth's crust, serves as a vital nutrient for bacterial growth. Beyond that, Fe-oxides also function as essential electron acceptors for iron-reducing microorganisms (Lovley et al., 1993; Vargas et al., 1998; Weber et al., 2006). The reduction of Fe(III) is associated with essential nutrient cycling processes in soils and sediments. For instance, poorly crystallized iron provides surface areas for organic carbon adsorption and coprecipitation leading to an iron-carbon complexation (carbon stablization and sequestration) and therefore, prevents increased carbon fluxes to the atmosphere (Eusterhues et al. 2013). However, microorganisms (mainly iron-reducing bacteria) can obtain energy through the reduction of Fe(III) to Fe(II), and consequently facilitate de-sequestering/consumption of the stored carbon. Thus, examination of iron-reducing bacteria will improve our understanding of carbon fluxes, and in a broader sense, aid in addressing global carbon and iron cycling.

Materials and Methods

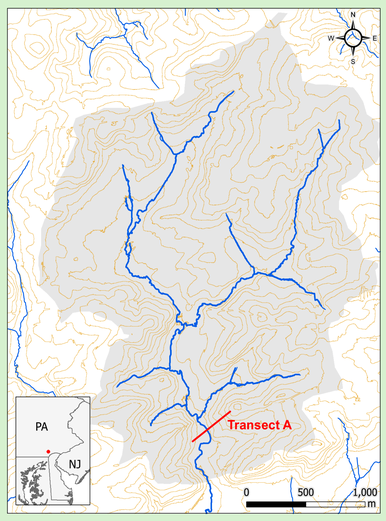

Fig. 1 White Clay Creek, a well studied and instrumented site at Christina River Basin Critical Zone Observatory.

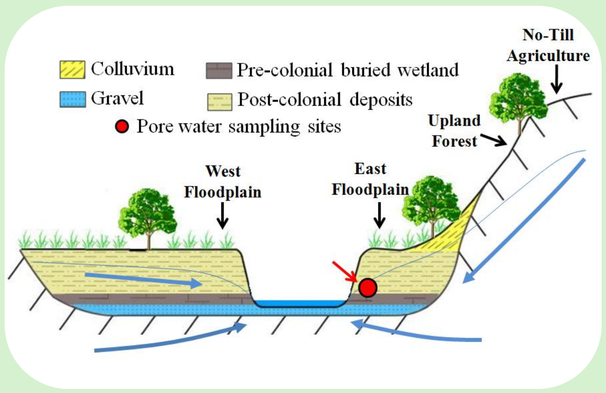

Fig. 2 A schematic map showing Transect A and the pore water sampling location.

Bacterial Culture Enrichment

Growth Medium

•Defined lactate, Fe-NTA medium with vitamins and minerals as previously described in Lovley et al., 1993.

Enrichment under Anaerobic Conditions

•Growth medium was purged with N_2-CO_2.

•Bottles were sealed with butyl rubber stoppers and secured with aluminum caps.

•Cultures showing significant growth were streaked onto Fe-NTA plates in glove box for single colony isolation.

DNA Extraction, PCR-DGGE, and Sequencing

DNA Extraction and Isolation

•Microbial genomic DNA from the isolates and enrichments were extracted using MoBio PowerSoil DNA Isolation kits.

PCR Amplification of 16S rRNA Genes

•For Sanger sequencing: primers 27F and 1492R

•For PCR-DGGE: 1070F and 1392R (GC)

Gel Electrophoresis

•PCR products were verified on a 1.5% agarose gel with a negative control.

Denaturing Gradient Gel Electrophoresis (DGGE)

•DNA fingerprinting was performed via PCR-DGGE, a vertical polyacrylamide gel with a 40-70% gradient (Muyzer et al., 1993; Kan et al., 2006).

Sanger Sequencing and Phylogenetic Analysis

•Clean sequences of 16S rRNA genes were blasted against GenBank.

•Phylogenetic tree was constructed with MacVector.

Data and Results

Isolates in Growth Media Bottles

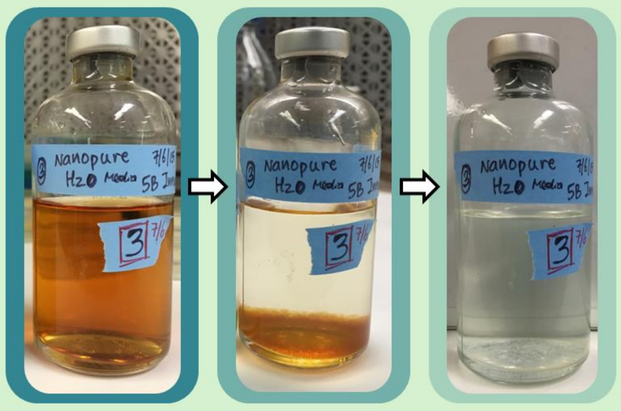

Fig. 3 Bacterial growth in Fe-NTA medium

•Growth of bacteria were monitored by observation of turbidity/cell density.

•Color change indicated microbial iron reduction capability.

PCR-DGGE

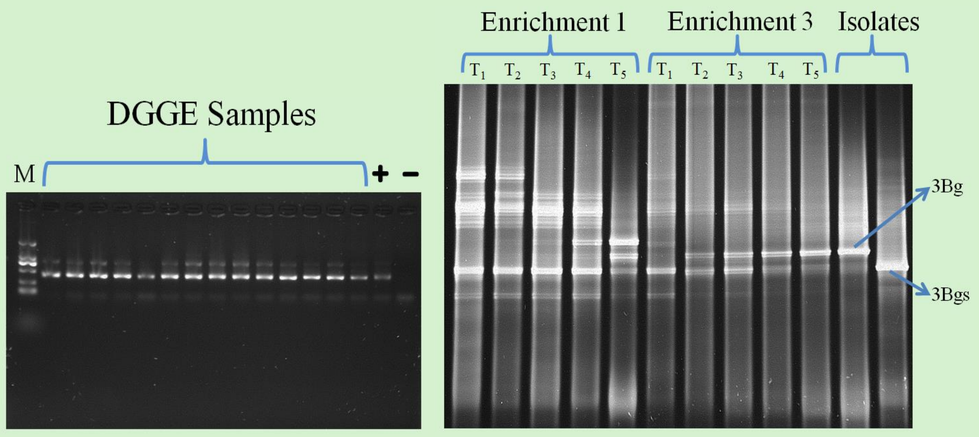

Fig. 4a PCR products on agarose gel

Fig. 4b DGGE gel image

•Population shifts were observed during the enrichment time series.

•Number of bands were reduced for both Enrichment 1 and 3 (Fig. 4b).

•Two dominant Fe-reducing bacteria were isolates from Enrichment 3 and represented distinct bacterial groups.

DNA Sequencing and Phylogenetic Analysis

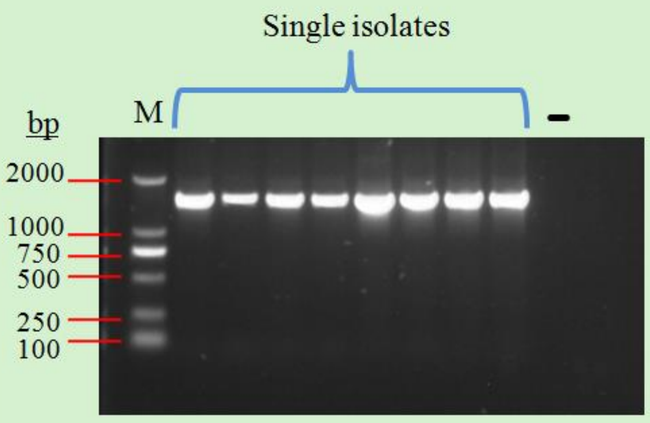

Fig. 5a PCR products used for sequencing

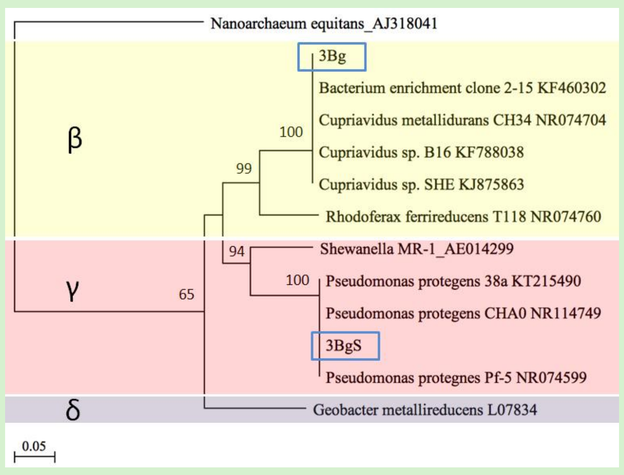

Fig. 5b Phylogenetic tree from sequence data

Epifluorescence Microscopy with SYBR Gold Staining

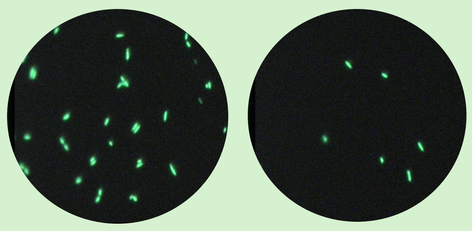

Fig. 6a Isolate 3Bg (*Cupriavidus* sp.)

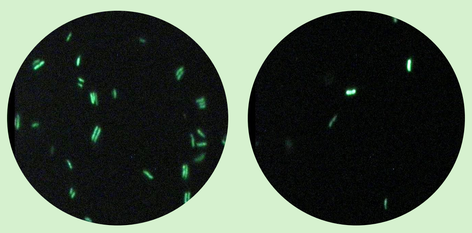

Fig. 6b Isolate 3Bgs (*Pseudomonas* sp.)

Discussion

•The population of iron-reducing bacteria is abundant within the soils of White Clay Creek.

•More than one group of iron-reducing bacteria was isolated from the sediments, suggesting the presence of multiple populations that can utilize iron.

•Future investigations include further characterization of the isolated bacteria and their impacts on the iron-carbon complexation.

References

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Acknowledgements

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